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# The epigenome of neurodegenerative disease: Novel strategies to treat dementia

Andre Fischer[1]

[1]European Neuroscience Institute, Laboratory for Aging and Cogntive Diseases, Grisebach Str 5, 37077 Göttingen, Germany[1]

Changes in gene expression in the brain may underlie cognitive deficits inherent to both normal aging and neurodegenerative disease. However, the mechanisms underlying pathological alterations in the brain transcriptome are little understood. Epigenetic mechanisms such as histone acetylation have been shown to be important for memory processes in the adult brain. There is now accumulating evidence that altered chromatin plasticity and histone acetylation are also involved in cognitive aging, neurodegeneration, and neuropsychiatric diseases. Histone deacetylase (HDAC) inhibitors exhibit neuroprotective and neuroregenerative properties in animal models of various brain diseases. As such, targeting the epigenome, for example via HDACs, seems to be a promising therapeutic strategy. In this presentation, I will discuss the current knowledge on HDAC proteins and the possible function of distinct histone modifications in neurological diseases. I will also address the interaction of histone-modifications with other epigenetic marks such as changes in the entire RNAome during disease pathogenesis. I will argue that better knowledge of those processes will aid in the development of diagnostic tools and help in designing potent treatment for neurological disorders.

# Neuronal mechanisms of attention in monkey visual cortex

John HR Maunsell[1], Marlene R Cohen[1]

[1]Harvard Medical School, Department of Neurobiology, 220 Longwood Avenue, 02115 Boston, USA

Visual attention improves perception for an attended location or feature and also modulates the responses of sensory neurons. In laboratory studies, the sensory stimuli and task instructions are held constant within an attentional condition, but despite experimenters' best efforts, the attention of a subject likely varies from moment to moment. Electrophysiological studies based on single neurons have been unable to use neuronal responses to identify attentional fluctuations and determine whether these are associated with changes in behavior. We show that an instantaneous measure of attention based on the responses of modestly sized neuronal populations in area V4 of rhesus monkeys can reliably predict large changes in the animal's ability to perform a difficult psychophysical task. Unexpectedly, this measure shows that the amount of attention allocated at any moment to locations in opposite hemifields is uncorrelated, suggesting that animals allocate attention to each stimulus independently.

# Memory consolidation during aging: The role of histone acetylation along gene-coding regions

Shahaf Peleg[1]

[1]European Neuroscience Institute Göttingen (ENI-G), Grisebachstr. 5, 37077 Göttingen, Germany

During aging, a gradual decline is frequently observed in the performance of cognitive and memory tasks. Importantly, aging is also the major risk factor for neurodegenerative diseases, which affect millions of people worldwide. The reasons for the decline in brain function during aging are however not well understood.

Previous studies suggest that remodeling of the chromatin structure via histone acetylation, a process modulating the availability of genes for transcription, may play a central role in memory formation. The acetylation of histones is regulated by two main classes of proteins, histone-acetyltransferases (HATs) and histone-deacetylases (HDACs). Interestingly, the inhibition of HDACs, has already been implicated with facilitated learning ability and increased neuronal plasticity.

We demonstrate that during memory consolidation, hippocamapal Histone 4 lysine (K) 12 acetylation levels is uniquely dysregulated in older mice when compared with younger mice. Based on the results obtained by using the ChIP-seq technique on a whole hippocampus tissue, we were able to propose a model by which histone acetylation contribute to age-associated memory impairment. In addition, we found that the administration of HDAC inhibitors, which elevate H4K12ac, is able to reinstate learning-induced gene expression and memory function in older mice. Our work reveals that H4K12ac may serve as a novel therapeutic target to treat age-associated memory impairment. Finally, our model of how H4K12ac is linked to gene expression dysregulation might account for various age-associated maladies.

# Non-invasive near-infrared fluorescence imaging of stroke pathophysiology

Jan Klohs[1]

[1]Institute for Biomedical Engineering, ETH & University of Zurich, Zurich, Switzerland
[2]Center for Stroke Research Berlin (CSB), Department of Experimental Neurology, Charité - University Medicine Berlin, Germany
[3]German Heart Center, Berlin, Germany

Light in the near-infrared (NIR) region between 700–900 nm can penetrate deep into living tissue, thereby offering a unique opportunity to use near-infrared fluorescence (NIRF) imaging techniques to detect and visualize fluorescent probes in the intact organism. The technique relies on the technical ability to spatially resolve and quantify fluorescence emission in-vivo. Here, we present the design and construction of a NIRF imaging system for small animals. The system can be operated in epi-fluorescence mode as planar NIRF imaging. In this mode, the fluorescence intensity distribution at the sample surface is analyzed. Using an in-vivo phantom we could show that planar NIRF imaging has a high detection sensitivity and is capable of detecting pmol amounts of fluorochromes. However, residual absorption leads to signal attenuation by one order of magnitude per centimeter of tissue. In addition, photons are severely scattered at depths exceeding 0.5 mm, rendering the extraction of accurate spatial information difficult. Quantitative analysis of fluorescence emission can be achieved using fluorescence molecular tomography (FMT). FMT uses sequential image acquisition schemes and application of an inversion code for three dimensional reconstruction of fluorochrome distribution. The reconstruction leads also to an improved image resolution of the order of 1 mm as compared to planar NIRF techniques, where image resolution is about 2-3 mm with no information of depth.

An increasing number of fluorescent probes emitting in the NIR have been developed. We present examples for the successful application of NIRF imaging to assess relevant pathophysiological processes in a mouse model of stroke (middle cerebral artery occlusion, MCAO). NIRF probes can be classified by their mechanism of contrast generation as non-specific, targeted and activatable probes. Non-specific fluorescent probes are usually NIR dyes which achieve contrast by distributing differentially in tissue based on the pharmacokinetic properties. We could show that NIRF-labeled albumin can be used for non-invasive detection of blood-brain barrier impairment after MCAO. However, circulating NIRF probe creates a high degree of background fluorescence making reducing attainable contrast. Targeted NIRF probes consist of a NIR dye and a targeting moiety and achieve contrast when probe is bound to the target while unbound probe fraction is cleared from the circulation with improved contrast. Using a fluorescently labeled monoclonal antibody against the CD40 receptor we were able to specifically visualize brain inflammation in mice after MCAO. Further gain in contrast can be made by the use of activatable probes. Activatable probes consist of fluorescent dye molecules in close proximity to each other, leading to dark quenching of the fluorescence. The fluorescence emission is recovered by enzyme-mediated cleavage. We have used a NIRF probe which can be activated by matrix metalloproteinases (MMPs). We have studied MMP activity at different stages after MCAO and to monitor treatment response with an MMP inhibitor.

Although, the non-linear dependence of fluorescence signal and depth makes quantification technically difficult and the scattering of the light limits the achievable resolution of the method, it is expected that NIRF imaging will continue to play a pivotal role in studying animal models of neurological disease and for the pre-clinical development of drugs.

# Visualizing circuits in the visual system

Joshua Richard Sanes[1]

[1]Harvard University, Center for Brain Science, 52 Oxford, 02138 Cambridge MA, USA

Formation of neural circuits requires that axons recognize appropriate cells, and even appropriate parts of cells, upon which to synapse. As an orderly and accessible part of the brain,the retina is well suited for analyzing mechanisms and molecules that underlie these processes, and for relating developmental events to adult circuit function. In the retina, amacrine and bipolar cells form synapses on retinal ganglion cells (RGCs) in the inner plexiform layer (IPL). The visual features to which different RGC subtypes respond depend on what inputs they receive is specific sublaminae in the IPL. We have therefore sought molecules that mark RGC subtypes and mediate lamina-specific connectivity. Candidates include members of the immunoglobulin superfamily, such as Sidekicks, Dscams and JAMs, and members of the cadherin superfamily, such as Class II and protocadherins. I will discuss our progress toward identifying and testing such candidates. I will also discuss how transgenic mouse lines generated in the course of these studies have allowed us to uncover cell types and circuits that were not observed in previous studies that treated RGCs as a single group.

# Neurobiology of Insect Acoustic Communication

Berthold Hedwig[1]

[1]Department of Zoology, Downing Street, Cambridge CB2 3EJ, UK

Insect acoustic communication is best known in cicada, grasshoppers, crickets and bush-crickets. These species use far field acoustic signalling for intraspecific communication, because mate finding is a challenge in dense and cluttered vegetation. Sound signals, produced by timbals or stridulatory organs can reach more than 100 dB SPL, cover sonic and ultrasonic frequencies, and are species-specific and genetically fixed. As sound has a transient nature, repetitive signal patterns are used to enhance the chances of being heard. In these insects, different hearing organs with a tympanic membrane and sensitive primary auditory afferents have evolved to pick up acoustic signals and to forward auditory activity to the central nervous system. Generally males produce a calling song to attract females or to evoke a female auditory response. In either case acoustic signalling guides a phonotactic approach towards the other sex. Consequently the neural basis of signal generation and auditory processing are in the centre of neurobiological research which during the last decades has especially focussed on crickets as a model system. So what are the neural mechanisms underlying acoustic communication?

In crickets singing behaviour is controlled by the brain via descending command neurons, which drive the singing motor network in the ventral ganglia. Song patterns are generated by rhythmic movements of the front wings and as the wing motoneurons are located in the 2nd thoracic ganglion it had been assumed that the same ganglion will also house the singing central pattern generator Exploring the CNS with intracellular recordings, however, revealed interneurons in the abdominal ganglia as key players for singing motor pattern generation. While singing, the males face a fundamental neurobiological problem as their own auditory pathway is exposed to the self generated sound pattern which could desensitize their hearing. Crickets, however, use a corollary discharge mechanism that reduces the response of auditory neurons during singing.

Female acoustic orientation behaviour has been analysed using trackball systems. The animals show hyper-acute directional steering and respond to changes in the direction of acoustic stimulation as small as 1 deg off their length axis. This acuity is reflected in corresponding tympanic membrane vibrations and afferent activity. At the level of thoracic auditory interneurons directional contrast is enhanced by reciprocal inhibition and auditory activity forwarded to the brain. Female phonotaxis is tuned to the temporal structure of the species-specific male calling song but what are the neural mechanisms underlying the recognition of the song? We identified a network of local auditory brain neurons. Some of these neurons exhibit a tuning towards the temporal pattern of the male's calling song based on sequences of excitatory and inhibitory synaptic interactions. In terms of auditory processing temporal filtering occurs at a very early stage within the brain. Pattern recognition finally must be linked to descending pre-motor interneurons that control phonotactic walking and steering.

# The memory function of sleep

Jan Born[1]

[1]Department of Neuroendocrinology, University of Lübeck, 23538 Lübeck, Germany

Whereas memories are encoded and retrieved optimally only when the brain is awake, the consolidation of memories requires an offline mode of processing as established optimally only during sleep. Declarative memories benefit particularly from slow wave sleep (SWS), whereas procedural and emotional aspects of a memory appear to benefit additionally from rapid eye movement (REM) sleep. Recent studies have elucidated some of the neurophysiological mechanims underlying the consolidation of memories during sleep, especially in the hippocampus-dependent declarative memory system. Consolidation in this system critically relies on the covert reactivation of newly encoded memories within hippocampal circuitry during SWS which likely stimulates the gradual transfer and redistribution of representations to the neocortex for long term storage. Memory reactivations in the hippocampus are driven by (<1Hz) EEG slow oscillations which are generated in neocortical networks, partly depending on the use of these networks for encoding of information during prior learning. By synchronizing the hippocampo-neocortical dialogue with specific activity from other brain regions, including thalamo-cortical spindle activity and locus coeruleus burst activity, slow oscillations enable persisting plastic changes underlying the long-term storage of memories in the neocortex.

# A neurobiological approach towards insect photoperiodism

Sakiko Shiga[1]

[1]Osaka City University, Graduate School of Science, 3-3-138, Sugimoto, Sumiyoshi, 558-8585 Osaka, Japan

The lives of most animals are organized on a seasonal schedule. To predict seasons a most reliable environmental signal is photoperiod. Animals adjust to seasonal variation in temperature, and food or water supply by responding to photoperiod. Photoperiodism controls seasonal development in various insects. Since Marcovitch in 1923 first showed insect photoperiodism in the strawberry root aphid, *Aphis forbesi*, which switches parthenogenesis to bisexual reproduction by responding to short days, a large number of papers have appeared on photoperiodic control of diapause and seasonal morphs. The minimal requirements for photoperiodism are photoreceptors, a photoperiodic clock and hormonal effectors. Although photoreceptors and hormonal effectors have been studied in many species, the neural mechanism underlying the photoperiodic clock has not been addressed. Bünning first pointed out in 1936 that endogenous circadian rhythms are involved in the measurement of day or night length in the photoperiodic clock. After a long break, just recently mutant analysis and RNA interference studies have shown in several species that circadian clock genes, *period*, *timeless* or *cycle*, are involved in photoperiodism. Yet important neurobiological questions are unsolved: How do circadian clocks contribute to day-length measurement on the cellular level? ; How is the number of short or long days counted in the brain? ; How do environmental signals control hormone releases, and what is the underlying neuronal circuitry? In *Drosophila melanogaster* circadian clock genes and neurons are well studied. However, its photoperiodic responses are shallow and therefore is difficult to assay. We are then foccusing on photoperiodism in the blowfly *Protophormia terraenovae*, combined with comparative studies using different species.

Ablation experiments showed that secretory pars lateralis neurons in the dorsal protocerebrum are important for diapause induction under short-days commonly in *P. terraenovae*, the bean bug *Riptortus pedestris*, and tobacco hornworm *Manduca sexta*. These pars lateralis neurons innervate neurohemal organs, suggesting that these neurons are involved in control of hormone production or release. Next we examined the roles of circadian clock neurons in photoperiodism. In *P. terraenovae*, five types of PERIOD-immunoreactive cells were found. When a type of PERIOD cells (s-$LN_v$s) were bilaterally ablated, flies became arrhythmic in behavior and did not discriminate photoperiod, suggesting that circadian clock neurons are involved in photoperiodism. In the s-$LN_v$s, PERIOD-immunoreactivity was highest in the nucleus at 12 h after lights-off and lowest 12 h after lights-on regardless of photoperiod. Thus, as in *D. melanogaster*, PERIOD nuclear translocation entrains to photoperiod and day-length information seems to be encoded in clock cells. Immuno-electronmicroscopy revealed synaptic connections from s-$LN_v$s to the pars lateralis neurons indicating that clock neurons could directly affect hormone release.

To summarize, our results suggest that circadian clock neurons, s-$LN_v$s, are involved in time measurements and may synaptically signal day-length information to the pars lateralis neurons for hormonal control. We have now to determine how the clock neurons switch the activity of pars lateralis neurons, and how the number of day-length is counted or stored in the brain.

# Symposia

S1 Molecular mechanisms controlling neurogenesis and tumorigenesis in the CNS stem cells

S2 Levels of olfactory plasticity in insects

S3 Perspectives of small-animal PET and SPECT imaging in neuroscience

S4 Principles of neural function – how theories inspire experiments

S5 Neuropeptides and endocannabinoids - Key players in the modulation of behavioral processes

S6 Motor neuron disease models: Loss of function or gain of toxic function ? Molecular mechanisms and therapeutic perspectives

S7 Adult neural stem cells in physiology and disease

S8 Peripheral mechanisms in olfaction

S9 Plasticity in the human visual system - Probing dysfunction with functional magnetic resonance imaging

S10 Information technology meets brain research - New developments in neuroinformatics

S11 Development of fear and anxiety in humans: Behavioural, cognitive and neural changes

S12 Epilepsy – a hyperexcitation syndrome with multiple causes

S13 Translational regulation in neurons and glial cells of the central nervous system

S14 Dynamic processes in the auditory system

S15 Light sensors in new light: A comparative and integrative view on photoreceptors, their function, differentiation and degeneration

S16 Barrel cortex function: From single cells to behaving animals

S17 Neurobiology of complex social behaviour: from bonding to autism

S18 ALS, Huntington's disease and Parkinson's disease: From molecular pathogenesis to target validation in aggregopathies

S19 Neural cell adhesion molecule NCAM and its post-translational modifications at the crossroad of signalling pathways and neural functions

S20 Cellular actions of neuropeptides and biogenic amines in invertebrates

S21 Optogenetics in neuroscience: From basic principles to applications

S22 Unravelling the activity-dependent mechanisms of network formation in the neonatal cortex

S23 The social brain - in health and disease

S24 How do neurodegenerative diseases develop and how to cure them: What can we learn from diverse animal models?

# Symposium

## S1: Molecular mechanisms controlling neurogenesis and tumorigenesis in the CNS stem cells

S1-1 Role of Tailless (Tlx) in neurogenesis and gliomagenesis
*Hai-Kun Liu*

S1-2 THE CELL- DEATH LIGAND CD95 HAS CONTEXT DEPENDENT ROLES IN NEUROLOGICAL DISORDERS, STEM CELL PHYSIOLOGY AND PRIMARY BRAIN TUMORS
*Ana Martin-Villalba*

S1-3 Identification of a transcriptional network that drives mesenchymal transformation in gliomas
*Maria Stella Carro, Wei Keat Lim Lim, Mariano Javier Alvarez, Robert J. Bollo, Xudong Zhao, Evan Y. Snyder, Erik P. Sulman, Sandrine L. Anne, Fiona Doetsch, Howard Colman, Anna Lasorella, Ken Aldape, Andrea Califano, Antonio Iavarone*

S1-4 Bone morphogenetic protein -7 release from endogenous neural precursor cells suppresses the tumourigenicity of glioblastoma stem cells.
*Michael Synowitz, Sridhar R. Chirasani, Alexander Sternjack, Peter Wend, Stefan Momma, Benito Campos, Ilaria M. Herrmann, Daniel Graf, Thimios Mitsiades, Christel Herold-Mende, Daniel Besser, Helmut Kettenmann, Rainer Glass*

S1-5 Neural precursor cells induce glioma cell-death via stimulation of TRPV1
*Rainer Glass, Jitender Kumar, Kristin Stock, Michael Synowitz, Stefania Petrosino, Roberta Imperatore, Ewan St. J. Smith, Peter Wend, Bettina Erdmann, Ulrike A. Nuber, Ulf Gurok, Vitali Matyash, Joo-Hee Wälzlein, Sridhar R. Chirasani, Gary R. Lewin, Luigia Cristino, Vincenzo Di Marzo, Helmut Kettenmann*

# Role of Tailless (Tlx) in neurogenesis and gliomagenesis

Hai-Kun Liu[1]

[1]German Cancer Reseasrch Center (DKFZ), Helmholtz Professorship Molecular Biology of the Cell I, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany

Malignant gliomas are the most common primary brain tumors and associated with frequent resistance to therapy as well as poor prognosis. Here we demonstrate that the nuclear receptor tailless (Tlx), which in the adult is expressed exclusively in astrocyte-like B cells of the subventricular zone, acts as a key regulator of neural stem cell (NSC) expansion and brain tumor initiation from NSCs. Overexpression of Tlx antagonizes age-dependent exhaustion of NSCs in mice and leads to migration of stem/progenitor cells from their natural niche. The increase of NSCs persists with age and leads to efficient production of newborn neurons in aged brain tissues. Tlx-overexpressing NSCs initiate the development of glioma-like lesions and gliomas and glioma development is accelerated upon loss of the tumor suppressor p53. We also demonstrate that Tlx transcripts are overexpressed in human primary glioblastomas, and the Tlx gene is amplified in some of the human brain tumors. Further we found that Tlx inhibits the expression of its target genes PTEN and p21 in NSCs. Inactivation of those two genes specifically in adult NSCs leads to glioma formation, which mimics the Tlx-overexpressing phenotype. Our study clearly demonstrates how NSCs contribute to brain tumorigenesis driven by a stem cell-specific transcription factor, thus identifies a crucial pathway for NSC maintenance and brain tumor initiation.

# THE CELL-DEATH LIGAND CD95 HAS CONTEXT DEPENDENT ROLES IN NEUROLOGICAL DISORDERS, STEM CELL PHYSIOLOGY AND PRIMARY BRAIN TUMORS

Ana Martin-Villalba[1]

[1]German Cancer Research Center, Molecular Neurobiology (G381), Heidelberg, Germany

No abstract available

# Identification of a transcriptional network that drives mesenchymal transformation in gliomas

Maria Stella Carro[1,2], Wei Keat Lim Lim[3,4], Mariano Javier Alvarez[4,5], Robert J. Bollo[9], Xudong Zhao[1], Evan Y. Snyder[10], Erik P. Sulman[11], Sandrine L. Anne[1], Fiona Doetsch[6], Howard Colman[12], Anna Lasorella[1,6,7], Ken Aldape[13], Andrea Califano[1,3,4,5], Antonio Iavarone[1,6,8]

[1]University of Freiburg, Department of General Neurosurgery, Breisacherstraße 64, 79106 Freiburg, Germany[2]Institute for Cancer Genetics [3]Department of Biomedical Informatics[4]Center for Computational Biology and Bioinformatics [5]Joint Centers for Systems Biology, [6]Department of Pathology,[7]Pediatrics [8] and Neurology, Columbia University Medical Center, New York, NY, 10032 [9] Department of Neurosurgery, New York University School of Medicine & NYU Langone Medical Center, New York, NY, 10016.[10]Burnham Institute for Medical Research, La Jolla, CA 92037 [11]Division of Radiation Oncology,[12]Department of Neuro-Oncology [13]Department of Pathology, M.D. Anderson Cancer Center, Houston, Texas 77030.

High-grade gliomas are the most common intrinsic brain tumors in adult and are essentially incurable. The mesenchymal sub-class of gliomas expresses genes linked to the most aggressive properties of tumors (migration, invasion and angiogenesis) and is associated with the worst prognosis. In this study, we aimed at the identification of transcription factors the drive the activation of the mesenchymal genes in high-grade gliomas. The ability to pinpoint key regulator of this signature is crucial in order to develop new therapeutic options in the treatment of high grade gliomas.

We have applied ARACNe (Algorithm for the Reconstitution of Accurate Cellular Network) to a panel of 176 tumor samples and have identified a small network of transcription factors connected to the mesenchymal signature. Computational and validation experiments have pointed two transcription factors (Stat3 and C/EBPß) as synergistic initiators and master regulators of the mesenchymal signature in gliomas. Ectopic co-expression of Stat3 and C/EBPß reprogram neural stem cells along the aberrant mesenchymal lineage, whereas elimination of the two factors in glioma cells leads to collapse of the mesenchymal signature and reduces tumor aggressiveness. In human gliomas, expression of Stat3 and C/EBPß predicts poor clinical outcome. Taken together, these results reveal that activation of a small module is necessary a suffiecient to initiate and maintain an aberrant phenotypic state in eukaryotic cells.

# Bone morphogenetic protein-7 release from endogenous neural precursor cells suppresses the tumourigenicity of glioblastoma stem cells.

Michael Synowitz[1,2], Sridhar R. Chirasani[2], Alexander Sternjack[3], Peter Wend[4], Stefan Momma[5], Benito Campos[6], Ilaria M. Herrmann[6], Daniel Graf[7], Thimios Mitsiades[7], Christel Herold-Mende[6], Daniel Besser[8], Helmut Kettenmann[2], Rainer Glass[2]

[1]Department of Neurosurgery, Charité University Hospital, 13353 Berlin, Germany. [2]Cellular Neuroscience-, [3]Cellular Immunology-, [4]Signal Transduction-, Epithelial Differentiation-, Invasion and Metastasis-, [8]Embryonic Stem Cells-Research Groups at the Max Delbrück Centre for Molecular Medicine (MDC), 13125 Berlin, Germany. [5]Restorative Neurology Group, Edinger Institute Frankfurt/ M., 60528 Frankfurt/ M., Germany. [6]Division of Neurosurgical Research, Department of Neurosurgery, University of Heidelberg, 69120 Heidelberg, Germany. [7]Zentrum für Zahn-, Mund-und Kieferheilkunde der Universität Zürich, Plattenstrasse 11, CH-8032 Zürich.

Glioblastoma cells with stem-like properties (glioblastoma stem cells) control brain tumour growth and recurrence. Here, we show that endogenous neural precursor cells perform an anti-tumour response by specifically targeting glioblastoma stem cells: In vitro, neural precursor cells predominantly express BMP7; BMP7 is constitutively released from neurospheres and induces canonical BMP signalling in glioblastoma stem cells. Exposure of human and murine glioblastoma stem cells to neurosphere-derived BMP7 induces tumour stem cell differentiation, attenuates stem-like marker expression, reduces self-renewal and the ability for tumour initiation. Neurosphere-derived or recombinant BMP7 reduces glioblastoma expansion from stem-like cells by down-regulating the transcription factor Olig2. In vivo, large numbers of BMP7-expressing neural precursors encircle glioblastoma in young mice, induce canonical BMP signalling in glioblastoma stem cells and can thereby attenuate tumour formation. This anti-tumour response is strongly reduced in older mice. Our results indicate that endogenous neural precursor cells protect the young brain from glioblastoma by releasing BMP7, which acts as a paracrine tumour suppressor that represses proliferation, self-renewal and tumour-initiation of glioblastoma stem cells.

# Neural precursor cells induce glioma cell-death via stimulation of TRPV1

Rainer Glass[1], Jitender Kumar[1], Kristin Stock[1], Michael Synowitz[1,2], Stefania Petrosino[3], Roberta Imperatore[4], Ewan St. J. Smith[5], Peter Wend[6], Bettina Erdmann[7], Ulrike A. Nuber[8], Ulf Gurok[9], Vitali Matyash[1], Joo-Hee Wälzlein[1], Sridhar R. Chirasani[1], Gary R. Lewin[5], Luigia Cristino[4], Vincenzo Di Marzo[3], Helmut Kettenmann[1]

[1]Cellular Neuroscience, [5]Molecular Physiology of Somatic Sensation, [6]Signal Transduction, Epithelial Differentiation, Invasion and Metastasis, [7]Central Facility for Electron Microscopy; at the Max Delbrück Centre for Molecular Medicine (MDC), 13125 Berlin, Germany.

[2]Department of Neurosurgery, Charité-Universitätsmedizin Berlin, 13353 Berlin, Germany.

[3]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, [4]Institute of Cybernetics, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli (NA), Italy.

[8]Stem Cell Gene Regulation, Lund University, SE-221 00 Lund, Sweden.

[9]Max Planck Institute for Molecular Genetics, 14195 Berlin, Germany.

Gliomas are incurable primary brain tumors. Here, we describe that neural stem and precursor cells (NPCs) exert a paracrine defense against gliomas. Gliomas have increased expression-levels of the transient receptor potential vanilloid subfamily member -1 (TRPV1) as compared to tumor-free brain. NPCs migrate to gliomas and induce tumor cell-death by releasing bioactive lipids which activate TRPV1. NPC-released TRPV1 agonists mediate cell-death of primary human glioblastoma cells and glioma cell lines, by triggering the activating transcription factor-3 (ATF3) controlled branch of the ER-stress pathway. In vivo, NPCs induce brain tumor cell-death and prolong survival in mouse models bearing wild-type but not TRPV1 knock-down tumors. NPC-mediated tumor suppression is restricted to the young brain, but can be recapitulated in the old brain by systemic administration of the synthetic vanilloid Arvanil, offering a new therapeutic strategy for glioblastoma.

# Symposium

# S2: Levels of olfactory plasticity in insects

S2-1 Not just hard-wired: Developmental plasticity in the *Manduca* olfactory system
*Lynne Ann Oland*

S2-2 Developmental plasticity and adult maturation of olfactory synaptic microcircuits in the mushroom bodies of the honeybee
*Claudia Groh, Ian Meinertzhagen, Wolfgang Rössler*

S2-3 Mating-induced differential sex-pheromone and plant odour processing in a male moth
*Romina Barrozo, Christophe Gadenne, Sylvia Anton*

S2-4 Structural plasticity in the honeybee brain related to memory formation.
*Jean-Marc DEVAUD, Benoît Hourcade, Damien Lefer, Thomas Muenz, Wolfgang Rössler, Jean-Christophe Sandoz*

S2-5 Adaptive dynamics on different time scales throughout the olfactory pathway enhances efficient coding of odor features
*Mark Stopfer, Joby Joseph, Stacey Daffron*

S2-6 Olfactory coding and olfactory learning in *Drosophila*: an optical imaging approach
*André Fiala*

# Not just hard-wired: Developmental plasticity in the *Manduca* olfactory system

Lynne Ann Oland[1]

[1]University of Arizona, Department of Neuroscience, PO Box 210077, 85721-0077 Tucson, USA

The olfactory pathway of the moth *Manduca sexta* provides an experimentally advantageous sensory system in which to study developmental and adult plasticity. The animal is large at interesting developmental stages, the sensory periphery and the olfactory centers in the CNS are readily accessed for surgery and for recording, it has robust odor-modulated behavior, and there is a wealth of data about the anatomy and physiology of both the developing and adult systems. Importantly, the moth olfactory (antennal) lobe has an organization that closely resembles that of the vertebrate olfactory bulb. Although it is somewhat simpler, there is sufficient complexity in both its neuronal and glial elements that it serves as a useful model. This talk will summarize the work of several laboratories that have focused on the moth as a model system.

From a developmental perspective, metamorphic adult development is a time during which many steps in development typically associated with embryogenesis occur, largely in the absence of neuronal activity. During this period, the system is sensitive to a variety of influences, including especially the ingrowth of sensory axons. Studies in *Manduca* during this period have revealed the critical importance of sensory input: (1) in the absence of receptor ingrowth, the normal glomerular structure of the neuropil and the normal tufted character of the dendritic arbors of antennal lobe neurons are absent; (2) male sex-specific glomeruli and behavior can be induced in a female host animal by transplanting a male antenna onto the female at the beginning of metamorphosis; (3) sensory axons must be present for a specified period for the glomerular structure of the neuropil to be sustained into adulthood; (4) the timing of sensory axon ingrowth is critical – too early leads to mistargeting, too late leads to abnormality in the shape and discreteness of glomeruli; (5) sensory axons induce changes in glial cells that in turn affect correct axonal fasciculation and stabilize glomeruli; and (6) sensory axons affect the development of the normal repertoire of currents in glial cells.

With its emergence from the pupal stage, the moth must engage in foraging and reproductive behavior, and must effectively tune its olfactory circuitry in response to its experience. Like other insects, the moth is capable of olfactory learning and that learning is influenced by both age and mating status. Ensemble recordings *in situ* during olfactory conditioning have revealed both associative and discriminative olfactory learning. The changes in network activity are correlated with feeding behavior and are persistent. Odor representations are dynamic, the recordings showing changes in the patterning of activity rather than simple increases in activity in the parts of the network representing a particular odorant.

Taken together with data gathered in other insects, the evidence of robust developmental and adult plasticity in the antennal lobes of *Manduca* makes clear that these less complex systems offer extensive opportunity to study both activity-independent and activity-dependent processes by which the olfactory system in particular, and the nervous system in general, develop and maintain the capability to modify their circuitry as needed to optimize function.

# Developmental plasticity and adult maturation of olfactory synaptic microcircuits in the mushroom bodies of the honeybee

Claudia Groh[1], Ian Meinertzhagen[2], Wolfgang Rössler[1]

[1]University of Würzburg, Department of Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany
[2]Life Sciences Centre, Dalhousie University, Halifax, NS, Canada B3H 4J1

Mushroom bodies (MBs), prominent paired structures of the central brain in insects, are higher brain centers of particular importance for olfactory processing, odor representation and association including learning and memory. Their main input neuropils are the calyces. Honeybees possess particularly large and doubled MB calyces that are divided into three anatomically distinct and clearly delineated sensory input regions. In this study we focused on structural plasticity of the input synapses in the MB calyx. These constitute characteristic synaptic complexes termed calycal microglomeruli (MG). The various levels of structural plasticity of the MB calyces we investigated include both developmental (postembryonic) and adult structural plasticity – in particular plasticity associated with brood care, environmental conditions, and age.

To be able to follow cellular and subcellular changes in the organization of calycal MG, presynaptic boutons of projection neurons (PNs) and postsynaptic compartments of MB intrinsic neurons, the Kenyon cells (KCs), were analyzed using various markers for synaptic proteins and cytoskeletal elements in combination with high-resolution laser scanning confocal microscopy. Serial -electron microscopic studies were also performed to investigate synaptic changes at the level of synaptic sites and connectivity.

Our developmental studies have revealed that both larval feeding and naturally occurring variations in pupal rearing temperature affect the initial numbers of MG in olfactory subregions of the adult MB calyx. At the level of behavior, adult bees reared at the lower range of naturally occurring brood temperatures performed less well in associative memory tasks, forage later than bees raised at higher temperatures, and differ in dance-communication performance. Next we asked whether and how sensory experience and age affect synapses of the MB calyx during adulthood. Combined f-actin and tubulin staining of KC processes revealed that the most drastic effect of natural sensory experience was a massive outgrowth of KC dendrites during the transition from nurse bees to foragers and, a concurrent presynaptic pruning of PN boutons. KC dendritic growth and PN-presynaptic pruning were associated with a volume increase of the MB calyx, as previously reported at the onset of foraging. What are the synaptic changes that occur at the pre- and postsynaptic site? Using serial-electron microscopy and 3D analyses, we investigate and quantify how changes in the numbers and density of MG during maturation are accompanied by changes in synaptic characters such as active zones or numbers of postsynaptic profiles and the associated changes in synaptic connectivity.

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# Mating-induced differential sex-pheromone and plant odour processing in a male moth

Romina Barrozo[1], Christophe Gadenne[1], Sylvia Anton[1]

[1]INRA, Unite Mixte de Recherches en Physiologie d'Insectes-Signalisation et communication, Centre de Recherches de Versailles-Route de ST Cyr, 78026 Versailles cedex, France. University of Buenos Aires, Dep. Biodiversity & Experimental Biology, Argentina.

Innate behaviours in animals can be modulated by factors such as the environment, experience or the physiological state. This behavioural plasticity originates from changes in the underlying neuronal substrate. A well-described form of plasticity is induced by mating. In both vertebrates and invertebrates, males experience a post-ejaculatory refractory period during which they avoid new females. In the male moth *Agrotis ipsilon*, mating induces a transient inhibition of responses to the female-produced sex pheromone. To understand the neural bases of this inhibition and its possible odour specificity, we carried out a detailed analysis of the peripheral and central olfactory system and the behaviour of male A. ipsilon, using sex pheromone, plant volatiles, and their mixture. We found that although mating changes the coding properties of central pheromone-sensitive neurons in the antennal lobe (the macroglomerular complex, MGC), thus affecting the post-mating behaviour, the sensory input remains constant. Besides, the post-mating inhibition does not affect the processing of a behaviourally attractive flower volatile, heptanal in the ordinary glomeruli (OG). When challenged with mixtures of sex pheromone and the flower volatile, additional evidence for differential processing of odours was found. Whereas synergism was observed in virgin males, the sex pheromone leads to inhibitory effects in mated males in the OGs, in correlation with behavioural changes. We propose the existence of a complex inhibitory system in which responses of males to the female sex-pheromone depend on their mating status but also on the presence of other biologically relevant odours (heptanal). We provide evidence for a transient odour-selective central mechanism leading to post-mating sexual inhibition in males, which is interpreted as a "refusal to respond", but not as exhaustion behaviour as described in vertebrates.

# Structural plasticity in the honeybee brain related to memory formation.

Jean-Marc DEVAUD[1], Benoît Hourcade[1], Damien Lefer[1], Thomas Muenz[2], Wolfgang Rössler[2], Jean-Christophe Sandoz[1,3]

[1]CNRS/Paul Sabatier University, Research Center on Animal Cognition, 118 route de Narbonne, 31062 Toulouse, France
[2]Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Würzburg, Germany
[3]present address: Evolution, Genome and Speciation (CNRS UPR 9034) Avenue de la Terrasse, 91198 Gif sur Yvette, France

Insects provide excellent models to analyse how neural networks underlie behaviour. In the recent years, their study has contributed to a great extent to improve our understanding of the neural bases of perception, learning and memory of odours. As honeybees (Apis mellifera) forage for food on flowers, they make associations between odours and nectar to identify food sources, and rely on the memory of such associations on later foraging trips. This associative memory can be studies in controlled laboratory conditions, as the animals can be conditioned to learn and remember an association between an odorant and sugar.
We have been focusing our research on the plastic changes affecting the olfactory centres when a stable olfactory memory is formed in such conditions. In particular, we have been looking for structural rearrangements in two main olfactory centres known for their role in olfactory learning and memory in the insect brain: the antennal lobes (ALs) and the mushroom bodies (MBs). The modular organization of these two neuropils allowed us to quantify changes affecting synaptic units in the brains of conditioned animals that had formed a stable memory and of controls without memory of the association. By comparing measurements from these different groups, we have been able to identify plastic changes in the neural networks of both brain regions.
In the ALs, the main synaptic units (glomeruli) undergo volume changes that are odorant-specific[1], while in the MBs the density - and probably the number - of synaptic boutons (microglomeruli) increases as memory is formed[2]. These results show that, as in Vertebrates, stable forms of memory are embedded in modifications of the structural organization of brain networks. We are now currently investigating the relationships between these memory traces in both brain regions, and we are looking for the possible implication of signalling pathways involved in synaptic plasticity and memory.

[1] Hourcade et al. (2009) Learn Mem. 16: 607-615.
[2] Hourcade et al. (2010) J. Neurosci. 30: 6461-6465

# Adaptive dynamics on different time scales throughout the olfactory pathway enhances efficient coding of odor features

Mark Stopfer[1], Joby Joseph[1,2], Stacey Daffron[1]

[1] US National Institutes of Health, NICHD, 35 Lincoln Dr., Bethesda, MD, 20892 USA

[2] present address: Center for Neural and Cognitive Sciences, University of Hyderabad, India

Animals often encounter odors as plumes, which can be characterized by their chemical identity and concentration, onset, ongoing chaotic temporal structure, and offset. Olfactory neural systems extract and encode these characteristics at multiple points, each of which has intrinsic limitations of dynamic range, temporal resolution and integration time. Adaptation, the ability of a system to adjust its behavior given its history, plays a critical role in forming neural representations of odor plumes. We tested the roles of adaptation in the locust by making simultaneous recordings from populations of neurons in the first three layers in the olfactory pathway (odor receptor neurons (ORNs), antennal lobe (AL) neurons, and their follower Kenyon cells (KCs)) while presenting reproducible, simulated odor plumes, and by exploring our results with a computational model.

Odor plumes evoke two forms of plasticity, one central and one peripheral, operating on different time scales. Slow-building, long lasting (~15min) facilitation within the circuitry of the AL ensures the plume's onset elicits the strongest signal, boosts the oscillatory output of the inhibitory local neurons (LNs), decreases the number of non-synchronized spikes in projection neurons (PNs), and leads to more reliable, odor-specific responses. Rapid-onset, transient (~5sec) sensory adaptation in ORNs predominates within plumes, quickly reducing overall input to the AL and output to the KCs, which then fire only sparsely. The two forms of plasticity interact with the dynamics of the AL so that the vast majority of spikes in KCs occur at the onset and then after the offset of the plume (although some KCs fire reliably and with odor specificity at particular points within the plume). This mechanism appears to efficiently extract information of particular importance to the animal.

Using a map based model we are analyzing how these various neural layers and processes interact and operate under different stimulation regimes. We model adaptation in the ORNs with a simple kinetic scheme based on common ligand receptor binding principles, and model the antennal lobe as an adaptive gain control system where adaptation of the feedback parameter, over many seconds, controls PN rate and synchrony. Together, these mechanisms largely determine the responses of KCs to odor.

# Olfactory coding and olfactory learning in *Drosophila*: an optical imaging approach

André Fiala[1]

[1]Georg-August-Universität Göttingen, Molecular Neurobiology of Behavior, c/o ENI, Grisebachstr. 5, 37077 Göttingen, Germany

Many animals are able to differentiate a large variety of odors with respect to their chemical identity and concentration. We have asked how different odor identities are represented in the brain of the fruit fly *Drosophila melanogaster* at two levels of processing: at olfactory sensory neurons and at olfactory projection neurons within the fly's antennal lobes. To this end, we have used the genetically encoded, FRET-based calcium sensor Cameleon 2.1 because of the minimization of movement artefacts due to its ratiometric properties, a good signal-to-noise ratio and high fluorescence intensity in the non-activated state. We find that odor-evoked activity patterns differ in terms of their similarity when olfactory sensory neurons and projection neurons are compared, as revealed by principal component analysis performed by Dr. Marc Timme's group. Interestingly, an odor pair that evokes clearly different calcium activity patterns at the level of sensory neurons appears to evoke very similar activity patterns at the level of second order projection neurons. These results correlate with behavioural learning experiments performed in Prof. Bertram Gerber's group showing that this odor pair is strongly generalized. We propose that olfactory computation within the antennal lobe might not only cause a fine discrimination of odors but can also cause a convergence of odor information from separated input channels into distinct classes. In collaboration with Marc Timme and Bertram Gerber we therefore investigate whether or not such a classification mechanism might be subject to learning-dependent modulation. In particular we investigate whether structurally similar odors that are generalized by fruit flies can be distinguished by the animals when differentially trained.

# Symposium

## S3: Perspectives of small-animal PET and SPECT imaging in neuroscience

S3-1 Focal changes of cerebral glucose metabolism during cognitive tasks in rats: PET imaging using [$^{18}$F]FDG
*Heike Endepols*

S3-2 Optical and SPECT imaging in experimental stroke research
*Nina Stemmer*

S3-3 In vivo SPECT-imaging of activity-dependent changes in regional cerebral blood flow in the rodent brain
*Jenni Neubert*

S3-4 Measurement of cerebral glucose consumption rate in pathologic tissue using FDG PET
*Heiko Backes*

S3-5 Non-invasive detection of amyloid plaques by combined multi-functional and morphological imaging in transgenic mouse models of Alzheimer's Disease
*Florian Christoph Maier, Andreas Schmid, Hans Wehrl, Gerald Reischl, Bernd Pichler*

S3-6 In vivo imaging of cerebral potassium metabolism in focal cerebral ischemia in rats using 201TlDDC-SPECT
*Jürgen Goldschmidt*

# Focal changes of cerebral glucose metabolism during cognitive tasks in rats: PET imaging using [$^{18}$F]FDG

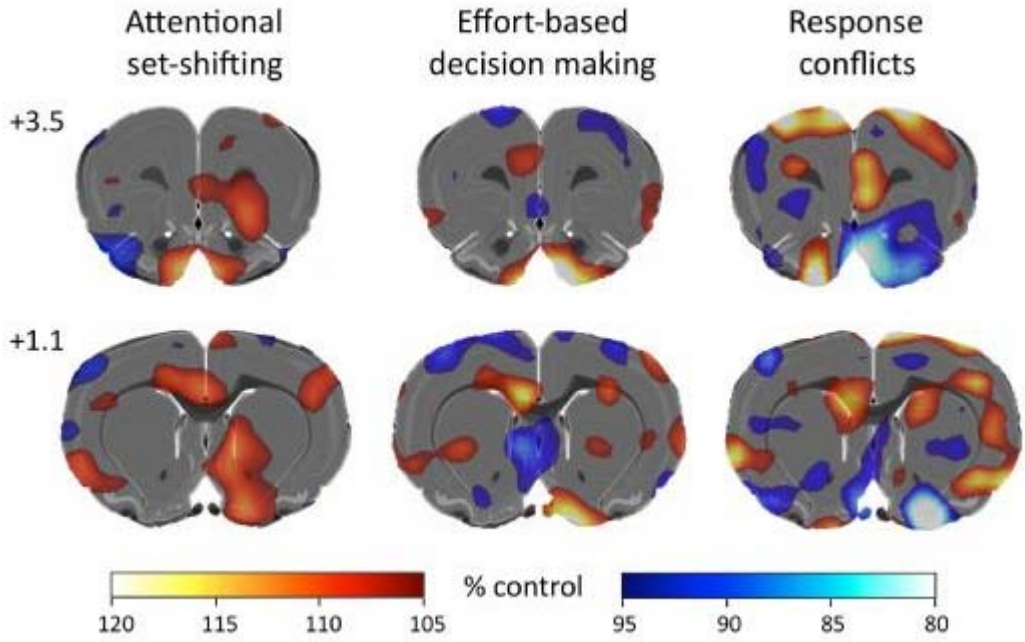
Heike Endepols[1]

[1]Max Planck Insitute for Neurological Research, Multimodal Imaging, Gleueler Str. 50, 50931 Köln, Germany

Performing cognitive tasks in combination with functional imaging in rats requires unrestrained animals, which is difficult if behavior and imaging data acquisition take place at the same time. Using the tracer [$^{18}$F]fluorodeoxyglucose (FDG) this problem can be overcome, because FDG is irreversibly trapped in metabolically active cells and can be imaged after tracer accumulation. In our cognitive paradigms we use a protocol where the behavioral task starts five minutes after intraperitoneal FDG injection (2 mCi) and is conducted for 30- 40 min in an operant chamber. Subsequently, the rat is anesthetized and placed in the PET scanner. The scan starts 60 min after FDG injection and lasts for 30 min. Because quantification of glucose metabolism is not possible with this setting, and even semiquantitative methods require at least one arterial blood sample [1], intensity normalization is performed with a carefully picked reference region. Normalized metabolic values obtained from the task are then compared with a control task, yielding percent increase or decrease of regional metabolic activity.
So far, we have combined metabolic FDG-PET with effort-based decision making [2], attentional set-shifting, and processing of response conflicts (Simon test). These tasks produced different patterns of positive as well as negative metabolic activity changes compared to a control condition (Fig. 1). However, we always found metabolic activation of prefrontal (prelimbic, infralimbic, orbitofrontal) and cingulate areas, basal ganglia, and hippocampus. We conclude that behavioral FDG-PET is a suitable method to image regional changes of metabolic brain activity related to cognitive functions in small animals.

Fig. 1: Grand average metabolic patterns developing during attentional set-shifting (n=8), effort-based decision making (n=8) and during processing and resolution of response conflicts (Simon task; n=4). Increases of metabolic activity compared to a control task are shown in shades of red, decreases of metabolic activity in shades of blue.

References:
[1] Schiffer et al. (2007) J Nucl Med 48: 277-287.
[2] Endepols et al. (2010) J Neurosci 30: 9708-9714.


Attentional set-shifting
Effort-based decision making
Response conflicts
+3.5
+1.1
% control
120
115
110
105
95
90
85
80

# Optical and SPECT imaging in experimental stroke research

Andreas Wunder[1,3], Ralph Buchert[2,3], Winfried Brenner[2,3], Ulrich Dirnagl[1,3]

[1]Charite - University Medicine Berlin, Dep. of Experimental Neurology, Center for Stroke Research Berlin (CSB), Charitéplatz 1, 10117 Berlin, Germany

[2]Charite - University Medicine Berlin, Dep. of Nuclear Medicine, Charitéplatz 1, 10117 Berlin, Germany

[3]Charite - University Medicine Berlin, Small Animal Imaging Center (SAIC) at CVK, Augustenburger Platz 1, 13353 Berlin, Germany

In clinical stroke, non-invasive imaging technologies such as Magnetic Resonance Imaging (MRI) and Computed Tomography (CT) are the mainstay of diagnostic imaging. However, these techniques are of limited use in characterizing pathological mechanisms. Examples include the delineation of the penumbra or "tissue at risk", which can only be approximated, or reliable information on inflammatory processes and other relevant cellular and biochemical processes, which impact on outcome after stroke. There is consensus that more specific imaging methods are urgently needed for improved selection of patients and stratification to specific therapy, as well as more reliable prediction and monitoring of outcome. New, highly specific imaging agents are currently developed for different imaging modalities. The markers are evaluated in animal models using dedicated small animal imaging systems. Among the imaging techniques used in stroke research, we will describe Single Photon Emission Computed Tomography (SPECT) in comparison to Positron Emission Tomography (PET) and some examples of non-invasive near-infrared fluorescence (NIRF) imaging. Both SPECT and NIRF are highly sensitive in detecting signal molecules, SPECT isotopes or NIRF dyes, respectively. However, compared to each other both have advantages and disadvantages.

We will present detailed information on the working principle of SPECT, the differences to PET and on the main differences between clinical and preclinical SPECT imaging.

Examples of optical imaging and some previous SPECT work will be presented to show how both techniques can be successfully used in small animal models of stroke and to illustrate the pros and cons of both techniques.

# In vivo SPECT-imaging of activity-dependent changes in regional cerebral blood flow in the rodent brain

Jenni Neubert[1]

[1]Leibniz Institute for Neurobiology, Auditory Learning and Speech, Brenneckestr.6, 39118 Magdeburg, Germany

Single-photon emission computed tomography (SPECT) has been used since several decades for imaging regional cerebral blood flow (rCBF) for diagnostic purposes in humans. SPECT-imaging of rCBF is based on the use of radioactively labeled lipophilic tracers, which cross the blood-brain barrier and accumulate in the brain in a blood-flow dependent manner. One of the most widely used flow-tracers for SPECT-imaging is 99m-Technetium hexamethylpropyleneamine oxime (99mTc-HMPAO). After crossing the blood-brain barrier the lipophilic 99mTc-HMPAO complex is rapidly converted to a hydrophilic 99mTc-compound that is trapped in the brain and shows no measurable redistribution.
99mTc-HMPAO-SPECT can be used in a similar manner as 18F-2-fluoro-2-deoxy-glucose positron emission tomography (18F-FDG-PET) for imaging spatial patterns of neuronal activity in the brain: Under physiological conditions blood flow as well as glucose metabolism are tightly coupled to neuronal activity and both tracers 99mTc-HMPAO and 18F-FDG accumulate in the brain in an activity-dependent manner. Both tracers are trapped in the brain after accumulation and in both cases tracer distributions remain stable and can be scanned after a certain time span of tracer uptake. There is no need for applying the tracers under restrained conditions inside a scanner. Quite different from functional MRI based on measuring BOLD signals both techniques 99mTc-HMPAO-SPECT and 18F-FDG-PET can be used, therefore, for imaging spatial patterns of neuronal activity that occur in awake behaving animals.

Thus far, however, little use has been made of 99mTc-HMPAO-SPECT for imaging activity-dependent changes in rCBF in the rodent brain. Small-animal SPECT-imaging has long been a somewhat neglected imaging modality with custom-made scanners present in only a few research labs. This is now changing, and dedicated small-animal SPECT-scanners have become commercially available providing spatial resolutions in the submillimeter range exceeding those of small-animal PET-scanners.
We here used a dedicated small animal SPECT/CT scanner for in vivo imaging of activity-dependent changes in rCBF in rodents using 99mTc-HMPAO as a tracer. We administered the tracer via chronically implanted jugular vein catheters in Mongolian gerbils and mice. We injected 99mTc-HMPAO intravenously within time spans of about five minutes during ongoing activity of the animals. We present images from rCBF in the different species under different experimental conditions focusing on intracranial self-stimulation and auditory learning. We show that the spatial resolution of 99mTc-HMPAO small-animal SPECT is sufficient to reveal activations of small brain regions such as the accumbens nucleus upon medial forebrain bundle stimulation in mice. 99mTc-HMPAO SPECT also reveals complex spatial patterns of neuronal activity in trained animals solving tasks. The technique is a promising new approach for in vivo imaging of spatial patterns of neuronal activity in unrestrained behaving animals.

# Measurement of cerebral glucose consumption rate in pathologic tissue using FDG PET

Heiko Backes[1]

[1]Max Planck Institute for Neurological Research, Medical Physics, Gleueler Str. 50, 50931 Cologne, Germany

Local cerebral glucose consumption can be determined in vivo with positron emission tomography (PET) using [18F]-fluoro-2-deoxyglucose (FDG) as tracer. Like glucose, FDG is transported into cells by glucose transporters and phosphorylated by hexokinase, but – in contrast to glucose – it is not further metabolised and therefore accumulates in metabolically active cells. However, FDG accumulation (e. g. in form of a standardized uptake value) is not always a good measure for glucose consumption rate. In tissue where the ratio of glucose transport and metabolism (and thereby the local lumped constant, i.e. the ratio of metabolic rate of FDG to that of glucose) is altered, FDG uptake may lead to erroneous results with respect to glucose consumption. With the help of tracer kinetic modeling the processes of transport and phosphorylation of FDG can be distinguished and alterations of the local lumped constant can be taken into account for the quantification of glucose metabolism.

We present a non-invasive method for the quantification of cerebral glucose consumption in rats and its application to acute ischemic tissue. In 5 male Wistar rats the middle cerebral artery was occluded inside the µPET scanner by flushing TiO2 spheres into the internal carotide artery following a 2-minute H2[15]O-PET measurement for determination of baseline cerebral blood flow (CBF). 45 minutes after induction of ischemia a second H2[15]O -PET scan was performed followed by a 60 minutes FDG -PET measurement. The integrated number of counts per volume from the H2[15]O-PET scan is a measure for local CBF. The FDG tracer input function is determined non-invasively from the time activity curve of the rat's muzzle. FDG kinetic rate constants are calculated by fitting a two-tissue-compartment model to the dynamic PET data. FDG kinetics is basically expressed by the transport rate constant K1 and the net influx rate constant Ki. Taking into account the differences in transport and metabolism of glucose and FDG, local glucose consumption rate is calculated as a function of K1 and Ki, instead of the classical calculation with a fixed lumped constant.

The acute ischemic tissue is characterized by a clear reduction of CBF, a decrease of the FDG transport rate K1, and an increase of the net influx rate constant Ki by up to a factor of two. K1 and CBF correlate significantly indicating that K1 is a good surrogate marker for CBF in hypo-perfused tissue. The glucose consumption rate calculated as a function of K1 and Ki shows a preserved glucose metabolism in the acute ischemic tissue. Thus, the alterations in the ratio of K1 and Ki cause an elevation of FDG uptake although glucose consumption rate is preserved.

We demonstrate that by kinetic modeling of FDG-PET data the glucose consumption rate can be determined even in pathologic tissue where the local lumped constant is changed. Furthermore, K1 is a good surrogate marker for CBF in hypo-perfused tissue, which may displace laborious H2[15]O PET measurements for the identification of tissue with low CBF.

# Non-invasive detection of amyloid plaques by combined multi-functional and morphological imaging in transgenic mouse models of Alzheimer's Disease

Florian Christoph Maier[1], Andreas Schmid[1], Hans Wehrl[1], Gerald Reischl[2], Bernd Pichler[1]

[1]University of Tuebingen, Laboratory for Preclinical Imaging and Imaging Technology of the Werner Siemens-Foundation, Roentgenweg 13, 72076 Tuebingen, Germany[2]University Hospital Tuebingen, Radiopharmacy, Tuebingen , Germany

Transgenic animal models of Alzheimer's Disease together with current small animal imaging instrumentation provide powerful tools to study disease characteristics and progression longitudinally in a non-invasive manner. Clinically well established markers like [11C]PIB and [15O]H2O are only poorly understood in the preclinical field of neuroimaging. As new therapeutical approaches towards a causative cure for Alzheimer's Disease are currently under development these biomarkers need to be further evaluated in transgenic mouse models. Thus our aims were the evaluation of [11C]PIB-, [15O]H2O-PET and Arterial Spin Labeling (ASL-MRT) in three different mouse models to follow plaque deposition and physiological changes longitudinally and non-invasively.

APPPS1, APP23 and Tg2576 mice with respective littermate controls were injected intravenously with 7.6±2.8 MBq [11C]PIB for measurement of amyloid deposition (specific activity > 50GBq/µmol) and with 28.5±3 MBq [15O]H2O for measurement of cerebral perfusion. Dynamic PET scans were performed for 1h p.i. and the mice were anesthetized with 1.5% isoflurane in 100% oxygen. In addition, 3D MR images and ASL were acquired for each mouse. The PET images were analyzed using cortical regions as target and the cerebellum as internal reference. The obtained time activity curves were processed with the Logan Plot and the simplified reference tissue model (SRTM) by Lammertsma. ASL-MRT data were analyzed with an in-house programmed Matlab routine using a simplified version of the Bloch equation. Furthermore, the animals were analyzed histopathologically.

[11C]PIB showed specific binding in cortical regions of transgenic APPPS1 (SRTM 0.32±0.18) and APP23 mice (SRTM 0.23±0.09) in comparison to littermate controls (APPPS1 co: SRTM 0.05±0.07, n=8, $p<0.05$, 9.5 months old; APP23 co: SRTM 0.01±0.01, n=6, $p<0.01$, 16 months old). In sharp contrast to this Tg2576 mice did not show any specific uptake (SRTM 0.02±0.02) and could not be differentiated from littermate control mice (SRTM 0.01±0.01, n=4, 18 months old) with [11C]PIB. However both APP23 and Tg2576 mice showed a decreased cerebral blood flow with [15O]H2O and ASL while APPPS1 mice did not show any differences.

These results clearly show the possibilities and the limitations of [11C]PIB, [15O]H2O and ASL -MRT for imaging amyloid plaques and the associated physiological changes. Furthermore, combined measurements using these imaging modalities allow both differentiation of transgenic and control animals as well as a clear separation between the three mouse lines. As aged transgenic APP23 and Tg2576 mice show both parenchymal and vascular plaques while APPPS1 mice are confined to parenchymal deposits there seems to be a strong connection between vascular amyloid and loss of cerebral perfusion. Thus [15O]H2O and ASL could be potential candidate imaging techniques to distinguish between neuritic and vascular type dementias in the preclinical field.

# In vivo imaging of cerebral potassium metabolism in focal cerebral ischemia in rats using 201TlDDC-SPECT

Jürgen Goldschmidt[1]

[1]Leibniz-Institute for Neurobiology, Auditory Learning and Speech, Brenneckestr. 6, 39118 Magdeburg, Germany

Focal cerebral ischemia is accompanied by severe alterations in potassium (K+)-metabolism in the affected region. Monitoring these alterations in vivo could be of substantial interest in preclinical research as well as for diagnostic purposes in clinical applications.
In principle, in vivo monitoring of K+-metabolism is possible using suitable isotopes of the K+-analogues rubidium or thallium. Due to the poor blood-brain barrier permeability of K+ and K+-analogues, however, little use has been made thus far of these tracers for imaging cerebral potassium metabolism.
We here present a novel approach for in vivo imaging of cerebral K+-metabolism in focal cerebral ischemia. We intravenously injected rats with the lipophilic chelate complex 201-thallium diethyldithiocarbamate (201TlDDC) after induction of focal cerebral ischemia by endothelin-mediated middle cerebral artery occlusion (MCAO). We monitored the 201Tl-distribution in the rat brains at various time points after 201TlDDC-injection using a small-animal SPECT/CT scanner.
We had previously shown using histochemical techniques that, after crossing the blood-brain barrier, Tl+ is released from TlDDC and that neurons take up the ion in an activity-dependent manner. We reasoned that, conversely, in cerebral ischemia due to breakdown of Na,K-ATPase activity and K+-gradients Tl+-uptake will be reduced in the infarcted area and that, with infarct progression, Tl+ will be lost from the tissue. We here show using SPECT-imaging that upon MCAO there is in fact a reduced 201Tl-uptake in vivo on the lesioned side as well as a continuous loss of 201Tl from the lesioned area during the first hours after onset of ischemia. The definite lesion is characterized by a marked reduction in 201Tl-content.
Our findings suggest that 201TlDDC is a useful tracer for SPECT-imaging of tissue viability, lesion growth and lesion size in focal cerebral ischemia. Using high-resolution multi-pinhole SPECT-imaging the patterns of 201Tl redistributions in rodent brains can be imaged with millimeter or submillimeter spatial resolutions. Small-animal 201TlDDC-SPECT can provide novel insights into the spatiotemporal dynamics of K+-metabolism in focal cerebral ischemia.

# Symposium

# S4: Principles of neural function – how theories inspire experiments

S4-1 Neural control of motor function in animals and robots
*Florentin Wörgötter, Poramate Manoonpong, Silke Steingrube, Marc Timme*

S4-2 Neural Control of Locomotion - from Joint Control to Adaptive Locomotor Behaviors
*Ansgar Büschges, Matthias Gruhn*

S4-3 Energy-Information Trade-Offs between Movement and Sensing
*Malcolm A MacIver*

S4-4 Exploring higher brain function with electrophysiology in behaving fruit flies
*Gaby Maimon*

S4-5 Adaptation in the fly visual system: efficient extraction of behaviorally relevant stimuli
*Rafael Kurtz*

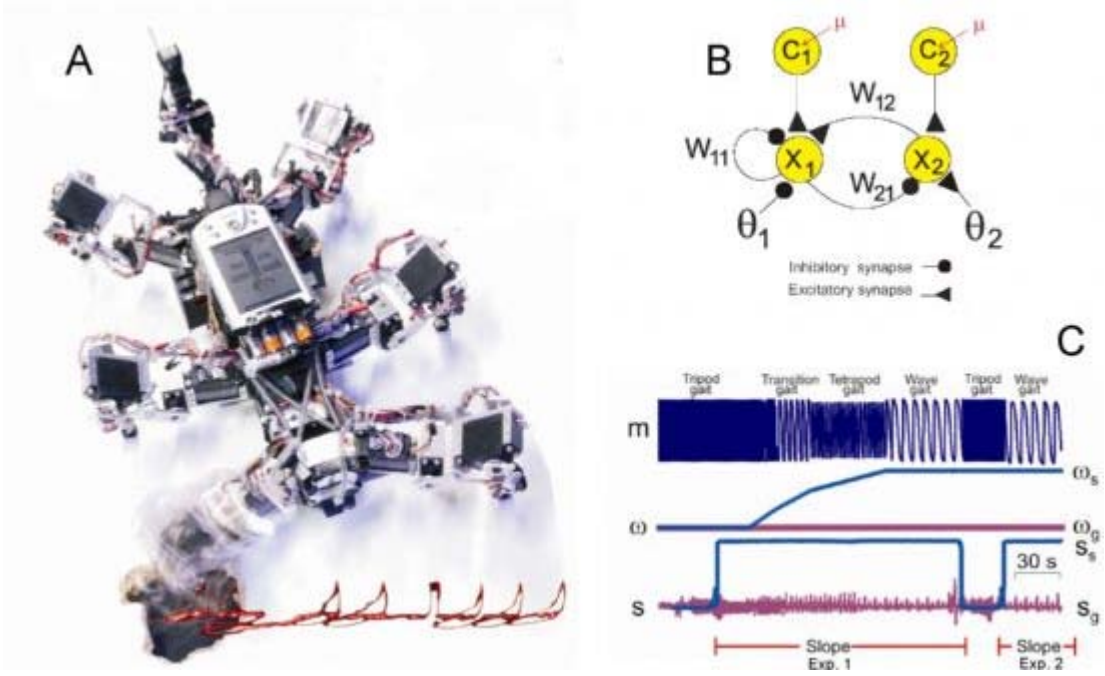
S4-6 Sensory processing with noisy spikes
*Jan Benda, Jan Grewe, Henriette Walz*

# Neural control of motor function in animals and robots

Florentin Wörgötter[1], Poramate Manoonpong[1], Silke Steingrube[1], Marc Timme[2]

[1]University Göttingen, Inst. Physics 3 - Biophysics, Computational Neuroscience, Friedrich Hund Platz 1, 37077 Göttingen, Germany
[2] Max Planck Inst. f. Dynamik und Selbstorganisation, Bunsenstr. 10, 37073 Göttingen, Germany

Controlling sensori-motor systems in higher animals or complex robots is a challenging combinatorial problem, because many sensory signals need to be simultaneously coordinated into a broad behavioral spectrum. To rapidly interact with the environment, this control needs to be fast and adaptive. Insects solve this problem by specific control networks which provide the required information processing for autonomous behavior. By contrast, current robotic solutions operate with limited autonomy and are mostly restricted to few behavioral patterns. In a recent contribution, we have introduced chaos control as a novel strategy to generate complex behavior of the autonomous hexapod robot AMOS-WD6 (Steingrube et al., Nature Physics, 6, 224-230, doi:10.1038/nphys1508). In this system, 18 sensors drive 18 motors using a two-neuron Central Pattern Generator circuit (CPG) in combination with simple neural control networks for signal shaping. Without sensor input, the CPG produces chaotic patterns. However, due to the fact that the robot operates in its environment, stimuli are always present and lead to the fast, adaptive stabilization of unstable periodic orbits by which certain gaits are selected. Signal shaping, using an additional velocity control and a phase generating network, leads then to the generation of eleven basic behavioral patterns (e.g., orienting, taxis, self-protection, various gaits) and their combinations. The complete network contains about 50 neurons only. With this the robot can navigate through complex terrain, free itself from traps, circumnavigate obstacle or climb across then, etc. The control signal quickly and reversibly adapts to novel situations and additionally enables learning and synaptic long-term storage of behaviorally relevant motor responses. For example the robot can learn to select useful gaits in different situations, like a slow energy saving gait when climbing or a fast, running gait when being "hunted". This study, thus, shows that it is possible to arrive at complex adaptive behavior purely by neuronal control and AMOS-WD6 is one of the first robots – if not the only one – that operates exclusively on neuronal principles which mimic parts of the circuitry of insects. The complexity of the control network remains small and the resulting behavior is versatile and natural. Thus, neural control provides a powerful yet simple way to self-organize a large set of behaviors in autonomous agents with many degrees of freedom.



# Neural Control of Locomotion - from Joint Control to Adaptive Locomotor Behaviors

Ansgar Büschges[1], Matthias Gruhn[1]

[1]Zoological Institute, Dept. of Animal Physiology, Zülpicher Str. 47b, 50694 Cologne, Germany

When animals move, the underlying motor output commands and the movements they generate are the result of complex neuronal and neuromuscular processes. Together, they enable the generation of behavior that, although seemingly automatic, remains perfectly adaptable to changes in the external world. Understanding the neural basis of movement contributes much to unraveling the function of the nervous systems of animals and humans alike, and is also important for research in robotics where the aim is to develop realistically performing autonomous machines (7). Walking is one of the most common means of locomotion in terrestrial animals. The underlying movements of the appendages arise from the interaction of the neuronal activity from three different sources: (i) descending brain signals for the selection, initiation and maintenance of locomotor behaviors, (ii) the activity of local central pattern generating networks and feedback from sensory neurons about movements and forces generated in the moving limbs and finally (iii) coordinating signals from neighboring appendages to ensure the proper coordination of all appendages. In the past two decades, substantial information has been gathered on how functional walking motor outputs are generated. Among the best investigated animals are several vertebrates and notably arthropods, such as crayfish, cockroach and the stick insect. The knowledge acquired on the stick insect leg muscle control system has even allowed the formulation of a basic neural controller architecture for single-leg stepping.
The talk will first review the current knowledge about the network organization and activity underlying the control of stick insect walking, both on the level of single leg control (1,3,6) and inter-leg coordination (2). We will then report on recent progress made in understanding the ways by which modifications in the motor output for walking are generated in order to allow adaptations in locomotor behavior (8). In this context, we will touch upon the generation of changes in stepping velocity (5,9), movement direction, and the generation of turns (4). The current knowledge will be placed in the greater scope of the symposium.

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# Energy-Information Trade-Offs between Movement and Sensing

Malcolm A MacIver[1]

[1]Northwestern University, Mechanical Engineering, Neurobiology and Physiology, 2145 Sheridan Rd, Tech B224, 60208 Evanston, USA

No abstract available

# Exploring higher brain function with electrophysiology in behaving fruit flies

Gaby Maimon[1]

[1]The Rockefeller University, Laboratory of Integrative Brain Function, 1230 York Ave / Mailbox 294, 10065 New York, USA

A good theory of a sensory system would explain why information about the outside world is reflected by the activity of neurons in just the observed format and not some other format. In visual neuroscience, data for such theories typically come from presenting stimuli to quiescent animals. In nature, however, animals tend to move around their environment. Locomotion has two important consequences for vision theory. First, natural stimuli will tend to have rich dynamics, prescribed by the statistics of how the retina and animal move. How such natural dynamics affect sensory coding has received some attention. Second, and perhaps less studied, is the possibility that the act of locomotion itself may change the state of the visual system, such that sensory responses become more suited for guiding locomotion and less suited for the visual needs of a stationary animal. To study the influence of locomotion on visual processing — and other higher brain processes down the road — we have developed a new electrophysiological recording system in the fruit fly, *Drosophila melanogaster*. We obtain whole-cell patch clamp recordings from genetically identified neurons simultaneously with behavioral measurements in the same fly. In our initial experiments, we have targeted the vertical-system visual neurons (VS cells) of the fly lobula plate, the third visual neuropile in the fly's brain. VS cells have been extensively studied in restrained animals and are thought to estimate self motion during flight so as to facilitate stabilizing reflexes. However, no one had recorded from VS cells during actual flight behavior. Our data revealed that VS cells show two prominent physiological modulations during flight: a tonic shift in the baseline membrane voltage and a strong boost of visually driven activity. Both these modulations are likely to change the chemically or electrically mediated synaptic output at the VS-cell terminals. When the animal stops flying, visual responses remain boosted for many seconds whereas the baseline membrane voltage returns to its pre-flight level immediately, suggesting that these two effects have separable underlying mechanisms. A similar gain change has now been observed in rodents, where cortical visual-neuron responses are stronger in mice walking on a treadmill as compared to quiescent animals. Further work will be needed to reveal the function of these gain changes. One possibility is that stronger visual responses might allow for more accurate or faster locomotion-specific behavior. Because electrical signaling is energetically costly, the brain may also save energy by keeping cellular responses at a minimum when neurons are not actively being used to drive behavior. These results demonstrate the importance of studying visual neurons, and formulating visual-system theories, not only in the context of naturalistic sensory input but also in the context of ethologically relevant locomotory output.

# Adaptation in the fly visual system: efficient extraction of behaviorally relevant stimuli

Rafael Kurtz[1]

[1]Bielefeld University, Neurobiology, Post Box 100131, 33501 Bielefeld, Germany

Motion detection is of utmost importance for organisms that rely on their visual sense for behavioral control. During locomotion continually changing global motion patterns are generated on the eyes, so-called optic flow, which is essential for optomotor course control. In addition to information about self-motion, optic flow contains information about the 3D-layout of the environment, resulting from relative motion between objects at different distance to the moving observer. Essential tasks, such as object detection and responses to conspecifics or predators are therefore largely based on the computation of optic flow.

The lobula plate tangential cells (LPTCs) of flies are a class of individually identifiable neurons, which process global dynamic visual input. By integrating the outputs of arrays of local motion-detecting elements in a specific way, different LPTCs are sensitive to distinct types of global motion patterns. However, rather than providing an accurate representation of motion velocity, it appears to be more relevant that certain features of the motion stimulus are extracted by these neurons. This is a difficult task in the presence of rapidly changing complex visual input, which a fly encounters during flight in heterogeneous environments.

In this talk it is outlined how synaptic integration and the ability to adapt their response characteristics to the instantaneous stimulus properties enables visual motion–sensitive LPTCs to remain sensitive to behaviorally relevant features of their complex dynamic input. It is analyzed in how far adaptation helps motion-sensitive neurons to decrease their acitvity without sacrificing their sensitivity to the appearance of novel stimuli.

# Sensory processing with noisy spikes

Jan Benda[1], Jan Grewe[1], Henriette Walz[1]

[1]Department Biology II, Ludwig-Maximilian Universität München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

The role of noise in neural signal processing is still a controversial topic. Often neural noise is considered as something detrimental that renders signal processing and detection more demanding. For example, upstream neurons need to average over many input neurons in order to extract a good representation of the encoded common signal.

In spiking neurons neural noise translates into variability of the timing of action potentials. Many theoretical studies show that there are positive aspects of noise-induced spike-timing variability, and that it can be used as an advantage. The most prominent example is sub-threshold stochastic resonance. Here, the addition of the right amount of noise drives the membrane voltage over the threshold and thus allows the neuron to respond to a sub-threshold stimulus. Less well known is the effect of super-threshold stochastic resonance. A population of noisy spiking neurons can transmit more information about a stimulus than a single noiseless neuron, because noise reduces redundancy in such a population of neurons.

Ultimately, sensory stimuli should not only be transmitted from one neuron to the other, but they need to be processed in order to extract relevant features. Fast and strong stimuli will be able to synchronize the response of a population of noisy neurons. A target neuron reading out synchronous spikes will thus encode different aspects of the stimulus compared to a neuron that responds to all input spikes.

After introducing these theoretical concepts we will show electrophysiological data from the electrosensory system of weakly-electric fish that demonstrate these ideas. In particular we find evidence for a parallel readout of synchronous and all spikes. The resulting different tuning properties match well with different types of behaviorally relevant stimuli, e.g. prey versus communication signals. Also, a comparison of electroreceptor neurons from the active electrosensory system with high spike-timing variablity and receptor neurons from the passive electrosensory system with low variablity reveals how noise is tuned to the different demands on a sensory system arising from distinct temporal stimulus properties.

# Symposium

# S5: Neuropeptides and endocannabinoids - Key players in the modulation of behavioral processes

S5-1 Neuropetide S: control of state-dependent properties in the amygdala in instances of stress and fear
*Hans-Christian Pape*

S5-2 Role of endocannabinoids in fear adaptation
*Carsten T. Wotjak*

S5-3 The role of a NPS receptor polymorphism in panic disorder and related endophenotypes
*Andreas Reif*

S5-4 Involvement of the endocannabinoid system in reward processing
*Miriam Schneider*

S5-5 Role of amygdaloid and cortical cannabinoid receptors in fear learning and memory
*Michael Koch, Sybille Kuhnert*

S5-6 Neuropeptide S as a potential target for the treatment of anxiety disorders
*Markus Fendt*

# Neuropetide S: control of state-dependent properties in the amygdala in instances of stress and fear

Hans-Christian Pape[1]

[1]Westfälische Wilhelms-Universität Münster, Institut für Physiologie I, Robert-Koch-Str. 27A, 48149 Münster, Germany

Fear is a crucial adaptive component of the behavioral repertoire that is generated in anticipation of or in response to stimuli which threaten to perturb homeostasis. Fear-relevant associations can be learned and consolidated as part of long term memory, and be extinguished through extinction learning. Acute or chronic stress can severely influence the balance of these processes, eventally leading to pathological alterations and anxiety disorders. Recent years have seen considerable progress in identifying relevant brain areas – such as the amygdala, the hippocampus and the prefrontal cortex - and key neuromodulatory mechanisms involved in balancing physiological and pathophysiological states of fear and anxiety.
The recently discovered Neuropeptide S (NPS) and its cognate receptor represent such a system of neuromodulation. On one hand, NPS increases wakefulness and arousal. On the other, NPS produces anxiolytic-like effects by reducing acute fear responses and by modulating long-term aspects of fear memory, like contextual fear and fear extinction. Underlying mechanisms involve the presynaptic control of excitatory transmitter release, particularly in synaptic contacts to defined subsets of GABAergic interneurons in the amygdala. The recent availability of a NPS-EGFP transgenic mouse line has revealed that NPS-expressing neurons in the brainstem possess distinctive intrinsic properties, respond in an excitatory fashion to corticotrophin releasing factor (CRF), and send axonal projections to widespread but defined targets in the subiculum, intralaminar thalamus and amygdala. Data from combined behaviroal and electrophysiological in vivo and ex vivo studies indicate that the NPS system is activated in stressful situations, controls hyperexcitability in the amygdala and can thereby overcome stress-induced impairment of fear extinction.
Overall, the NPS system appears to be critically involved in state-setting properties of network functions in the amygdala in instances of stress and fear.

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# Role of endocannabinoids in fear adaptation

Carsten T. Wotjak[1]

[1]Max-Planck-Institut für Psychiatrie

It belongs to our phylogenetic and ontogenetic heritage that we are alarmed in potentially dangerous situations. The important it is to recognize a potential threat, the important it is to adequately react to it. In most people, initial alarm responses and subsequent adaptive behaviors are well balanced. However, certain human beings fail to show adequate adaptation, in particular after stressful or traumatic experiences. Under those circumstances, adaptive processes may become maladaptive and promote the development of psychopathologies, such as depression, phobias, panic disorder or post-traumatic stress disorder. In the recent years, the endocannabinoid system of the brain has emerged as a new player in orchestrating hormonal and behavioral coping with aversive encounters. This talk will summarize some of our recent findings obtained in mice and rats about the role of the cannabinoid receptor type 1 (CB1) in (i) the relief of phobic-like fear, (ii) the buffering of panic-like responses and (iii) the extinction of avoidance behavior.

# The role of a NPS receptor polymorphism in panic disorder and related endophenotypes

Andreas Reif[1]

[1]University of Würzburg, Department of Psychiatry, Füchsleinstr. 15, 97080 Würzburg, Germany

Despite having a substantial heritability, the genetic basis of anxiety disorders is still largely unknown. Approaches to overcome this issue are genome-wide association and linkage studies, but also to test candidate genes from animal studies not only in categorical case-control association studies but also for an influence on intermediate phenotypes of anxiety. To do so, two large national research networks have been established. First, the Panic-Net (www.panic-net.de) aims to investigate mechanisms of disease in panic disorder with respect to CBT response, neuroimaging, and a behavioral avoidance test (BAT) which was accomplished in a large set of patients. Second, a Collaborative Research Center on fear, anxiety, and anxiety disorder (http://sfbtrr58.uni-muenster.de/) bridges basic studies in animals and translational research in healthy volunteers, who are investigated by means of several neuropsychological paradigms. In both studies, the effects of genetic variation in systems, governed by animal experiments, are tested. By combining both networks, a powerful sample is created which enables to scrutinize molecular determinants of panic disorder with respect to disease mechanisms. As a proof of principle, we analyzed the consequences of a functional variant (rs324981) in the gene encoding the NPS receptor, as the NPS system has recently been suggested to play an important role in rodent anxiety. The functional NPSR A/T (Asn107Ile) variant (rs324981) was investigated for association with (1) panic disorder with and without agoraphobia, (2) dimensional anxiety traits, (3) autonomic arousal level during a behavioral avoidance test and (4) brain activation correlates of anxiety-related emotional processing and (5) fear conditioning. The more active NPSR rs324981 T allele was found to be associated with panic disorder in the female subgroup of patients in both samples as well as in a meta-analytic approach. The T risk allele was further related to elevated anxiety sensitivity, increased heart rate and higher symptom reports during a behavioral avoidance test as well as decreased activity in the dorsolateral prefrontal, lateral orbitofrontal and anterior cingulate cortex during processing of fearful faces in patients with panic disorder. During fear conditioning, higher fear ratings were observed in risk allele carriers along with increased activation of the dACC/dmDFC during the acquisition suggesting that risk allele carriers display by overinterpretation of fear reactions. Taken together, the present results provide converging evidence for a role of NPSR gene variation in panic disorder potentially via heightened autonomic arousal, distorted processing of anxiety-relevant emotional stimuli and overinterpretation of fear-related stimuli.

# Involvement of the endocannabinoid system in reward processing

Miriam Schneider[1]

[1]Central Institute of Mental Health, Psychopharmacology, University of Heidelberg, Mannheim, Germany

Rewards are crucial for survival of an individual and its genes and they support elementary processes such as drinking, eating, social contact and reproduction. Reward-related behaviors are very complex and the term "reward" includes a variety of different connotations that are mainly linked to appetitive emotions, the hedonic impact and reward motivation, but also include learning and extinction processes, anticipation as well as expectation of rewarding stimuli.

Dysfunctions of reward processing and altered reward perception are linked to various neuropsychiatric disorders, such as addiction, depression and schizophrenia, and a detailed knowledge on neurocircuits and mechanisms involved in reward processing is therefore crucial for the development of treatment and prevention strategies.

Along with the dopaminergic and the endogenous opioid system, the endocannabinoid system has emerged recently as a key neurochemical mediator of reward processes. It is well known that cannabinoids can induce euphoric and rewarding effects in humans and animals and growing evidence indicates that the endocannabinoid system modulates various aspects of drug and non-drug reward.

With the present study we have investigated the modulatory effets of the endocannabinoid system on hedonic perception and consummatory behavior of alcohol and a palatable non- drug reward (sweetened condensed milk) during various developmental stages in rats, including adolescence, adulthood and aging. The data indicate an interesting modulatory impact of the endocannabinoid system throughout the entire lifetime of a laboratory rat.

# Role of amygdaloid and cortical cannabinoid receptors in fear learning and memory

Michael Koch[1], Sybille Kuhnert[1]

[1]Brain Research Institute, Neuropharmacology, POB 330440, 28334 Bremen, Germany

Cannabinoid (CB1) receptors are abundant in cortical and limbic structures suggesting an important role of the endocannabinoid system in the modulation of emotional and cognitive processes. In fact, recent work using transgenic mice and behavioral pharmacology in rodents has supported this contention, especially with respect to fear learning and memory. We here investigated in rats the role of CB1-receptors in the basolateral amygdala (BLA) and medial prefrontal cortex (mPFC), respectively, on fear memory formation and extinction. We measured the effects of local microinfusions of the CB1-receptor agonist WIN-55,212 (WIN) or the CB1-receptor antagonist AM-251 (AM) on acquisition, consolidation, retrieval and extinction of fear using the fear-potentiated startle paradigm.

No effects on fear acquisition of WIN or AM were found in the BLA or mPFC. Intra-BLA WIN, but not AM impaired fear consolidation and retrieval. Intra-mPFC AM reduced fear consolidation, and mPFC infusion of WIN reduced retrieval of the fear memory trace. Fear-extinction was compromised by AM infused into the mPFC.

Our data indicate that fear memory consolidation and retrieval, as well as extinction are regulated differentially by amygdaloid and cortical CB1-receptors. These data add to a growing literature on the modulation of learning and memory by cannabinoids and complement recent findings in CB1-receptor knock-out mice.

# Neuropeptide S as a potential target for the treatment of anxiety disorders

Markus Fendt[1]

[1] Novartis Institutes for BioMedical Research, Neuroscience Research, WSJ-386.3.28, 4056 Basel, Switzerland

Recent human genetic studies demonstrated an association of a human neuropeptide S (NPS) receptor variant with over-interpretation of fear reactions and a role of this gene variation in panic disorders. The important role of NPS in emotion is supported by a number of animal studies showing that NPS is involved in innate and learned fear suggesting that the NPS receptor may be a potential target for the treatment of anxiety disorders. In recent studies, we investigated the effects of (a) NPS injections and (b) NPS receptor deficiency in different behavioral paradigms which are relevant for neuropsychiatric research. We found that NPS injections into the amygdala, a crucial brain site for conditioned fear, dose-dependently block the expression of conditioned fear and that this effect is independent of NPS effects on locomotor activity. Surprisingly, mice with a NPS receptor deficiency only had a weak or a very modest phenotype in behavioral paradigms of innate and learned fear. However, spontaneous locomotor activity was clearly affected in these mice. Taken together, our studies support literature data showing that NPS and NPS receptors are involved in the modulation and/or processing of fear responses. This indicates that NPS receptor agonists may be useful to treat anxiety disorders. However, since NPS is also involved in other brain processes, NPS receptor agonists might also induce unwanted behavioral effects.

# Symposium

## S6: Motor neuron disease models: Loss of function or gain of toxic function? Molecular mechanisms and therapeutic perspectives

S6-1 ALS Genetics Untangled
*Ashley Jones*

S6-2 A zebrafish model to study the pathogenesis and treatment of ALS
*Wim Robberecht*

S6-3 TDP-43 and FUS: Novel players in motor neuron disease
*Manuela Neumann*

S6-4 Studying toxicity of ALS-causing mutant SOD1 by analyzing SOD1 obligate dimers
*Albrecht M. Clement, Christian Behl*

S6-5 Axonal RNA Transport in Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis
*Michael Sendtner*

S6-6 Update on translational research and treatment strategies in ALS
*Albert Christian Ludolph*

# ALS Genetics Untangled

Ashley Jones[1]

[1]MRC Centre for Neurodegeneration Research, King's College London, UK

It is commonly held that 5% of all ALS cases are familial, the implication being that these are inherited and the other 95% are not. Recent advances in our understanding of genetic mechanisms are revealing that the difference between familial and sporadic disease is to a large extent artificial (although convenient) and that ALS, like many other diseases, shows complex inheritance.

Over the last 18 years we have moved from a single gene known to cause 2% of ALS, *SOD1*, to several, with many loci awaiting further examination and several genes with suggestive findings from association studies. Pathways leading to motor neuron death are coming to light, and coupled with the rapid progress of genetic technology and the collaborative spirit among the ALS genetics community, it seems reasonable to be optimistic that the underlying mechanisms leading to ALS will be revealed at least in part by genetics.

The current state of ALS genetic knowledge will be reviewed with the evidence for the involvement of the various implicated genes.

# A zebrafish model to study the pathogenesis and treatment of ALS

Wim Robberecht[1]

[1]VIB - Leuven, Neurobiology, Herestraat 49, 3000 Leuven, Belgium

Amyotrophic lateral sclerosis remains a fatal neurodegenerative disease of the motor neurons. We have generated a zebrafish model to identify genes that can modify the phenotype in the fish. Expression of mutant SOD1 or mutant TDP -43 induces an axonopathy in the zebrafish consisting of reduced axonal outgrowth and aberrant branching. This model was used to study a library of morpholinos in order to identify those that rescue the phenotype. Furthermore, it allows to rapidly screen for the effect of proteins or compounds on the toxic gain of function of the ALS causing mutant proteins.

# TDP-43 and FUS: Novel players in motor neuron disease

Manuela Neumann[1]

[1]University of Zurich, Neuropathology, Schmelzbergstr. 12, 8091 Zurich, Switzerland

Formation of abnormal, ubiquitinated protein aggregates are the characteristic hallmark lesions in most neurodegenerative disorders. For most neurodegenerative diseases the ubiquitinated proteins building these inclusions have been identified several years ago, like tau in Alzheimer's disease and a-synuclein in Parkinson's disease. However, there were two neurodegenerative disorders remaining, namely frontotemporal dementia with ubiquitinated inclusions (FTLD-U) and amyotrophic lateral sclerosis (ALS), were the ubiquitinated proteins in the inclusions remained unknown until recently, when two novel players, namely TDP -43 and FUS have been discovered as disease proteins in these conditions.

In 2006, the transactive response (TAR) DNA binding protein with Mr 43 kD (TDP-43) was identified as the pathological protein in the majority of FTLD- U (now referred to as FTLD-TDP) and most cases of amyotrophic lateral sclerosis (ALS), thereby providing strong evidence that FTLD and ALS are part of a clinicopathological spectrum of diseases. Subsequently, mutations in TARDBP, the gene encoding TDP-43, were identified in familial ALS and FTLD, emphasizing that TDP -43 dysfunction is directly involved in disease pathogenesis and neurodegenerative processes in these conditions. TDP -43 is a DNA/RNA -binding protein which becomes abnormally phosphorylated, truncated and ubiquitinated in disease process. Notably, the presence of TDP-43 inclusions is consistently associated with a dramatic reduction of nuclear TDP-43 expression suggesting both, loss of function and gain of function, as potential pathomechanisms in TDP-43 proteinopathies.

The discovery of TDP-43 prompted researchers to prioritise genetic analysis to functionally related genes in other familial forms of ALS and led to the identification of mutations in the fused in sarcoma (FUS) gene, encoding another DNA/RNA binding protein, as a cause of familial ALS. Subsequent analysis demonstrated FUS also as accumulating protein in rare subtypes of FTLD (FTLD-FUS), thereby reinforcing the concept that FTLD and ALS are closely related conditions.

The discoveries of TDP-43 and FUS imply alterations in RNA/miRNA processing as a key event in the pathogenesis of ALS and FTLD and opened new avenues for research, although the underlying pathomechanisms leading to TDP-43 or FUS mediated neurodegeneration remain unresolved so far. Future efforts need to focus on the establishment of valid in vivo models, and identification of disease -relevant mRNA and miRNA targets for both proteins.

# Studying toxicity of ALS-causing mutant SOD1 by analyzing SOD1 obligate dimers

Albrecht M. Clement[1], Christian Behl[1]

[1]University Medical Center Mainz, Institute for Pathobiochemistry, Duesbergweg 6, 55128 Mainz, Germany

Although mutations in the SOD1 gene have been identified to cause ALS more that 15 years ago, the toxic property of mutant SOD1 has not yet been identified. It is established that glia cells contribute to the disease, but the detrimental action of mutant SOD1 in the different cell types, either by protein aggregation, untypical enzymatic activity, mitochondrial dysfunction, or toxicity due to aberrant secretion, is not understood. Nevertheless, it is believed that elucidating the toxic properties of mutant forms of SOD1 could not only be a key issue to understand the pathomechanisms of some familial forms of the disease, but could give also hints for deciphering the molecular basis for the onset and the progression of sporadic cases. Based on the analysis of double transgenic mice expressing mutant SOD1 and SOD1(WT) it is proposed that SOD1(WT) might be involved in mutant SOD1 toxicity. Double transfection experiments and subsequent fluorescent lifetime based FRET and biochemical analysis revealed that SOD1(WT) does not primarily co-aggregate with mutant SOD1. In contrast, SOD1(WT) is able to reduce mutant SOD1 aggregation by heterodimerization. In order to study if the formation of heterodimers between mutant and SOD(WT) is of functional importance, we generated obligate SOD1 dimer proteins. By expressing obligatory SOD1 dimers in cellular as well as C.elegans animal models we could demonstrate that toxicity of mutant SOD1 is uncoupled from their aggregation potential. In further experiments we characterized the biochemical and biophysical properties of obligatory dimer proteins representing the two major classes of SOD1 mutants. These analyses of dimer proteins might be a key to disclose the toxic properties of mutant SOD1.

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# Axonal RNA Transport in Spinal Muscular Atrophy and Amyotrophic Lateral Sclerosis

Michael Sendtner[1]

University of Wuerzburg, Institute of Clinical Neurobiology, Versbacher Str. 5, 97078 Wuerzburg, Germany

Human motoneuron diseases are characterized by loss of motor endplates, axonal degeneration and cell death of motoneurons. The identification of responsible gene defects for familial ALS, spinal muscular atrophy (SMA) and spinal muscular atrophy with respiratory distress (SMARD) has pointed to distinct pathophysiological mechanisms responsible for the various forms of the disease. Evidence from mouse models suggests that enhanced vulnerability and sensitivity to proapoptotic stimuli is only responsible for some but not all forms of motoneuron disease(1). Reduced levels of the survival motoneuron (SMN) protein, which are responsible for SMA lead to disturbed RNA processing in motoneurons. A prominent phenotype of SMN deficient motoneurons is reduced axon elongation in the absence of defects that result in reduced motoneuron survival (2,3). In particular, the axonal transport of the mRNA for ß-actin is severely reduced. The SMN protein is part of a complex in the cell body that assembles UsnRNP particles. These U snRNP particles are central constituents of the spliceosome. In addition, the SMN protein is part of another complex in axons and axon terminals of motoneurons. This complex is distinct from the classical SMN complex, and it includes mRNA transport proteins, in particular the hnRNP-R protein (1,4). The hnRNP-R protein binds directly to ß-actin mRNA, and both cell culture experiments in which hnRNP-R expression is reduced and in vivo studies point to an essential role of SMN/hnRNP-R interaction for axonal translocation of ß-actin mRNA.

The consequence is a severe depletion of ß-actin-protein in axon terminals, resulting in disturbed axon elongation, reduced growth cone size and functional deficits in neurotransmission that are caused by disturbed integration and clustering of voltage-gated calcium channels in axon terminals (3). The deficit in clustering of voltage-gated calcium channel in growth cones of SMN-deficient motoneurons is accompanied by a significant reduction of spontaneous Ca2+ transient frequency. In mild models of SMA, this defect can be compensated by enlargement of motor units (5). PTEN signalling pathways restore local ß-actin by stimulating local protein synthesis (6). Current research in our lab focuses on the development of new therapeutic approaches (7), based on new cell culture techniques to visualize local translation of mRNAs in axon terminals of cultured motoneurons, and development of techniques that allow biochemical analysis and characterization of mRNA transport complexes in isolated motoneurons (8). In addition, new mouse models are generated (9) that allow analysis of axon abnormalities and disturbed synaptic function at the neuromuscular endplate in vivo by multiphoton microscopy.

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# Update on translational research and treatment strategies in ALS

Albert Christian Ludolph[1]

[1]Department of Neurology, University of Ulm

The goal of treatment strategies for ALS can be both, prevention and modification of the natural history of the disease itself.
Although initial steps have been taken, in particular in disease models, preventive strategies in humans are predominantly hampered by the lack of knowledge of the preclinical stages of ALS. However, both, preventive and disease-modifying strategies can be studied in disease models. The value of these models was hotly debated in the recent decade since translational research frequently failed. However, before deciding on useful and less useful models methodological standards for preclinical trials had to be established. Presently, a number of animal (not only rodent) models are available or are currently being developed. Based on the established methodological standards, it is a research priority to show which animal model is the most predictive for human drug development.

In principle, treatment approaches can be based on genetic and pathogenetic knowledge. Therapeutics based on genetics (for example well-defined mutations) are hampered by the genetic heterogeneity of ALS. Current clinical trials with antisense nucleotides for the most frequent human mutations, mutations in the gene for the superoxide dismutase (SOD1), will show as a proof of principle whether these treatments will have a chance to be successful. However, treatment of the more frequent so-called sporadic forms will be based on pathogenetic insights which are still in their early stages. A compeletely different approach is based on the clinical success of the benzothiazole riluzole; currently it seems that the further development of structurally related compounds may be advantageous.
Taken together, translational research of the future should focus on the elucidation of the pathogenesis, including preclinical stages of ALS both in experimental animals and in humans, clarify whether the reduction of transcription of specific genes could be useful for therapeutics and further develop the positive effect of successful drugs, such as benzothiazoles.

# Symposium

## S7: Adult neural stem cells in physiology and disease

S7-1 ForNeuroCell: Bavarian Consortium for “Adult Neural stem cells”
*Juergen Winkler*

S7-2 Research Network "Integration of Stem Cell Derived Neurons"
*Dieter Chichung Lie*

S7-3 Functional Relevance of Adult Neurogenesis
*Alexander Garthe*

S7-4 Voluntary and forced metamorphosis of astroglia into neurons
*Benedikt Berninger, Christophe Heinrich, Felipe Ortega, Sánchez Rodrigo, Giacomo Masserdotti, Marcos Costa, Magdalena Götz*

S7-5 Adult neurogenesis in Parkinson's disease
*Beate Winner*

S7-6 Neurogenesis and proliferative cellular plasticity after brain ischemia
*Christoph Redecker*

# ForNeuroCell: Bavarian Consortium for "Adult Neural stem cells"

Juergen Winkler[1]

[1]University Hospital Erlangen, Division of Molecular Neurology, Schwabachanlage 6, 91054 Erlangen, Germany

There is an urgent need for the development of new treatments for neurodegenerative diseases such as Parkinson's (PD) and Alzheimer's disease (AD). These neurological disorders cause long-term and severe impairment affecting the professional as well the daily life of patients. During the last decades there is a tremendous development in regard of the understanding of the underlying molecular mechanisms, however, the major obstacle remains, that still no causal therapy exists at present. To overcome these challenges the development of innovative and long lasting therapies are an urgent need.

Stem cells based neuronal replacement strategies for the treatment of neurological diseases conceptually aim at the functional replacement of neural cells, which are lost in the course of the disease. One of the most exciting findings in neuroscience over the last decades was the identification of neural stem and precursor cells in the adult brain (aNSC's). These cells of the brain maintain the exceptional capacity to divide and to mature into neurons, astrocytes and oligodendrocytes. There are two regions within the adult CNS where these aNSCs reside, the hippocampus and the subventricular zone. Stem cell based therapy for the treatment of neurodegenerative diseases can be distinguished in two approaches: (i) replacement through transplantation and (ii) neuronal replacement through recruitment of endogenous neural stem cells. Due to the fact, that aNSCs exist in the mammalian brain and have the capacity to generate new neurons, the Bavarian Consortium *ForNeuroCell* tries to develop along these concepts stem cells based therapies based on aNSCs. By combining the expertise of basic neuroscience, neurology, neuropathology, neuroimaging as well as neurophysiology, it is the ultimate goal of *ForNeuroCell* via modulation and targeted differentiation of aNSCs to induce regenerative processes in the CNS in order to functionally restore deficits associated with these diseases. The following topics are pursued: (1) Identification of signalling pathways to modulate and maintain aNSCs; (2) Reprogramming and targeted differentiation of endogenously present aNSCs; (3) Preclinical testing of aNSCs in acute and chronic disease models; (4) Development of imaging technologies to detect endogenous and transplanted stem cells in the living mammalian brain. The Bavarian Network *ForNeuroCell* consists of 10 projects of the following universities: Friedrich -Alexander-University Erlangen-Nuremberg, Ludwig-Maximilans-University Munich, Technical University Munich, University Regensburg, Julius-Maximilans-University Wuerzburg, and the Helmholtz Centre Munich.

*ForNeuroCell* and these academic centres envision aNSCs based therapeutic approaches for disorders like PD and AD ultimately translating these concepts into the clinic.

# Research Network "Integration of Stem Cell Derived Neurons"

Dieter Chichung Lie[1]

[1]Helmholtz Zentrum München, Institute of Developmental Genetics, Ingolstädter Landstrasse 1, 85764 München-Neuherberg, Germany

Stem cells hold great promise for repair of the central nervous system. A major challenge for stem cell based replacement therapies is the functional integration of stem cell derived neurons into compromised networks. The BMBF - funded research network

# Functional Relevance of Adult Neurogenesis

Alexander Garthe[1]

[1]Regenerationsgenomik, Zentrum für Regenerative Therapien Dresden, Dresden, Germany

Adult hippocampal neurogenesis lifelong produces low numbers of new granule cells that add to the mossy fiber connection between the dentate gyrus and CA3. The hypothesis is that new neurons are critical to the function of the dentate gyrus and several specific functions have been hypothesized. We follow the idea that the new neurons allow an activity-dependent optimization of the mossy fiber tract in order to cope with situations of novelty and complexity. The precursor cells that are stimulated by activity and training might build up a "neurogenic reserve" that allows flexible response to cognitive challenges in the course of life.
By now many studies have investigated the function of the hippocampus after adult neurogenesis had been suppressed or ablated. Gain-of-function experiments, in contrast, have been rare. Physical exercise and environmental enrichment both increase adult neurogenesis and are additive in their effect. However, despite similar end results they appear to have specific sub-effects on adult neurogenesis. Are these differences also found in behavioral tasks that assess neurogenesis-specific functions? We use a modification of the classical Morris water maze that allows the analysis of the strategies used by the mice to navigate to the hidden platform. Thereby we can ask how new neurons might increase cognitive flexibility during the acquisition and reversal period of the task. Are the results of the two gain-of-function paradigms complementary to the loss-of-function experiments? From the behavioral consequences of both positive and negative regulation of adult neurogenesis a first synthesis can be attempted of what the new neurons are good for. One key consequence of the findings is that "regulation" by activity and "function" of the new neurons have to be regarded as connected.

# Voluntary and forced metamorphosis of astroglia into neurons

Benedikt Berninger[1], Christophe Heinrich[1], Felipe Ortega[1], Sánchez Rodrigo[1], Giacomo Masserdotti[1], Marcos Costa[1], Magdalena Götz[1]

[1]Institute of Physiology, LMU München, Physiological Genomics, Schillerstrasse 46, 80336 München, Germany

In my presentation, I will discuss neurogenesis from astroglia from two perspectives, namely the physiological process of how astroglia-like stem cells generate neurons in the adult subependymal zone (SEZ) and how we can instruct bona fide non-neurogenic astroglia such as parenchymal astroglia from the cerebral cortex towards neurogenesis.

To observe cell division and lineage progression of astroglia-like stem cells, we isolated cells from the adult SEZ and cultured them without growth factors at low density. We demonstrate here that SEZ cells in this culture system are primarily neurogenic and progress through stereotypic lineage trees comprising asymmetric stem cell divisions, symmetric transit-amplifying divisions and final symmetric neurogenic divisions. Stem cells, identified by their astro/radial glial identity and their slow-dividing nature, were observed to generate asymmetrically and fast dividing cells that maintained an astro/radial glia identity. These in turn gave rise to symmetrically and fast dividing cells that lost the glial hallmarks, but had not yet acquired neuronal features. Moreover, we found that cell growth correlated with the number of subsequent divisions of SEZ cells and their progeny, with slow-dividing astro/radial glia exhibiting most substantial growth prior to division. The fact that in the absence of exogenously supplied growth factors and signals provided by the local niche such neurogenic lineage progression takes place in a highly stereotypic fashion, suggests that it is to a significant degree cell-intrinsic or pre-programmed at the beginning of the lineage.

In contrast, astroglia from the postnatal cerebral cortex are intrinsically non-neurogenic but can be reprogrammed in vitro to generate neurons following forced expression of neurogenic transcription factors, thus opening new avenues towards a potential use of endogenous astroglia for brain repair. We show that strong and persistent expression of neurogenic fate determinants driven by silencing-resistant retroviral vectors instructs astroglia from the postnatal cortex in vitro to mature into fully functional, synapse-forming neurons. Importantly, the neurotransmitter fate choice of astroglia-derived neurons can be controlled by selective expression of distinct neurogenic transcription factors: Forced expression of the dorsal telencephalic fate determinant neurogenin-2 (Neurog2) directs cortical astroglia to generate synapse-forming glutamatergic neurons; in contrast, the ventral telencephalic fate determinant Dlx2 induces a GABAergic identity. Moreover, neuronal reprogramming of cortical astroglia is not restricted to postnatal stages, but can also be achieved from terminally differentiated astroglia of the adult cerebral cortex following injury-induced reactivation.

# Adult neurogenesis in Parkinson's disease

Beate Winner[1,2]

[1]Nikolaus-Fiebiger Zentrum für Molekulare Medizin, Nachwuchsgruppe III, Glücksstr. 6, 91054 Erlangen, Germany

[2]Salk Institute, Laboratory of Genetics, 10010 North Torrey Pines Road, 92037 La Jolla, CA, USA

The pathological hallmark of sporadic Parkinson's disease is the neuronal accumulation and aggregation of human wild-type a -synuclein (hWT a -syn). In addition to the substantia nigra and other brain regions, hWT a-syn aggregates are found in the olfactory bulb and hippocampus. New neurons are constantly generated and functionally integrated in these brain regions. In hWT a -syn transgenic mice, the number of newborn neurons is decreased in the adult hippocampus and in the SVZ/ olfactory bulb system. The impact of hWT a-syn in different a-syn transgenic mice will be discussed.

# Neurogenesis and proliferative cellular plasticity after brain ischemia

Christoph Redecker[1]

[1]Jena University Hospital, Hans Berger Clinic for Neurology, Erlanger Allee 101, 07747 Jena, Germany

The adult brain responds to brain lesions such as stroke with an increased proliferation of endogenous progenitor cells in the subgranular zone of the dentate gyrus adding a significant number of new neurons to the hippocampal network. Pharmacological interventions but also rehabilitative training like environmental enrichment, wheel running or skilled training further stimulate postischemic neurogenesis, correlating with a better functional outcome in sensorimotor but also cognitive tasks. The vast majority of these newborn neurons showed the typical morphology of granule cells and functional recruitment during specific behavioural tasks. Within the direct surround of the ischemic lesion the proliferative response is even more complex, comprising a close interaction between reactive microglia, astrocytes, and distinct populations of proliferating cells expressing the immature neuronal marker doublecortin. Rehabilitative training again modulated these complex cellular events, but neurogenesis is missing in the perilesional area. However, application of growth factors like BDNF or a combination of anti-inflammatory drugs and behavioural training favour the generation of some new neurons and may create some new strategies to improve functional recovery after ischemic brain damage.

# Symposium

# S8: Peripheral mechanisms in olfaction

S8-1 OLFACTION TARGETED
*Peter Mombaerts*

S8-2 Mammalian olfaction: from genes and cells to system function and pathology
*Frank Zufall*

S8-3 Mammalian olfactory chemosensors: from genes to behavior
*Ivan RODRIGUEZ*

S8-4 Encoding and processing of olfactory information in neural circuits
*Silke Sachse, Veit Grabe, Antonia Strutz, Marco Schubert, Bill S. Hansson*

S8-5 A cellular module for single-molecule sensitivity in sperm
*U. Benjamin Kaupp*

S8-6 Evolutionary aspects of sensory perception
*Sigrun I. Korsching*

# OLFACTION TARGETED

Peter Mombaerts[1]

[1]Max Planck Institute of Biophysics, Frankfurt, Germany

The mammalian olfactory system is increasingly recognized as an attractive model system for studying the formation of neural circuits during development. An olfactory sensory neuron expresses just one the of ~1000 odorant receptor genes in the mouse genome. Axons of neurons that express a given odorant receptor gene coalesce into a few of the ~1800 glomeruli in the olfactory bulb of the mouse. A mapping problem is thus posed: ~1000 populations of neurons, each expressing a particular odorant receptor gene, must be sorted onto ~1800 glomeruli. A productive approach to study axonal coalescence is targeted mutagenesis of odorant receptor genes, to express axonal markers in neurons expressing a given odorant receptor gene. These experiments have revealed that the odorant receptor is a critical determinant of axonal coalescence into glomeruli. My talk will summarize recent results of our research on odorant receptor gene choice and axonal wiring.

# Mammalian olfaction: from genes and cells to system function and pathology

Frank Zufall[1]

[1]University of Saarland School of Medicine, Physiology, Kirrbergerstr., 66421 Homburg, Germany

Our group is interested in elucidating the general organization of the mammalian sense of smell, using a systems-oriented approach that combines analyses at the molecular and cellular level with approaches aimed at understanding circuits and behavior (1). A specific focus of the group is to gain insight into those mechanisms that mediate chemical communication in mammals (2). More recently, we have found a gene that allows to link olfactory mechanisms in mice with those in humans. This provides an important example of how the study of general olfactory mechanisms in mice can lead to insight into the pathophysiology of olfaction in humans.

(1) Munger, S.D., Leinders-Zufall, T. and Zufall, F., Subsystem organization of the mammalian sense of smell. Annu. Rev. Physiol. 71, 115-140 (2009).

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# Mammalian olfactory chemosensors: from genes to behavior

Ivan RODRIGUEZ[1]

[1]University of Geneva, Dpt. of Genetics and Evolution, 30 quai Ernest Ansermet, 1204 Geneva, Switzerland

Mammalian species rely on their olfactory system to adequately interact between individuals and with their surroundings. At the core of this complex sensory system are large superfamilies of specialized G -coupled receptors, which are present at the interface between the outside and the inside worlds, on dendrites of olfactory sensory neurons of the main olfactory or vomeronasal epithelia. Surprisingly, these receptors play multiple roles. They first define the agonist profile of a given sensory neuron. But they also regulate chemoreceptor gene expression, in the sense that they are involved in the maintenance of the monogenic and monoallelic transcription that characterizes olfactory receptor gene expression. This limited transcription defines how narrowly tuned a given sensory neuron is. Third, olfactory chemoreceptors are involved in the precise convergence of like-axons in the olfactory bulb, a confluence that represents the first step of olfactory coding. Chemoreceptors thus not only define olfactory neural circuits, but are directly implicated in their establishment and functioning. Some of these circuits, due to the nature of the chemoreceptors they express, appear highly specialized. This seems to be the case of a group of vomeronasal sensory neurons that transcribe members of a small family of formyl peptide receptor genes. These genes are, like most olfactory chemoreceptor genes, characterized by monogenic transcription and a punctate expression pattern in the sensory neuroepithelium. In vitro expression of the corresponding receptors provides sensitivity to disease/inflammation-related ligands. The same molecules activate vomeronasal neurons in vivo. Taken together, these observations suggest that the neural circuit expressing formyl receptor proteins may play a function associated with the identification of pathogenic states, or with the discrimination of pathogens.

# Encoding and processing of olfactory information in neural circuits

Silke Sachse[1], Veit Grabe[1], Antonia Strutz[1], Marco Schubert[1], Bill S. Hansson[1]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology

We are investigating how odors are coded and processed in the *Drosophila* brain to lead to a specific odor perception. The basic layout of the first olfactory processing centers, the olfactory bulb in vertebrates and the antennal lobe (AL) in insects, is remarkably similar. Odors are encoded by specific ensembles of activated glomeruli (the structural and functional units of the bulb-lobe) in a combinatorial manner. The vinegar fly *Drosophila* melanogaster provides an attractive model organism for studying olfaction, as it allows genetic, molecular and physiological analyses. We are performing calcium as well as chloride imaging to decipher basic principles involved in olfactory transduction, coding, processing and perception of odors. We will present our recent insights into olfactory coding strategies yielded by morphological and functional analysis of the different neuronal populations, i.e. sensory neurons, local interneurons and projections neurons, present in the *Drosophila* antennal lobe.

# A cellular module for single-molecule sensitivity in sperm

U. Benjamin Kaupp[1]

[1]Stiftung caesar, Molecular Sensory Systems, Ludwig-Erhard-Allee 2, 53175 Bonn, Germany

Sperm of the sea urchin *Arbacia punctulata* respond to a single molecule of chemoattractant released by eggs. The mechanism underlying this extreme sensitivity is unknown. Crucial signaling events in the response of sperm to the chemoattractant include the synthesis of the intracellular messenger guanosine 3',5'-monophosphate (cGMP) and the ensuing membrane hyperpolarization due to the opening of $K^+$-selective cyclic nucleotide-gated (CNGK) channels.

Calibrated photolysis of caged cGMP shows that = 45 cGMP molecules are required for a single-molecule response. This corresponds to a change in the total cGMP concentration of 45 nM in the flagellum. The changes in free concentration are much smaller due to buffering of cGMP by phosphodiesterase or other cGMP-binding sites. The CNGK channel can respond to such small cGMP changes because it is exquisitely sensitive to cGMP ($K_{1/2}$ = 25 nM) and activated in a non-cooperative fashion.

The structure of the CNGK channel differs from that of classical CNG channels of photoreceptors and olfactory neurons. These channels form heterotetramers composed of different subunits. In contrast, like voltage-activated $Ca_v$ and $Na_v$ channels, the CNGK polypeptide consists of four homologous repeat sequences. Each repeat harbors all the functional motifs of a classical CNG channel subunit. Disabling each of the four cyclic nucleotide-binding sites through mutagenesis revealed that binding of a single cGMP molecule to repeat 3 was sufficient to activate the CNGK channel. Thus, CNGK has developed an activation mechanism that is different from that of classical CNG channels, which requires the cooperative binding of several ligands and operates in the micromolar rather than the nanomolar range. At nanomolar concentrations and in small compartments such as cilia, when only few molecules are available, the simultaneous binding of several ligand molecules to the same channel is a rare event; thus cooperative activation would impair rather than enhance channel sensitivity. This study provides a framework that might be also relevant for pheromone signaling in vertebrate and insect olfaction.

# Evolutionary aspects of sensory perception

Sigrun I. Korsching[1]

[1]Institut für Genetik, Biocenter, Universität zu Köln, Zülpicher Str. 47a, 50674 Köln, Germany

The perception of odors triggers many behaviors throughout the animal kingdom, from food localisation and predator avoidance to many forms of intraspecies communication, including for purposes of reproduction. Olfactory receptors mediate the primary interaction of the olfactory brain with the external world. Any odor stimulus is initially represented as activation of one to many different olfactory receptors. The discovery of large families of olfactory receptor genes in recent years has enabled the study of the sense of smell at the molecular level.

Using an bioinformatic approach, we have delineated the olfactory receptor gene repertoires in lower vertebrates, including fish and amphibians. Species-specific rapid evolution in some olfactory receptor gene families, e.g. *taar* genes and *v2r* genes contrasts with a large degree of evolutionary conservation in others, e.g. the *v1r*-like ora genes of bony and cartilaginous fish. Several features of the latter gene family do not conform to the expectations for olfactory receptor genes, including multiexonic genes, an unusual genomic localisation and an atypical 'one neuronal population – one receptor' mode of expression.

Deorphanization of olfactory receptor genes and interspecies comparisons show a lesser degree of conservation for the molecular basis of odor detection, the olfactory receptor genes than for the odors themselves. It appears that physiologically relevant and evolutionary conserved odors are being recognized by different components of the olfactory receptor repertoire in different species.

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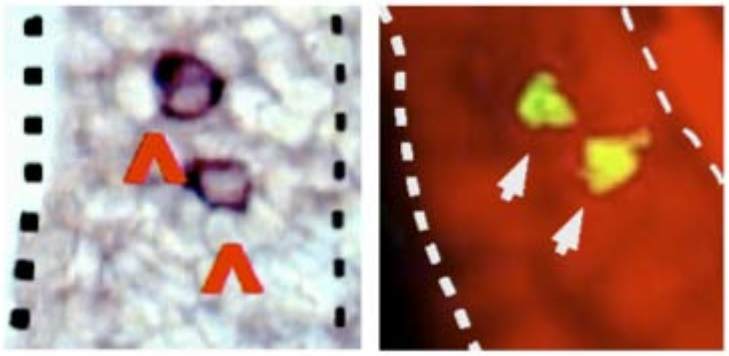
Hussain A, Saraiva LR, Korsching SI., Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts, Proc Natl Acad Sci U S A 106, 4313-8 (2009).

*Olfactory receptor expression pattern and odor-induced activity pattern*
Left, *in situ* hybridization for a TAAR receptor shows sparse expression in the olfactory sensory surface (aka nose) typical for olfactory receptor genes. Right, TAAR ligand-induced neuronal activity shows very similar sparse pattern of activated neurons, nuclear dye (red) visualizes the tissue.



# Symposium

## S9: Plasticity in the human visual system - Probing dysfunction with functional magnetic resonance imaging

S9-1 Can the visual cortex remap retinal input when the retina is lesioned?
*Antony Morland*

S9-2 Neuroplasticity in the visual cortex of patients with macular degeneration: evidence from fMRI.
*Mark W. Greenlee*

S9-3 Pre- and postnatal plasticity in the visual cortex – bilateral visual field maps in a patient with only one hemisphere
*Lars Muckli*

S9-4 Population receptive fields and plasticity
*Serge O. Dumoulin*

S9-5 Congenitally abnormal V1 input – insights into the self-organisation of the human visual system
*Michael B. Hoffmann*

S9-6 Functional plasticity in adult human cortex in response to an extreme alteration of visual input
*Alyssa A Brewer, Brian Barton, Ling Lin*

# Can the visual cortex remap retinal input when the retina is lesioned?

Antony Morland[1]

[1]University of York, Department of Psychology, Heslington, YO10 5AX York, United Kingdom

The occipital lobe of the human brain contains multiple maps of the retina. We tested whether or not these visual maps remap visual information following damage to the retina that occurred as a result of juvenile and age-related macular degeneration. We presented phase-encoded checkerboard stimuli (expanding ring and rotating wedges) during acquisition of T2* weighted magnetic resonance images to 16 patients and 24 controls. In a region of interest analysis, we found that signals in regions of the brain that would have normally represented the damaged retina did not exceed those in a control region. Moreover, the signals did not exceed those in controls in whom we simulated a central scotoma. We also predicted the area of cortex that should be active on the basis of the size of the patients' scotomata that were mapped with microperimetery. For the patient group, the measured active area did not exceed the area predicted on the basis of the scotomata. Our results indicate that the visual areas of the brain are limited in their capacity to reorganize when retinal damage occurs in adulthood, in contrast to the reorganization observed when retinal disease is present at birth.

# Neuroplasticity in the visual cortex of patients with macular degeneration: evidence from fMRI.

Mark W. Greenlee[1]

[1]Institute of Experimental Psychology, University of Regensburg

In patients with central scotomas, a large part of visual cortex is not adequately stimulated. Patients often use a new eccentric fixation area on intact peripheral retina ("preferred retinal locus" – PRL) that functions as a pseudo-fovea. We tested 19 patients with central scotomas (5-20 degrees in diameter) due to retinal dystrophy (Stargardt disease, cone-rod dystrophy) in a visual search task. In this task the letter "L" had to be detected between several "T" distractors arranged radially in the peripheral visual field. Additionally, stability of eccentric fixation and saccadic behaviour were determined. Patients with stable fixation had shorter latencies and needed less correction steps in the saccadic task, further they showed a significantly better performance in the visual search task than patients with unstable fixation. Patients more frequently detected targets when they fell into the area around pseudo-fovea compared to other positions in the visual field. The benefit of a stable pseudo-fovea is also visible in the pattern of fMRI results. When the target stimulus appeared at the position of the pseudo-fovea, we could find significant clusters of higher activation in retinotopic areas of the visual cortex corresponding to the respective PRL. This differential brain activation was not observed in healthy controls.

# Pre- and postnatal plasticity in the visual cortex – bilateral visual field maps in a patient with only one hemisphere

Lars Muckli[1]

[1]University of Glasgow, Centre for Cognitive Neuroimaging (CCNi), Institute of Neuroscience and Psychology, 58 Hillhead Street, G12 8QB Glasgow, United Kingdom

The prenatal development of retinotopic maps is regulated by two basic principles: gradients of molecular markers determine map orientations and waves of spontaneous activity determine neighbourhood relations. We were able to observe the developed retinotopic maps of a 10 year old girl who was born with only one cerebral hemisphere (Muckli et al 2009). The retinal ganglion cells from her normal developed left eye project entirely to the left thalamus and the left cerebral hemisphere, where full-field visual maps emerged. Patient AH's condition was congenital and distinct from patients who regained visual field loss after hemispherectomy early in life (Werth 2006). AH's precise retinotopic folding patterns in V1, V2 and LGN provide evidence for the underlying developmental mechanisms based on molecular markers and on activity- dependent cues. In V1, smooth and continuous maps from contra - and ipsilateral hemifields overlap each other, whereas in ventral V2 and V3 ipsilateral quarter field representations invaded small distinct cortical patches. In V1, the overlapping patterns of both visual hemifields were mirror symmetric, which is indicative off a mapping mechanism that aligns corresponding ephrin gradients of the nasal and lateral retina. Distinct patches in V2, V3 and thalamus reveals a surprising flexibility of the developmental mechanisms responsible for map formation.

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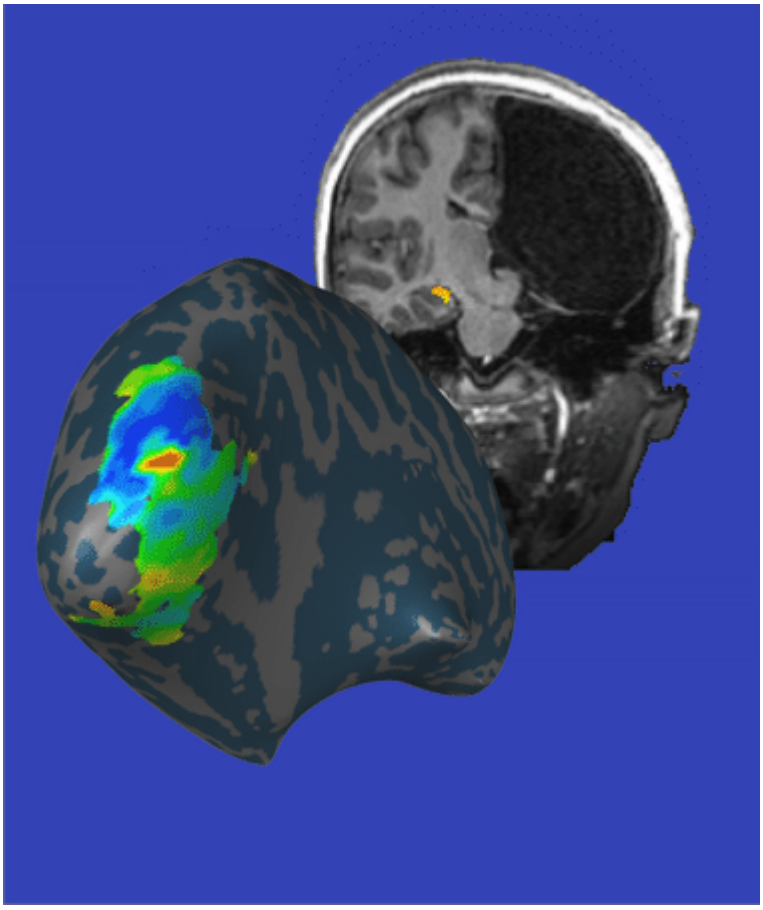
2. Werth R. (2010) Visual functions without the occipital lobe or after cerebral hemispherectomy in infancy. Eur J Neurosci. 2006 (10):2932-44.



# Population receptive fields and plasticity

Serge O. Dumoulin[1]

[1]Utrecht University, Experimental Psychology, Helmholtz Institute, Heidelberglaan 2, 3584 CS Utrecht, Netherlands

We introduced a new functional magnetic resonance imaging (fMRI) data -analysis method for estimating the neuronal population receptive field (pRF) properties in human visual cortex [1]. We show that in healthy subjects these pRF sizes vary in a systematic way between and within different visual field maps [1,2]. The pRF size estimates are consistent with invasive human and monkey electrophysiological measurements. The pRF method is non-invasive and can be applied to a wide range of conditions when it is useful to link fMRI signals in the visual pathways to neuronal population receptive fields.

We have applied our pRF measurements, in two extremely rare neuro -ophthalmologic cases: a subject born without an optic chiasma [3] and a case of sight salvage in adult life [4]. We describe highly atypical visual field representations in the otherwise functional visual system of the achiasmic subject. In the case of sight salvage in adult life, repairing the retinal image has not restored normal cortical organization, seven years post-surgery. The continuing deficits in his cortical organization may explain why this patient's visual performance remains substantially impaired. We confirm remarkable plasticity at an early stage of development but our results indicate very limited plasticity in the adult visual cortex.

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[3] Prakash, Dumoulin, Fischbein, Wandell, Liao (2010) J Neuro-Ophthalmology. 30: 45–48.
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# Congenitally abnormal V1 input – insights into the self-organisation of the human visual system

Michael B. Hoffmann[1]

[1]Magdeburg University, Ophthalmology, Visual Processing Lab, Leipziger Str. 44, 39120 Magdeburg, Germany

Different types of misrouting of the optic nerves can provide large scale abnormal input to the visual cortex: In albinism part of the temporal retina projects erroneously to the contralateral hemisphere, while in hypochiasmia part of the nasal retina projects erroneously to the ipsilateral hemisphere. Due to these congenital malformations of the optic chiasm, processing in the early visual cortex is in albinism and in hypochiasmia not only dedicated to the representation of the contralateral, but also of a sizable proportion of the ipsilateral visual field. This makes these conditions a powerful model to study principles of re- and self-organisation in the human visual system. Here, a series of studies will be presented, detailing the projection abnormality, its cortical organisation, its relevance for visual perception, and its consequences on processes of visuo-motor integration in humans. Results: In albinism the representation abnormality (i) extends on average eight degrees into the temporal retina, (ii) varies in its extent between subjects independent of visual acuity and nystagmus amplitude, but dependent on pigmentation defect, (iii) is organised as a retinotopic map in the primary visual cortex, (iv) is relevant for visual perception, and (v) leaves major lateralisation patterns in the primary motor cortex and the somato-sensory cortex unaffected. (vi) In both albinism and hypochiasmatic subjects, the abnormal cortical representation of the ipsilateral visual field is superimposed as a retinotopic mirror-symmetric overlay onto the normal cortical retinotopic representation of the contralateral visual field. In conclusion, in both conditions, i.e., enhanced and reduced crossing at the optic chiasm, the abnormal input to the visual cortex does not undergo a topographic reorganisation of the geniculo-striate projection. These findings indicate that conservative cortical mapping of large-scale abnormal input to the lateral geniculate nucleus is a general principle to make the abnormal visual input available for higher-tier cortical processing in primates. This is in contrast with the heterogeneity of solutions reported in various non-primate animal models of misrouted optic nerves.

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# Functional plasticity in adult human cortex in response to an extreme alteration of visual input

Alyssa A Brewer[1], Brian Barton[1], Ling Lin[1]

[1]University of California, Irvine, Department of Cognitive Sciences, 3151 Social Sciences Plaza, UCI, 92697 Irvine, USA

INTRODUCTION: In this study, we exploit the dynamic nature of posterior parietal cortex to examine cortical functional plasticity induced by a complete reversal of visual input in normal adult humans. A number of studies have investigated visuomotor adaptation to altered visual input by wearing inverting or reversing prism spectacles (e.g., Stratton, 1897, Miyauchi et al., 2004). Behavioral adaptation to inverting or reversing prism spectacles has been shown by several studies to develop within just a few days (e.g., Linden et al., 1999). However, corresponding results from cortical measurements have been controversial. Using retinotopic fMRI measurements, we now demonstrate changes within multiple visual field maps of normal adult humans following the extreme alteration of visual input produced by left-right reversing prism goggles.

Data from adult barn owls suggest that after long-term adaptation to a large shift in visual input from prisms the altered representations of visual space persist (Linkenhoker and Knudsen, 2002). Here we also investigate whether there is a difference in the timing or degree of a second adaptation to the left-right visual field reversal in adult humans after long-term recovery from the initial adaptation period.

METHODS: Subjects wore left-right reversing prism spectacles continuously for 14 days. These same subjects then returned for a 4-day re-adaptation to the reversed visual field 1-9 months later. Throughout the experimental periods, subjects underwent a daily battery of visual and visuomotor behavioral tasks. Every few days we measured the BOLD responses to retinotopic stimuli comprised of wedges and rings. For each subject, we defined the baseline organization of the occipital and parietal visual field maps using phase-encoded traveling wave analysis of these data. Then we measured receptive field alterations within these maps across time points, using the population receptive field (pRF) method (Dumoulin and Wandell, 2008).

RESULTS/CONCLUSIONS: The results from the first 14-day experimental period highlight a systematic and gradual shift of visual field coverage from contralateral space into ipsilateral space in parietal cortex throughout the prism adaptation period. After returning visual input to normal, nearly all maps shift back to their state at baseline by the next day. These measurements allow us to identify the cortical regions subserving the dynamic remapping of cortical representations in response to altered visual perception. These results enhance our understanding of the potential for functional plasticity in human visual cortex.

After the second, 4-day experimental period, the data demonstrate a faster time course for both behavioral and cortical re-adaptation. By the end of this much shorter re-adaptation period, the measurements again show a shift of visual field representation from contralateral towards ipsilateral visual space in parietal cortex. These measurements of cortical visual field maps in subjects with severely altered visual input demonstrate that the changes in the maps produced by the initial long prism adaptation period persist over an extended time.

# Symposium

# S10: Information technology meets brain research - New developments in neuroinformatics

# Motor systems oscillations: a case study in complexity

Stuart N. Baker[1]

[1]Newcastle University, Institute of Neuroscience, Framlington Place, NE2 4HH Newcastle, United Kingdom

The primate motor system exhibits characteristic oscillations, which differ from those investigated in other brain regions in frequency and possible function. Beta band oscillations (~25Hz) appear only during steady contractions, and are synchronised between sensorimotor cortex and the periphery (muscle). Application of standard spectral methods (coherence) suffices to demonstrate such corticomuscular coupling, but the detailed results prove difficult to interpret. More advanced methods such as directed coherence (Granger causality) lead to the conclusion that oscillations traverse a sensorimotor loop, not only propagating from cortex to muscle, but also back along sensory pathways. This provides tantalising clues as to the function of this activity.

Physiological tremor has a component at around 10Hz. Multiple mechanisms conspire to generate this; it can present a severe limitation to fine motor control. In our recent work, we demonstrated experimentally that excitatory interneurons in the primate spinal cord fire in antiphase with cortical inputs at ~10Hz. This allows phase cancellation of ~10Hz rhythms in motoneurons and reduction of tremor amplitude. Using computational modelling, we also showed that recurrent inhibition generated by Renshaw cells (a class of inhibitory spinal cord interneuron) acts to remove 10Hz components from the motor command. We speculate that recognising the existence of active tremor cancellation within spinal circuits may be important in understanding some pathological tremors.

Investigating oscillations in the motor system requires a close synergy between experiment, computational data analysis, and neural modelling. Experiments are difficult and costly to perform; the raw data generated can often be of use outside the originally intended purpose. CARMEN is a novel platform to facilitate sharing of both data and analysis methods. This was produced by a collaboration involving 19 diverse investigators from 12 UK universities. The talk will conclude with a brief overview of the capabilities of the CARMEN platform. Much of the data on oscillations described during the talk is publically available via CARMEN, for those interested in testing different analytical techniques.

# Structural connectivity at your fingertips

Rembrandt Bakker[1], Rolf Kötter[1,2]

[1]Radboud UMC Nijmegen, Donders Inst. for Brain, Cognition and Behaviour, P.O. Box 9101, 6500 HB Nijmegen, Netherlands
[2]Rolf Kötter sadly passed away on June 9, 2010

Uncovering the large-scale human connectome is a hot topic in the neurosciences, stimulated by a recent large NIH funding initiative, and accelerated by recent developments in MRI-based techniques; fiber tracking algorithms bear the promise of producing a graph of the structural connectome, albeit limited in scope to intercortical, undirected white matter fiber tracts. For layer-specific tracts, intracortical resolution and directed graphs, one has to fall back on axonal tracing experiments. These have been performed in animal studies for over a century, with the disadvantage that the procedure is lethal and only a few tracer injections can be done per animal. While this type of experiment offers unparallelled detail, it is an enormous neuroinformatics challenge to retain that level of detail after combining tracing results obtained from thousands of individual brains.

The first and major step has been to combine the results of hundreds of published tracing studies into a single database. For nonhuman primates, the largest such resource is CoCoMac (Collations of Connectivity data on the Macaque brain), built up under the guidance of Rolf Kötter†.

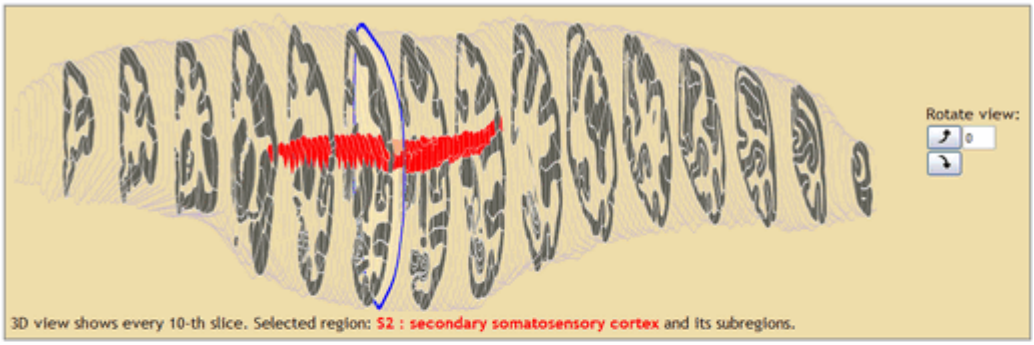
The next challenge is to bring these results into a single, standard atlas space. Tracing studies report the injection and labeled sites by name, typically taken from a histochemistry-based brain partitioning scheme. To transform these into spatial coordinates, the names need to be mapped from their native naming system to one that has an associated atlas space, i.e., for which a name-to-coordinate mapping is available. The name-to-name service is provided by CoCoMac itself, using objective relational transformation (ORT, [1]). For the name-to-coordinate mapping we rely either on the Rhesus monkey atlas by Paxinos, Huang and Toga [2] which is part of CoCoMac, or on the atlas registration services provided by Caret [3].

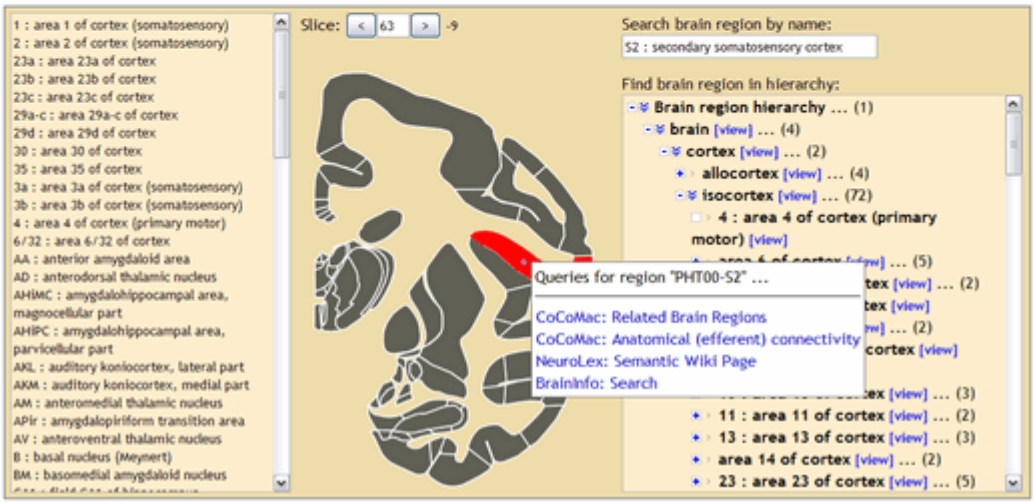
The third crucial aspect is to bring these services out to the neuroscience community. Progress is more often than not determined by the speed and ease at which complex techniques are available for non-expert users. To this end we have joint forces with the International Neuroinformatics Coordinating Facility (INCF) and built the Scalable Brain Atlas (SBA), an interactive atlas viewer which brings brain ontologies and atlas spaces together in a single web browser window (scalablebrainatlas.incf.org, no java required). With the SBA's built-in CoCoMac plugin, it is possible to launch CoCoMac queries and display connectivity results in a few intuitive mouseclicks. Deep-links to the CoCoMac website guide the expert user through the processing steps required to arrive at the resulting connectivity estimates, and allow downloads of raw connectivity data.

The fast online retrieval of CoCoMac connectivity required an overhaul of ORT and the creation of a conflict resolution algorithm, invoked when the name-to-name service yields ambiguous results. It relies on Bayesian reasoning to eliminate statements with low probabilities of being true. A new development is the creation of a wiki-inspired interface which allows CoCoMac to be kept up to date by the user community, without the supervision of its founder.

[1] Stephan KE, Zilles K, Kötter R (2000). Philos Trans R Soc Lond B Biol Sci. 355(1393):37-54
[2] Paxinos G, Huang X-F, Toga AW (2000). Academic Press: ISBN 0-12-358255-5
[3] Van Essen DC, et. al. (2001). JAMIA 8(5):443-459.

## Rhesus Monkey - Paxinos et al. 2000



# BUILDING A BRAIN OF VISIBLE CELLS

Mark H Ellisman[1]

Stephen Larson, James Bouwer, Steven Peltier, Chris Aprea, Ilya Zaslavsky, Sarah Maynard, Monica Berlanga, F. Imam, Jeff Grethe, Tom Deerinck, Eric Bushong, Maryann Martone, and Mark Ellisman; National Center for Microscopy and Imaging Research (NCMIR), Center for Research in Biological Systems, San Diego Supercomputer Center, California Institute for Information Technology and Telecommunications, University of California San Diego, La Jolla, CA, USAUCSD, Neuroscience, 9500 Gilman Dr. MC0608, 92093-0608 La Jolla, USA

A host of new technological tools for data acquisition and data sharing have been integrated and are beginning to deliver new open access brain data resources that can be explored across the full range of scales, from genomics to networks of neuronal systems. What remains unknown about networks of cellular connections in the brain provides significant drivers for development of these new capabilities. The brain is fundamentally multi-scale with functions dependent upon molecular and cellular synaptic associations, cytoarchitectural patterns of local connectivity, and long-range connectivity between brain regions - with ramifications to virtually all other organs of the body. Very restricted molecular modifications or defects can lead to alterations in the structure and function of brain tissue at all these levels, making integrated views and tools for study of the nervous system extremely valuable.

Despite rapid progress in development of new experimental methods, our ability to simultaneously study the brain across all these scales remains quite limited. Experimental methodologies available today reveal only limited views of nervous system organization. Many research groups are now working to expand data acquisition and computational methods to facilitate more wide spread use of multiscale data in biomedical research. These cooperatively constructed facilities containing pooled and integrated data are allowing researchers to generate and test hypotheses that involve brain systems spanning molecular to tissue scales and to analyze and study the nervous system across spatial dimensions spanning nanometers to centimeters.

New experimental technologies, including new microscopy methods, are now able to reveal new aspects of organization within these scales, yet the development of software tools to synthesize these data into more coherent models of brain structure and function has lagged behind. To help close this gap the NCMIR has created a client-server platform, the Whole Brain Catalog (WBC) (http://wholebraincatalog.org/) around the mouse brain, an extensively studied animal model system for investigations into human disease. The WBC allows data from brain regions to molecules to be represented in a common space, drawing on data deposited at local as well as geographically distant data repositories. It allows researchers to upload their own data into a standard anatomical framework using many standard formats such as 3D meshes of subcellular scenes and of brain region territories, large 2D image data sets from EM and light level microscopy, simulation-suitable marked up clusters of neurons or Neurolucida neuronal reconstructions, and solved protein structures - in formats deposited in the Protein Data Bank (PDB). These data are registered to the WBC and a standard atlas, the “Waxholm Space”, space recently developed as a standard conceptual framework for the mouse brain by the neuroscience community under the auspices of the International Neuroinformatics Coordinating Facility (INCF).

Several of NCMIR’s large scale image data collection resources are now streaming multiscale data about mouse animal models of disease and normal mice into the WBC. These include wide field reconstructions of cells in the nervous system using ultra-wide field laser scanning light microscopy, serial block-face scanning electron microscopy, ultra-wide field electron microscopy and tomography for whole cell reconstructions. These tools are applied to biomedical research projects including those pertaining to models of Parkinson’s disease, cancer and epilepsy as part of the collaborative project / driving biomedical research project portfolio of the NCMIR which shape technological research and development at the Center. Most notably, extremely large volumes of data are already being fed into the WBC using a new 3D EM technique being refined at NCMIR. This method combines the new high throughput scanning EM method, “serial block -face scanning electron microscopy (SBFSEM)”, developed by Winfried Denk and coworkers in Heidelberg with NCMIR-developed methods for high contrast staining and molecule-specific marking to facilitate correlated light and electron microscopy.

This Web-based resource also incorporates the 3D geometry of the Allen Reference Atlas structures of the mouse brain, with a NCMIR research collaborators images taken from the Cell Centered DataBase (CCDB) and integrated with collections from GENSAT mice and example mouse models of Parkinson’s disease. The brain models of the WBC can be easily rotated, panned and zoomed in real time allowing interactive exploration.

Zooming into the brain reveals further detail such as 3D representations of neurons, 3D representations of cellular substructure and so forth, like supramolecular complexes found in EM tomograms of neuropil or deposited in the PDB. Images of unlimited size can be warped and aligned to atlases; and images of unlimited size can be viewed. Thus this new tool, the Whole Brain Catalog, provides a window into the most extensive integrated source of neuroscience information by connecting its 3D multiscale client environment to the NIH Blueprint-supported effort, the "Neuroscience Information Framework" (NIF). This WBC – NIF integration is accomplished through NIF's collaborative ontology management system, NeuroLex that has been built on top of the same software that runs Wikipedia. From anywhere in the 3D brain window, and at any level of zoom, the user can "right click" to NIF to be spatially and semantically linked to known and cataloged information pertaining to the site within the brain which the user has brought into their viewing window.

# What contrast did you use? - Automated handling of metadata.

Jan Grewe[1,2], Thomas Wachtler[1,3], Jan Benda[1,2]

[1]Ludwig-Maximilians-Universität München, Department Biologie II, Großhadener Strasse 2, 82152 Planegg- Martinsried, Germany; [2]Bernstein Center for Computational Neuroscience Munich, BCCN Munich, Germany;[3]German Neuroinformatics Node, Ludwig-Maximilians Universität München, Germany

Acquisition, analysis, management, and sharing of electrophysiological data concerns not only the recorded voltage and current traces, but also requires metadata that provide information about stimulus conditions, settings of the recording setup, experimental procedures, etc. Similar to the diversity of formats used for storing recorded data, there are numerous ways in which metadata are stored. The hand-written lab book as the most common and simple way to record metadata is, however, not well accessible to data processing software. Systems for electronic management of metadata often use a (meta)data model that assumes a very detailed and specific structure and context. Within a lab this specificity is usually not a problem. However, when it comes to sharing data with other laboratories or even with a larger community, in particular considering the upcoming initiatives of public databases, the lack of interoperability of different data models may become a major obstacle.

We present a very general and simple approach to the problem of metadata sharing that is merely based on key-value pairs grouped in hierarchically organized sections. This tree is a generic structure to store arbitrary data that is independent of any specific data model or database schema. Through terminologies metadata can be structured and standardized for ensuring interoperability while at the same time not restricting the content, thus ensuring immediate flexibility to record any metadata necessary.

We exemplify the automated handling of metadata by our research on the role of noise in processing sensory information. The study is based on a comparison of response properties of two types of electroreceptors (active and passive electrosensory system) of different species of weakly electric fish (*Apteronotus albifrons*, *Apteronotus leptorhynchus*). Our recording tool (RELACS by JB) automatically annotates the recorded data with all metadata it knows about the stimulus, the recording settings, the experimental subject, the recording conditions etc. This information is imported into our database (LabLog by JG) which we use to keep track of the recorded data and to retrieve datasets. We show that receptors of the active electrosensory system show a much larger response variability and population heterogeneity than the ones of the passive system. With information-theoretic measures we characterize the impact of neuronal noise on the tuning of the two types of electroreceptors to dynamical stimuli. The comparison of electroreceptor neurons with high spike-timing variability (active system) and those with low variability (passive system) further reveals how the noise is tuned to the different demands on the respective system.

# What can we learn from multielectrode recordings?

Gaute T. Einevoll[1]

[1]Norwegian University of Life Sciences, Mathematical Sciences and Technology, POBox 5003, 1432 Aas, Norway

Mathematical modelling relies on experimental data to make progress, both to constrain and to test the models. A common experimental method has been in vivo single-unit extracellular recordings: when a sharp electrode is placed sufficiently close to the soma of a particular neuron, the recorded potential reliably measures the firing of individual action potentials in this neuron. This information is contained in the high-frequency part of the recorded potentials. The low-frequency part, that is, the local field potentials (LFP), has proved much more difficult to interpret and has typically been discarded.

Other experimental methods, particularly methods that measure population-level activity in vivo, are needed to facilitate development of biologically relevant neural network models. Large -scale electrical recordings using various types of multielectrodes, i.e., electrodes with many contacts, are one such option. As techniques for such recordings are rapidly improving, there is a need for new methods for extraction of relevant information from such data. Here we present results from projects in our group aimed at elucidating the link between recorded extracellular potentials and the underlying neural activity.

Extracellular potentials in the brain are in general due to complicated weighted sums of contributions from transmembrane currents in the vicinity of the recording electrode. The transmembrane currents accompanying various types of neural activity can be calculated by means of multicompartment models. Given these transmembrane currents, the extracellular potential can in turn be calculated using a electrostatic forward-modelling formula based on the quasistatic version of Maxwell's equations (Holt & Koch, J Comp Neurosci 6:169, 1999) . Several projects from our group where this forward-modelling scheme has been utilized will be presented, including:

(A) investigation of how neural morphology and electrical parameters affect the shape and size of extracellular action potentials (Pettersen & Einevoll, Biophys J 94:784, 2008),

(B)investigation of the frequency spectra and spatial range of the LFP generated by single neurons (Linden et al, J Comp Neurosci 2010),

(C)investigation of the relationship between the LFP and multi-unit activity (MUA) with the underlying neural activity in an activated columnar population of pyramidal neurons (Pettersen et al, J Comp Neurosci 24:291,2008),

(D) development of the iCSD method for estimation of current source-density from LFP recordings (Pettersen et al, J Neurosci Meth 154:116, 2006),

(E) introduction of laminar population analysis (LPA) where stimulus-evoked laminar-electrode data from rat barrel cortex are analyzed in a scheme where the MUA and LFP are jointly modelled using physiological constraints (Einevoll et al, J Neurophysiol 97:2174, 2007), and

(F) extraction of thalamocortical and intracortical network models based on laminar-electrode data from barrel cortex and simultaneous recording of thalamic firing activity recorded in the homologous barreloid (Blomquist et al, PLoS Comp Biol 5:e1000328, 2009)

# Simulation Science for Neuroscience

Henry Markram[1]

[1]Blue Brain Project, Brain Mind Institute, EPFL

Science traditionally rests on two branches of knowledge generation; experiment and theory. With the massive growth in the volume of data and fragments of knowledge of a system as complex as the brain, these two pillars are no longer enough. Organizing all the data to make it accessible to all is a major first step that started in the 1990's. With this development, it became clear that mathematical, statistical and computer science approaches could be applied to this data to derive new insights on the organizing principles of the brain. Correlation-based science began to emerge. The potential of this form of science to understand how all the data and knowledge fit together has become increasing obvious and has justified large-scale initiatives to gather more data, more standardized and more quantitative. Correlation-based science has, for example, become a primary strategy for post-genomic science as researchers attempt to correlate genetic variations with phenotypic variations. Correlation-based science however generates theories, which remain speculative until causation can be demonstrated. The academic domains of Physics, Chemistry & Engineering crossed this problem some time ago and used computer models to gather and integrate as much data and knowledge of a system as possible and then to explore causal relations (e.g. simulating nuclear events, cars, airplanes), in many cases completely replacing theory and experiment. Simulation science ideally should use bottom up models because the primary goal is to understand the function of the elements of the system and trace the chains of causality leading to the emergent properties. With accurate models, not only are predictions more pertinent and accurate, but the causal chains become more transparent. Developing simulation science for the neurosciences is a major challenge because it demands the convergence of virtually all domains of science and medicine ? at the experimental and theoretical levels. The convergence is achieved through a strategy called Integrative Biology and enabled by the rapid growth in supercomputing power. Simulation science is an inevitable end result in the life sciences as all fragments of data and knowledge become integrated and accounted for, eventually completely replacing experimental and theortical science and becoming a mere engineering tool. Until then, simulation science forces a strategy that can systematically pierce through the complexities of the brain to systematically reveal its principles of design and operation.

# Symposium

## S11: Development of fear and anxiety in humans: Behavioural, cognitive and neural changes

S11-1 Cue and Context Conditioning in Virtual Reality
*Andreas Mühlberger, Heike Ewald, Evelyn Glotzbach, Christian Tröger, Paul Pauli*

S11-2 Defensive Mobilization in Anxiety Disorder Patients
*Alfons O. Hamm*

S11-3 Neural changes in anxiety disorders and possible modulatory treatment approaches
*Andreas J. Fallgatter, Lena Ernst, Ann-Christine Ehlis, Sarah Tupak*

S11-4 On the relationship between emotion and cognition
*Luiz Pessoa*

S11-5 Corticolimbic Interaction in Anxiety – Influence of Genetic Variants
*Katharina Domschke, Patricia Ohrmann, Miriam Braun, Thomas Suslow, Jochen Bauer, Walter Heindel, Harald Kugel, Volker Arolt, Andreas Reif, Jürgen Deckert*

S11-6 Molecular Determinants of Fear Conditioning and Extinction
*Raffael Kalisch*

# Cue and Context Conditioning in Virtual Reality

Andreas Mühlberger[1], Heike Ewald[1], Evelyn Glotzbach[1], Christian Tröger[1], Paul Pauli[1]

[1]University of Würzburg, Department of Psychology, Marcusstr. 9-11, 97070 Würzburg, Germany

Human research on fear conditioning nearly exclusively examined fear conditioning of fear cues (often pictures or abstract stimuli). Context fear conditioning as well as the effects of safety cues and safety contexts were rarely studied, presumably because of difficulties to manipulate and control context stimuli. Advances in computer technology and especially in virtual reality (VR) are used to overcome this deficit and allow assessing both physiological as well as behavioral responses in an ecological valid environment.

Thus, we established a VR based paradigm to examine cued and context fear conditioning in humans in analogy to animal research. Behavioural and physiological responses triggered by cues and contexts were assessed as well as associations of these responses with genetic markers. Specific goals were to examine the effects of fear-conditioned contexts and of fear-conditioned cues modulated by contexts.

The aim of a first study was to examine context conditioning in a virtual reality environment in general and the effect of pre-exposure to the to-be-conditioned context in particular. To accomplish this, a VR paradigm presented via a head mounted display was used in which one of two rooms was contingently paired with an aversive electric shock as an UCS. Participants were randomly either exposed to the later context conditioning environment (n = 20) or to a neutral VR environment (n = 20) one day before the actual context conditioning experiment. To assess fear conditioning ratings of valence, arousal, and anxiety and on a physiological level startle reflex, skin conductance response, and heart rate were used. In accordance to the hypothesis, a successful context conditioning could be shown across subjective and physiological indicators. More importantly, a pre-exposure didn't prevent a successful context conditioning but failed to make any impact.

A second study focused on behavioral consequences of context conditioning. One context was paired with an electric shock (fear context), whereas in the other context no shock was administered (safety context). After the acquisition phases evaluative ratings for valence, arousal and anxiety were obtained for each context. At the end of the experiment a test of avoidance behavior was conducted. Participants choose two out of three contexts (a neutral context was added) consecutively, which they wanted to visit once again. As expected, most participants avoided to select the fear context. We also found clear evidence for evaluative conditioning in these participants. On the contrary, participants who avoided the safety context failed to show evaluative conditioning. These results demonstrate that avoidance behavior is strongly connected to evaluative conditioning.

Further studies on genetic markers that might influence cue or context conditioning as well as underlying neuronal circuits measured by fMRI will be presented. Overall we conclude that virtual reality is a new research paradigm that will allow investigating associative learning processes in highly experimentally controlled and ecologically valid environments and thus fill the gap between laboratory research in human and the relevance for human being in daily live.

# Defensive Mobilization in Anxiety Disorder Patients

Alfons O. Hamm[1]

[1]Dept. of Biological and Clinical Psychology, University of Greifswald

Currently diagnosis of emotional disorders is based on patients phenomenological symptom reports, often accomplished by interviews standardized according to the diagnostic categories and criteria represented in the DSM-IV or ICD-10. One problem of this approach, however, is that the patients do not fit into the precise list of symptoms and criteria. As a consequence there is extensive co-morbidity of several mental disorders (which is rather the rule than the exception) and diagnostic categories are rather overspecified so that in reality patients suffer from symptoms that are often not so finely differentiated as the DSM grid on which they have to be mapped (e.g., limited symptom panic attacks). The most central problem of this approach, however, is that due to the effort to maximize interdiagnostician agreement, i.e., reliability, validity is unintentionally sacrificed.

The current presentation will introduce a neuroscience perspective for classifying mental disorders, using dysfunctions of the emotional systems as an example. Starting from elucidating the neural networks of the mammalian defense system translational data will be presented suggesting comparable neural networks of defensive reactivity in humans. It will be shown, however, that a multi-measure approach is essential to disentangle different processes that are involved in human defensive reactivity (e.g. encoding of threat relevant information and the dynamics of defensive response mobilization). Finally data on emotional reactivity in patients with anxiety and borderline personality disorders will be presented suggesting a common mechanism for developing anxious and depressed psychopathology.

# Neural changes in anxiety disorders and possible modulatory treatment approaches

Andreas J. Fallgatter[1], Lena Ernst[1], Ann-Christine Ehlis[1], Sarah Tupak[2]

[1]University of Tuebingen, Dept. of Psychiatry and Psychotherapy, Osianderstr. 24, 72076 Tuebingen, Germany

Current research in patients with anxiety disorders suggests functional deficits within the prefrontal cortex, which result in reduced inhibition of the amygdale, in turn accounting for increased fear perception in these clinical populations. Principally, such a pathophysiological model would allow to positively influence the supposed prefrontal hypofunction via repetitive transcranial magnetic stimulation (rTMS) in a facilitating mode (high-frequency stimulation). Such a facilitating treatment may strengthen the inhibitory activity of the prefrontal cortex, resulting in a better control of panic -associated amygdale hyperactivity and, in turn, in a reduction of panic attacks.

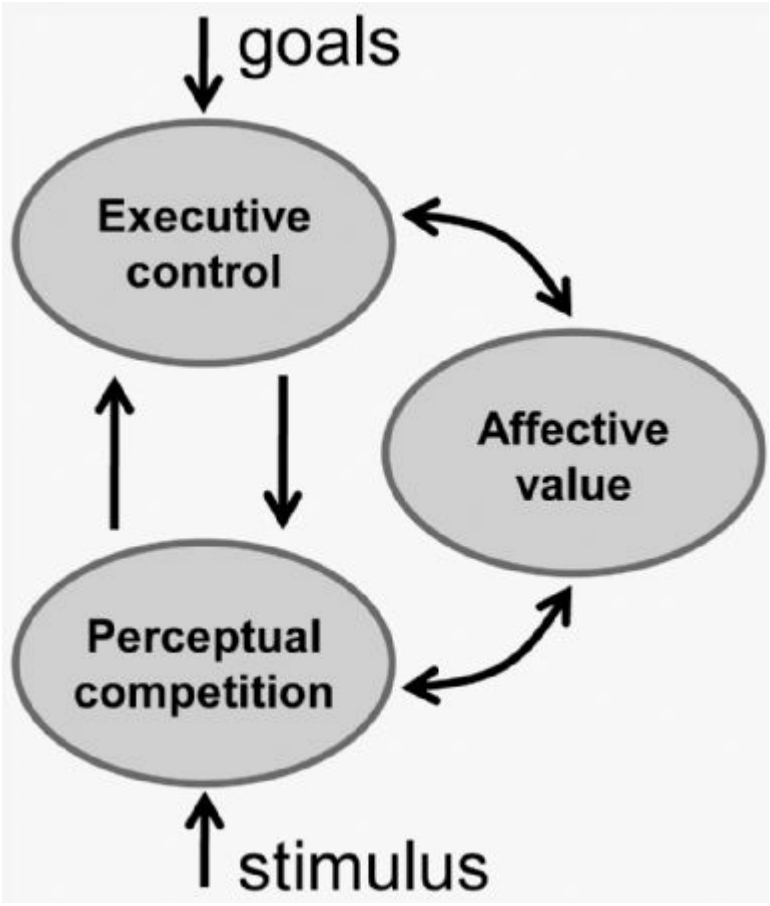
Based on this theoretical framework, we propose the application of a non-invasive optical imaging technique (Near-Infrared Spectroscopy; NIRS) for the measurement of anxiety-related hypoactivity of the prefrontal cortex, and for the control of a treatment success on the brain metabolic level. In a single patient with panic disorder we could detect a pattern of hypoactivity in the prefrontal cortex during an emotional Stroop task. Facilitating rTMS treatment (15 sessions within three weeks, add-on) was associated with (1) an improvement of prefrontal activity in the emotional Stroop task and (2) with the absence of further panic attacks. This is the first report of a NIRS-guided and –controlled facilitating rTMS treatment of a patient with a panic disorder. Given the excellent clinical applicability of the methods, the combination of NIRS and rTMS might have the potential to establish new treatment options in psychiatry aiming on the modulation of pathological regional brain activity patterns.

# On the relationship between emotion and cognition

Luiz Pessoa[1]

[1]Indiana University, Department of Psychological and Brain Sciences, 1101 E10th Street, 47405 Bloomington, IN, USA

The current view of brain organization supports the notion that there is a considerable degree of functional specialization and that many regions can be conceptualized as either 'affective' or 'cognitive'. Popular examples are the amygdala in the domain of emotion and the lateral prefrontal cortex in the case of cognition. This prevalent view is problematic for a number of reasons that will be discussed. It will be argued that complex cognitive–emotional behaviors have their basis in networks of brain areas, none of which should be conceptualized as specifically affective or cognitive. Central to cognitive–emotional interactions are brain areas with a high degree of connectivity called hubs (e.g., amygdala), which are critical for regulating the flow and integration of information between regions. Examples of cognitive-emotional interactions to be discussed include the visual processing of affective stimuli, and how threat processing (e.g., threat of shock) influences executive functions, such as response inhibition and response conflict. These examples will be used to motivate a so-called dual competition framework in which emotional information influences competition processes both at the perceptual and the executive levels (see Figure). Finally, it will be argued that although the processing of emotional information is indeed privileged, there are no specialized circuits or pathways per se for doing so. In particular, empirical data will be reviewed that challenge the notion that affective stimuli are processed independently of attention and awareness. In a related fashion, data will be reviewed that question the existence of a subcortical "low road", namely a colliculo-pulvino-amygdalar pathway, that is particularly effective at carrying affective information. Instead, an alternative scheme, called the multiple waves model, will be described that attempts to provide a better explanation of existing data based on fast and parallel cortical pathways.



# Corticolimbic Interaction in Anxiety – Influence of Genetic Variants

Katharina Domschke[1], Patricia Ohrmann[1], Miriam Braun[1], Thomas Suslow[1], Jochen Bauer[1], Walter Heindel[2], Harald Kugel[2], Volker Arolt[1], Andreas Reif[3], Jürgen Deckert[3]

[1]University of Muenster, Department of Psychiatry, Albert-Schweitzer-Strasse 11, 48149 Muenster, Germany
[2]University of Muenster, Department of Radiology, Albert-Schweitzer-Strasse 33, 48149 Muenster, Germany
[3]University of Wuerzburg, Department of Psychiatry, Fuechsleinstrasse 15, 97080 Wuerzburg, Germany

Anxiety disorders and panic disorder in particular are highly heritable diseases with an estimated heritability of around 50%. Association with variants in candidate genes of the serotonergic, dopaminergic and recently the neuropeptide S systems has been reported in these disorders and associated dimensional phenotypes such as anxiety sensitivity. In order to further elucidate the functional impact of these findings, relevant genetic variants were investigated for association with neuronal activation correlates of emotional processing as an intermediate phenotype of panic disorder.

Regional brain activation upon presentation of fearful faces (Ekman and Friesen, 1976) was investigated in 20 patients with panic disorder by means of fMRI at 3T. Probands were genotyped for the functional 5-HT1A -1019C/G, COMT val158met and NPSR A/T (Asn107Ile) polymorphisms.

In response to fearful faces, higher activation in the amygdala was observed in patients carrying at least one COMT 158val allele, while a significantly decreased activation of the ventromedial and dorsolateral prefrontal cortex, orbitofronal and anterior cingulate cortex was associated with the 5-HT1A -1019G/G genotype and NPSR T allele carriers, respectively.

The present data provide preliminary evidence for a role of the functional 5-HT1A -1019C/G, COMT val158met and NPSR A/T (Asn107Ile) polymorphisms in pre-/orbitofrontal frontal cortex and limbic system activation in response to emotional faces in panic disorder. The increased vulnerability to panic disorder in subjects carrying the respective risk genotypes might be due to an altered serotonergic, dopaminergic or neuropeptide S tonus in relevant brain regions leading to impaired processing of anxiety-related stimuli. This might be particularly conferred by a genetically determined alteration of the corticolimbic interaction with a lowered threshold of amygdala excitability along with an impaired inhibition of amygdala activation by the pre-/orbitofrontal cortex.

# Molecular Determinants of Fear Conditioning and Extinction

Raffael Kalisch[1]

[1]Institute for Systems Neuroscience, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany

Combination of behavioral analysis and neuroimaging with genetics and pharmacology has led to several important breakthroughs in human fear research in the past few years. The talk will summarize the most significant advances and present new data in the fields of conditioning and extinction. A particular focus will be on fear and extinction memory consolidation, based on the assumption that memory consolidation processes are the best predictors for future fear-related behavior and for long-term remission following exposure-based therapy of anxiety. Molecular analysis will be integrated with imaging-driven neural network analysis in an effort to sketch a coherent picture of the neurobiological foundations of fear learning.

# Symposium

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# INTRODUCTION TO CLINICAL AND NEUROPATHOLOGICAL FEATURES OF TEMPORAL LOBE EPILEPSY

Carola Haas[1]

[1]University of Freiburg, Experimental Epilepsy Research, Freiburg, Germany

No abstract available

# Possible roles for hippocampal neuron loss and synaptic reorganization in temporal lobe epileptogenesis

Robert S Sloviter[1]

[1]University of Arizona College of Medicine, Department of Pharmacology, 1501 N. Campbell Ave, 85724 Tucson, USA

The mechanisms that cause acquired temporal lobe epilepsy are unknown. Suspected mechanisms include neuron loss and synaptic reorganization, and a variety of other secondary changes. Determining which abnormalities precede and could cause spontaneous hippocampal-onset seizures is problematic because commonly-used chemoconvulsant-based animal models exhibit extreme variability and minimal evidence of hippocampal epileptogenesis. Continuous monitoring of both behavior and granule cell layer activity in awake rats after perforant pathway stimulation-induced status epilepticus has recently revealed that granule cells reliably generate spontaneous field depolarizations, population spikes, and epileptiform discharges in the first days post-injury, prior to the development of mossy fiber sprouting and other delayed changes, and that initial seizures are focal and minimally detectable by behavioral observation alone. In addition, every spontaneous behavioral seizure onset is preceded by spontaneous granule cell population discharges, confirming that these animals reliably exhibit hippocampal-onset seizures. These findings indicate that the "latent period" between injury and clinical epilepsy is not a silent, seizure-free, "pre-epileptic" state that awaits the development of an epileptogenic secondary mechanism. That is, hippocampal epileptogenesis appears to be coincident with initial neuron loss, rather than delayed secondary processes, and we suggest that hippocampal epileptogenesis that causes focal seizures is a rapid process distinct from the more complex events that lead to the occurrence of the first clinically obvious generalized seizure.

We hypothesize that extensive neuron loss in the entorhinal cortex disrupts the functional separation of Layer II entorhinal "grid cells," causing abnormal synchronous discharges that invade the dentate gyrus. This, in turn, produces population spikes and epileptiform discharges in granule cells disinhibited by injury-induced hilar neuron loss. Long delays between injury and generalized behavioral seizures, when they occur, may involve a "kindling" process in which initially subclinical focal seizures gradually overcome downstream barriers to seizure spread. In addition, clustering of spontaneous seizures and the duration of the interictal period, i.e. seizure frequency, could be powerfully influenced by both the inhibitory effects of mossy fiber sprouting (aberrant innervation of initially denervated and deactivated inhibitory neurons), and the upregulation of GAD67 and GABA, which seizure activity produces specifically in granule cells. Clearly, the roles of cell loss, synaptic reorganization, glial abnormalities, and altered expression of transmitters, receptors, and channels remain to be addressed in animal models that reliably exhibit confirmed hippocampal-onset seizures. Neuroprotection in the immediate post-injury period, and prolonged anti-kindling therapy, might be the most effective anti-epileptogenic strategy.

# Excitation-inhibition balance and epilepsy

Andreas Draguhn[1]

[1]Institute of Physiology and Pathophysiology, Neurophysiology, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

Traditionally, epileptic seizures have often been described as network states arising from an imbalance between excitation and inhibition within the affected networks. While not entirely untrue, however, this concept does not give justice to the recent advances in our understanding of synaptic inhibition and its role in normal and pathological network synchronization.

We will focus on GABAergic signaling in the rodent hippocampus to demonstrate the plethora of mechanisms involved in excitation-inhibition-balance and synchrony. The neurotransmitter GABA exerts both tonic and phasic actions on different cell populations in the hippocampus. Recent work indicates that the tonic action of GABA is most important for setting general excitability levels. Phasic synaptic actions of GABA are major organizers of the patial and temporal activity flow in hippocampal networks. They control neuronal discharges during aroused states and, thereby, enhance signal-to-noise-ratio in sparsely coding networks. They also synchronize excitability in large populations of neurons, setting the frequency of global network oscillations by the time course of synchronous inhibitory postsynaptic potentials. By selectively inhibiting different input or output pathways of hippocampal subfields, different inhibitory interneurons can support different network states and control the spread of activity between different hippocampal and cortical suibfields.

Plasticity of the GABaergic system is of particular importance for ictogenesis and epileptogenesis, i.e. for the short-term and long-term maladaptive processes leading to recurrent seizures. We will discuss three examples of such adaptive processes at different time scales: i) GABaergic synapses can change presynaptic transmitter concentration within seconds following changes in local network activity. Recent work from our and other laboratories shows that enhanced synaptic activity increases the uptake of GABA and its precursor molecules glutamate and glutamine. This, in turn, leads to an increase in inhibitory efficacy, providing a negative feedback loop for overall network activity. ii) Few episodes of pathologically enhanced network activity in vitro are sufficient to decrease inhibitory efficacy, probably by shifting the reversal potential of postsynaptic GABA receptors in depolarizing direction. iii) Finally, chronic seizure activity results in selective loss of interneurons and changes in synaptic activity of different neuronal subtypes within hippocampal networks, ultimately changing signal flow and excitation-inhibition balance and neuronal synchrony.

In summary, the microphysiological, cellular and network properties of GABAergic signaling are highly diverse, complex and plastic. Repeated episodes of enhanced activity tend to induce pathological changes in GABAergic mechanisms, increasing the probability of further hyper-synchronous network states. On the other hand, enhanced insight into the molecular, cellular and network mechanisms of inhibition Might open new avenues for therapeutic interventions.

# Transcriptional up-regulation of the T-type calcium channel CaV3.2 promotes epileptogenesis

Albert J. Becker[1]

[1]University of Bonn Medical Center, Dept. of Neuropathology, Sigmund Freud Str. 25, 53105 Bonn, Germany

Pronounced changes in intrinsic excitability have been observed in the pilocarpine-status epilepticus (SE) model of temporal lobe epilepsy (TLE), consisting of a conversion of regular firing to burst- firing hippocampal CA1 pyramidal neurons. This conversion likely plays a role in the transition from the interictal to the ictal state in TLE, because a subset of burst-firing neurons constitutes forerunners of epileptiform events observed in vitro. We have therefore investigated the cellular mechanisms of aberrant burst firing in detail. We found that the increased propensity for burst discharges in SE-experienced neurons is mainly due to functional up-regulation of T-type Ca2+ currents. Real -time RT -PCR experiments showed that the increase in Ca2+ currents is associated with transcriptional increase of the Cav3.2 but not Cav3.1 or Cav3.3 T-type Ca2+ channel subunits. Analysis of Cav3.2 promoter activation suggests a role for a Zn2+-dependent mechanism. Patch-clamp experiments in SE-experienced CA1 pyramidal neurons from mice lacking (Cav3.2-/- mice) or having (Cav3.2+/+ mice) Cav3.2 subunits, revealed that Cav3.2 -/- mice do not express an increase in T -type Ca2+ current density, as is found in Cav3.2+/+ animals. Subsequently, we have analyzed Cav3.2 -/- and littermate controls after pilocarpine induced-SE by telemetric EEG -/video-monitoring. We observed no significant differences in the severity of pilocarpine-induced SE between Cav3.2 -/- and Cav3.2+/+ mice. The frequency and severity of chronic seizures was significantly attenuated in chronic epileptic Cav3.2 -/- vs. +/+ mice (n=5 for both groups). Neuropathological sequelae of SE, such as segmental neuron loss and mossy fiber sprouting, were significantly reduced in Cav3.2-/- mice. These data indicate that the up- regulation of Cav3.2 after SE, and the associated dramatic changes in discharge behavior promote the initiation of seizure activity and neuronal cell death in chronic epilepsy.

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# A vascular cause of intractable epilepsies?

Mireille LERNER-NATOLI[1], Melanie MORIN[1], Valerie RIGAU[1], Frederic DE BOCK[1], Joel BOCKAERT[1]

Institute of Functional Genomics, Dpt Neurobiology, 141 rue de la Cardonille, F-34094 MONTPELLIER, FRANCE

Growing body of evidence supports the notion that BBB disruption contributes directly to epileptogenicity. Indeed, the extravasation of blood cells promotes inflammatory and immune responses, whereas serum proteins and ions that accumulate in the parenchyma induce osmotic unbalance, edema, alterations of local blood flow and changes in glial function. These environmental modifications are known to increase neuronal excitability and toxicity.

In human epileptic tissue (surgically removed from patients with intractable temporal lobe epilepsy) and in rodent models, we found that BBB degradation was associated with a significant neo-vascularisation. This pathological angiogenesis was confirmed by the over-expression of VEGF in neurons and astrocytes and of its receptor VEGFR-2 in endothelial cells. Further investigations carried out on an original in vitro model showed that epileptiform activity was sufficient to induce tight junction disassembly and neo-vascularisation via VEGF/VEGFR-2 activation. We suggest that recurrent seizures promote angiogenic processes in the chronic focus, keeping BBB permeable, thus reinforcing epileptogenicity.

That is the reason why we postulate that anti-angiogenic strategies, able to protect or repair the BBB, could reduce epileptogenicity and break the vicious circle of angiogenesis/epileptogenesis.

# Role of neurogenesis in temporal lobe epilepsy

Ute Häussler[1], Lena Bielefeld[1], Ulrich P. Froriep[2,3,4], Jakob Wolfart[3], Carola A. Haas[1,2]

[1] Experimental Epilepsy Research, Dept. of Neurosurgery,University of Freiburg, Freiburg, Germany
[2] Bernstein Center Freiburg, Freiburg, Germany
[3]Dept. of Microsystems Engineering - IMTEK, University of Freiburg, Freiburg, Germany
[4]Neurobiology and Biophysics, Faculty of Biology, University of Freiburg, Freiburg, Germany
[5]Cellular Neurophysiology, Dept. of Neurosurgery, University of Freiburg, Freiburg, Germany

The subgranular zone (SGZ) of the dentate gyrus is one of the few brain regions where the formation of new neurons takes place even in the adult. In particular, status epilepticus has a stimulating effect on neurogenesis in the SGZ. The role of newly born neurons in the epileptic hippocampus is, however, a matter of ongoing controversy.
To address this issue, we used the intrahippocampal kainate mouse epilepsy model, which recapitulates the main features of mesial temporal lobe epilepsy in humans: recurrent focal seizures, granule cell dispersion and selective cell death in the hippocampus. Following the focal injection of kainate into the hippocampus, we performed multi-site in vivo local field potential recordings along the septotemporal axis of the kainate-injected and in the contralateral hippocampus and quantified the strength of status epilepticus (SE) and recurrent epileptiform activity (EA). In addition, we used bromodeoxyuridine injections to monitor proliferative activity, immunohistochemical analysis to determine cell fate and patch-clamp recordings to investigate the functional integration of newly born granule cells after SE.
We show that following kainate injection into the septal hippocampus, SE spread along the septotemporal axis of both hippocampi, with stronger intensity at temporal and contralateral sites. Similarly, cell proliferation was strongly increased in the temporal ipsilateral and entire contralateral SGZ, giving rise to immature granule cells, which functionally integrated into the hippocampal network, as shown by perforant path stimulation. In contrast, in the septal portion of the KA-injected hippocampus, proliferation was increased in the hilus, but gave rise to glial cells instead of new neurons. Notably, intrahippocampal recordings at three weeks after injection revealed that the septotemporal position where strongest EA was measured coincided with the area of transition from lost to increased neurogenesis. This suggests a pro-epileptogenic effect of neurogenesis and the integration of newborn neurons, e.g. by alteration of network connectivity.

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# Symposium

# S13: Translational regulation in neurons and glial cells of the central nervous system

S13-1 Identification of dendritically-localized mRNAs in hippocampal neurons
*Iván Cajigas, Tristan Will, Georgi Tushev, Güney Akbalik, Andreas Strehl, Erin Schuman*

S13-2 FRAGILE X MENTAL RETARDATION PROTEIN REGULATES PROTEIN LEVELS IN POSTSYNAPTIC DENSITIES
*Stefan Kindler, Janin Schütt, Katrin Falley, Dietmar Richter, Hans-Jürgen Kreienkamp*

S13-3 The CPEB-Associated Cytoplasmic Polyadenylation Apparatus Regulates mRNA-Specific Translation in Dendrites and Synaptic Plasticity
*Tsuyoshi Udagawa*

S13-4 Putative role for CPEB1-mediated mRNA translation in neurons and glia
*David G. Wells*

S13-5 Expression and function of the CPEB2-4 subfamily in astrocytes, NG2 glia and microglia
*Martin Theis*

# Identification of dendritically-localized mRNAs in hippocampal neurons

Iván Cajigas[1], Tristan Will[1], Georgi Tushev[1], Güney Akbalik[1], Andreas Strehl[1], Erin Schuman[1]

[1]Max Planck Institute for Brain Research, Department of Synaptic Plasticity, Max von Laue Strasse 3, 60438 Frankfurt am Main, Germany

It is clear that de novo protein synthesis has an important function in synaptic transmission and plasticity. A substantial amount of work has shown that mRNA translation in hippocampal neurons is spatially controlled and that dendritic protein synthesis is required for different forms of long-term synaptic plasticity. In spite of this, the populations of mRNAs that are localized to dendrites remain elusive. Recent studies of process-localized transcripts show little overlap in the mRNAs identified, suggesting that there are many more transcripts to be discovered. We have undertaken a transcriptomics approach in order to comprehensively characterize the mRNAs resident in the synaptic neuropil. The results of our analyses will be presented.

# FRAGILE X MENTAL RETARDATION PROTEIN REGULATES PROTEIN LEVELS IN POSTSYNAPTIC DENSITIES

Stefan Kindler[1], Janin Schütt[1], Katrin Falley[1], Dietmar Richter[2], Hans-Jürgen Kreienkamp[1]

[1]University Medical Center Hamburg-Eppendorf, Institute for Human Genetics, Martinistr. 52, 20146 Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf, Center for Molecular Neurobiology, Martinistr. 52, 20146 Hamburg, Germany

Functional absence of fragile X mental retardation protein (FMRP) causes the fragile X syndrome (FXS), a hereditary form of mental retardation characterized by a change in dendritic spine morphology. The RNA-binding protein FMRP has been implicated in regulating postsynaptic protein synthesis. Here, we have analyzed whether the abundance of scaffold proteins and neurotransmitter receptor subunits in postsynaptic densities (PSDs) are altered in the neocortex and hippocampus of FMRP deficient mice. Whereas the levels of several PSD components are unchanged, concentrations of Shank1 and SAPAP scaffold proteins and various glutamate receptor subunits are altered in both adult and juvenile knockout mice. With the exception of slightly increased hippocampal SAPAP2 mRNA levels in adult animals, altered postsynaptic protein concentrations do not correlate with similar changes in total and synaptic levels of corresponding mRNAs. Thus, loss of FMRP in neurons appears to mainly affect the translation and not the abundance of particular brain transcripts. Semi-quantitative analysis of RNAs levels in FMRP immunoprecipitates showed that in the mouse brain mRNAs encoding PSD components, such as Shank1, SAPAP1-3, PSD-95 and the glutamate receptor subunits NR1 and NR2B are associated with FMRP. Luciferase reporter assays performed in primary cortical neurons from knockout and wild-type mice indicate that FMRP silences translation of Shank1 mRNAs via their 3' untranslated region. Activation of metabotropic glutamate receptors relieves translational suppression. As Shank1 controls dendritic spine morphology, our data suggest that dysregulation of Shank1 synthesis may significantly contribute to the abnormal spine development and function observed in brains of FXS patients.

# The CPEB-Associated Cytoplasmic Polyadenylation Apparatus Regulates mRNA-Specific Translation in Dendrites and Synaptic Plasticity

Tsuyoshi Udagawa[1]

[1]University of Massachusetts Medical School, Molecular Medicine, 373 Plantation Street, 01605 Worcester MA, USA

Translational control of mRNAs in dendrites is essential for specific forms of synaptic plasticity and learning and memory. The RNA binding protein CPEB regulates local translation of target mRNAs in dendrites. Here we identify poly(A) polymerase Gld2, deadenylase PARN, and translation inhibitory factor neuroguidin (Ngd) as components of the polyadenylation apparatus critical to CPEB-dependent translation in dendrites. Synaptic stimulation promotes CPEB phosphorylation and PARN expulsion, permitting Gld2-dependent polyadenylation and translation of target mRNAs. shRNA-mediated depletion of Gld2 in vivo attenuates mature synaptic spine number and protein synthesis -dependent long -term potentiation (LTP) in hippocampal dentate gyrus, whereas depletion of Ngd increases mature spine number and LTP. Moreover, Gld2 stimulates synthesis of the plasticity protein NMDA receptor subunit NR2A in dendrites while Ngd inhibits it. These data demonstrate that Gld2 and Ngd, members of the cytoplasmic polyadenylation apparatus, exert opposing actions on spine morphogenesis, local translation of NRA and plasticity at hippocampal synapses.

# Putative role for CPEB1-mediated mRNA translation in neurons and glia

David G. Wells[1]

[1]Yale University, Molecular, Cellular & Developmental Biology, 260 Whitney Ave, 06520 New Haven, USA

Localization of proteins to specific cellular compartments is critical to cell function. Mounting evidence now suggests that regulation of the transport and translation of mRNA in neurons plays an important role in growth cone guidance and synaptic plasticity. One mechanism for regulating mRNA translation in neurons is through the mRNA-binding protein cytoplasmic polyadenylation element binding protein (CPEB1). We have begun to examine the role of CPEB1 in both neurons and glia. Our initial work in neurons led us to believe that CPEB1 may regulate a cohort of mRNAs involved in cytoskeletal dynamics, ultimately affecting cell shape. I will focus on recent work showing that CPEB1 regulates cell motility and morphology in astrocytes and neurons, respectively. In both astrocytes and neurons, ß-catenin mRNA is regulated by CPEB1 but the signaling pathway to activation of CPEB1 appears to be distinct. The preponderance of the data supports a role for CPEB1 in regulating astrocyte motility, however, there is evidence that CPEB1 could have multiple roles in astrocytes. The signaling mechanisms as well as the functional implications of CPEB1-mediated ß-catenin synthesis in both neurons and astrocytes will be discussed.

# Expression and function of the CPEB2-4 subfamily in astrocytes, NG2 glia and microglia

Martin Theis[1]

[1]Universität Bonn, Institut für Zelluläre Neurowissenschaften, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

Much is already known about the role of CPEBs in neurons, yet the investigation of CPEB expression and function in glia is just beginning. With expression studies in cultured cells, single cell RT-PCR following electrophysiological characterization and FACS purification from fluorescently labelled transgenic mice, we have identified the expression pattern of the CPEB2-4 subfamily in glial subpopulations of the hippocampus. While microglia and NG2 glia show high transcript levels of CPEBs, we observed lower expression of CPEBs in astrocytes. What may be the reason for the differential expression in the various glial cell types? One clue came from studies of the Richter lab, showing that one function of CPEB-1 in cultured fibroblasts is growth control by preventing translation of small amounts of myc mRNA. NG2 cells indeed express typical astrocytic transcripts (GLT-1, GLAST, Cx43) but do not express corresponding functional glutamate transporter currents or gap junction coupling. We hypothesized that in NG2 cells, CPEBs may prevent the translation of these typical astrocytic mRNAs. With co-immmunoprecipitation experiments we confirmed Cx43 as a CPEB target. We previously observed that overexpression of CPEB3 in neurons decreased the basal protein synthesis of the CPEB targets GluR2 and beta -catenin. We followed a similar approach for astrocytes and overexpressed CPEB3 in astroglia by way of a GFAP-tTA transgene. Induction of CPEB3 overexpression in adult mice led to a decrease in Cx43 protein, but not mRNA expression. Cx43 is a major astrocytic gap junction protein, and consistently we observed decreased interastrocytic tracer coupling and decreased adult neurogenesis known to depend on Cx43 expression in neural stem cells. We follow a similar approach to study CPEB function in microglia by way of an Iba1-tTA transgene.

# Symposium

## S14: Dynamic processes in the auditory system

S14-1 Functional development of hair cell ribbon synapses
*Walter Marcotti*

S14-2 Molecular Physiology of Sound Encoding at the Hair Cell Ribbon Synapse
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S14-3 Synaptic inputs and coincidence detection in nucleus laminaris of the barn owl
*Catherine Emily Carr, Go Ashida, Paula Kuokkanen, Sahil Shah, Richard Kempter, Kazuo Funabiki, Hermann Wagner*

S14-4 Tonotopic Refinement of an Inhibitory Auditiory Map
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S14-5 Molecular specifications underlying the development and function of auditory brainstem regions: a global proteomic approach
*Eckhard Friauf, Bernd Kaltwaßer, Thomas Schulenborg, Christian Moritz*

S14-6 Monitoring activity-driven changes in the (phospho)proteome of plasma membrane proteins in the auditory brainstem
*Jens Schindler, Maren Weber, Juanying Ye, Ole Nørregaard Jensen, Hans Gerd Nothwang*

# Functional development of hair cell ribbon synapses

Walter Marcotti[1]

[1]University of Sheffield, Biomedical Science, Western Bank, S10 2TN Sheffield, United Kingdom

The exquisite temporal acuity of the mammalian auditory organ largely depends on the transfer characteristics of ribbon synapses in inner hair cells (IHCs), the primary sensory receptors of the mammalian cochlea.
Pre-hearing IHCs (before about postnatal day 12, P12, in most rodents) do not respond to sound but instead generate spontaneous calcium-dependent action potentials (APs: Marcotti et al. 2003 J. Physiol. 548: 383-400). Intracellular calcium signalling associated with APs is thought to regulate a variety of cellular responses involved in the cell's functional differentiation and/or maturation (Kros et al. 1998 Nature 394: 281-4), as proposed for the exocytotic machinery (Johnson et al. 2007 J. Physiol. 583: 631-46). Neurotransmitter release from pre- and post-hearing IHCs is controlled by calcium influx through L-type CaV1.3 calcium channels (Platzer et al. 2000 Cell 102: 89-97), the properties of which vary as a function of age and the cell's position along the cochlea. Since IHCs respond differently before and after the onset of hearing and as a function of frequency position, we investigated how IHC voltage responses, calcium channels and synaptic vesicle exocytosis change in order to meet the cell's requirements.
Electrophysiological responses from IHCs were studied as a function of postnatal development (from P1 to P69) and position along the cochlea of altricious rodents. All recordings were performed using acutely dissected cochleae maintained at 35-37°C in perilymph-like extracellular solution containing 1.3 mM calcium.
We demonstrated that a differential expression of IHC characteristics allows them to be optimal configured to efficiently process developmental signals during immature stages and sound stimuli in adult animals.

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# Molecular Physiology of Sound Encoding at the Hair Cell Ribbon Synapse

Tobias Moser[1]

[1]Otolaryngology and Center for Molecular Physiology, Universitätsmedizin Göttingen- HNO-Klinik, Göttingen, Germany

Sound encoding at the synapse between inner hair cells and spiral ganglion neurons in the mammalian cochlea operates with submillisecond temporal precision, drives neural spiking at hundreds per second over hours and covers sound pressures that vary by six orders of magnitude. Sound encoding is mediated by specialized synapses: the hair cell ribbon synapses. When hair cells transduce a sound driven mechanical stimulus into an electrical signal, voltage-gated $Ca^{2+}$ channels open and the $Ca^{2+}$ influx triggers exocytosis of glutamate filled vesicles at their ribbon synapses. It becomes evident that the hair cell ribbon synapse employs a molecular machinery that is highly specialized and in part distinct from that of small CNS synapses. Each of the 5-20 active zones drives spiking in one spiral ganglion neuron in the absence and presence of acoustic stimulation. High and sustained rates of synaptic transmission require very efficient means of vesicle cycling. Interfering at any step to the hair cell vesicle cycle results in auditory dysfunction, be it vesicle docking, priming, fusion or endocytic membrane retrieval. Moreover, the synapses of one hair cell differ from one each other in structure and function in order to collectively cover the broad range of sound pressures with the limited dynamic range of encoding at each individual synapse.

# Synaptic inputs and coincidence detection in nucleus laminaris of the barn owl

Catherine Emily Carr[1], Go Ashida[1], Paula Kuokkanen[2], Sahil Shah[1], Richard Kempter[2], Kazuo Funabiki[3], Hermann Wagner[4]

[1]University of Maryland, Biology, College Park, USA
[2]Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Germany
[3]Systems Biology, Osaka Bioscience Institut, Japan
[4]Institute for Biology II, RWTH Aachen, Germany

Synaptic mechanisms underlie the computation and representation of interaural time difference (ITD) in the auditory system. In the barn owl, excitatory inputs, phase locked to frequencies up to 8 kHz, generate ITD sensitive responses in the neurons of the barn owl's nucleus laminaris (NL). Bilateral excitatory inputs from the nucleus magnocellularis (NM) converge on NL such that axons from the ipsilateral NM enter NL dorsally, while contralateral axons enter from the ventral side, forming maps of ITD that are tapped by NL neurons that act as coincidence detectors. The afferents and their synapses on NL neurons generate an oscillatory, tone induced evoked potential, or "neurophonic," that varies systematically with position in NL, and can be used to derive maps of best ITD.

From dorsal to ventral within NL, best ITDs shift from far contralateral space to locations around 0µs and into ipsilateral space. Earlier recordings of the neurophonic in NL suggested that iso-delay contours ran parallel to the dorsal and ventral borders of NL (Sullivan and Konishi, 1986). This map is orthogonal to that seen in chicken NL, where a single map of ITD runs from around 0µs ITD medially to far contralateral space laterally (Köppl and Carr, 2008; Joseph and Hyson, 1993). Yet the trajectories of the NM axons are similar in owl and chicken (Young and Rubel, 1983). We therefore developed analytical techniques to measure delays in barn owl's neurophonic response. Clicks were used to measure conduction time, while lesions mark the 0µs iso-delay contour in multiple penetrations along an iso-frequency slab. We found iso-delay contours were not parallel to the borders of NL, but instead the representation of 0µs shifted systematically from dorsal to ventral within an iso-frequency slab. Along an iso-delay contour, latency increased systematically from medial to lateral in NL, for both ipsi-and contralateral axons.

The high frequency component (> 3 kHz) of the neurophonic appears to originate from the synaptic input to NL from NM. Furthermore, the neurophonic exhibits a high amplitude sinusoidal oscillation in response to tonal stimulation (Kuokkanen et al., 2010). The intracellular recordings from NL neurons also show an oscillation of the membrane potential at the stimulus frequency (Ashida et al, 2008). This oscillation is thought to originate from phase-locked inputs from NM fibers and underlies the sensitivity to ITD. However, how presynaptic, synaptic and postsynaptic properties affect the formation of the oscillatory intracellular potential and the extracellular neurophonic potential remains unclear. We have therefore used theoretical analyses and modeling of this circuit to reveal the parameter dependence of the oscillatory membrane potential in NL, in order to understand the synaptic mechanisms underlying the computation and representation of ITD.

# Tonotopic Refinement of an Inhibitory Auditiory Map

Karl Kandler[1]

University of Pittsburgh, Otolaryngology, 3501 Fifth Avenue, Rm 10016, PA 15261 Pittsburgh, USA

Before sensory experience can drive neuronal activity in the developing brain, spike activity is often dominated by spontaneous activity in the form of burst-like patterns. In the auditory system, such pre-hearing burst-like activity is generated in the cochlea from which it is transmitted to the auditory nerve and central auditory pathways. Although it is generally assumed that these spontaneous activity patterns guide the maturation of central auditory circuits, direct evidence for this hypothesis is lacking.

Before hearing onset, the GABA/glycinergic brainstem pathway from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO), undergoes tonotopic refinement by synaptic silencing and strengthening of maintained connections. After hearing onset, this functional refinement is followed by an anatomical refinement in the form of axonal pruning.

To investigate whether these refinement processes depend on specific patterns of activity bursts, we characterized the maturation of MNTB -LSO connectivity in mutant mice lacking expression of the a9 subunit of the nicotinergic acetylcholine receptor (a9nAchR). These knock-out (KO) mice lack functional cholinergic efferent transmission to cochlear hair cells, which in turn may modify cochlear activity pattern. Results demonstrate that the temporal fine -structure of MNTB spontaneous activity before hearing is significantly altered in KO mice compared to wild-type (WT) mice. Using functional mapping of the MNTB-LSO connectivity before hearing onset with focal glutamate uncaging, we found that in a9 KO mice the functional refinement of this pathway, achieved by combined synaptic silencing and strengthening, is significantly impaired. This indicates that the temporal pattern of pre-hearing spike activity is crucial for the tonotopic refinement of functional MNTB-LSO connectivity.

As a9 KO mice have normal hearing, we next asked whether deficits in functional refinement before hearing onset affect axonal pruning occurring after hearing onset. To this end, we used quantitative reconstructions of individual MNTB axonal terminals in the LSO of developing WT and KO mice. We found normal topographic organization of MNTB-LSO axonal terminals in the KO mice around hearing onset (P10-P12) indicating that altered pre-hearing activity does not affect axonal growth and anatomical MNTB-LSO connectivity. However, subsequent pruning of MNTB axons in KO mice was profoundly impaired despite hearing experience, resulting in decreased tonotopic precision at P21 in KO mice compared with WT mice.

In summary, our results show that tonotopic refinement of the inhibitory MNTB-LSO pathway involves two major, distinct steps. Before hearing onset, refinement is governed by synaptic silencing and strengthening without axonal pruning. This functional refinement requires precise temporal patterns of spontaneous spike activity. After hearing onset, the MNTB-LSO pathway is refined by axonal pruning, which depends on the presence of preceding functional refinement. We speculate that spontaneous activity-driven functional MNTB-LSO maps provide the templates for anatomical pruning after hearing onset.

# Molecular specifications underlying the development and function of auditory brainstem regions: a global proteomic approach

Eckhard Friauf[1], Bernd Kaltwaßer[1], Thomas Schulenborg[1], Christian Moritz[1]

[1]Animal Physiology Group, Department of Biology, University of Kaiserslautern, 67663 Kaiserslautern, Germany

Spatial and temporal differences between anatomically and functionally distinct tissues are reflected by the dynamic composition of the proteomes. Characterizing those in large scale is a powerful approach that can provide comprehensive insights into the specific underlying mechanisms. Today, proteomics enables the identification and relative quantification of differences of hundreds of proteins from minute amounts of tissues. This allows the exhaustive comparison of small tissues and the analysis of ontogenetic changes. In order to assess similarities, as well as differences, between anatomically and functionally distinct regions in the central auditory system, we have analyzed the protein profile of three prominent centers of the rat auditory brainstem, the cochlear nuclear complex (CN), the superior olivary complex (SOC), and the inferior colliculus (IC). The protein repertoires of these three centers were further compared to the protein repertoire of the rest of the brain (subsequently called "Rest"). By employing 2D-DIGE and MALDI-MS, we analyzed the subproteome of cytosolic proteins. We found 644 protein spots in a total of 1,864, which displayed significant level differences between any of the regions. When comparing the CN with the SOC, only 36 spots (2%) showed significant differences, indicating a great similarity between the two regions. On the other hand, the greatest difference occurred in the comparisons CN vs. Brain (24%) and SOC vs. Brain (23%). Interestingly, the IC, a major hub in the auditory brainstem, was closer to the Rest (5%) than to the IC and CN (10%). Details of the study are on a poster by Moritz *et al*.

In a second series of experiments, we analyzed temporal dynamics within the auditory brainstem: ontogenetic changes in the proteome of the SOC and the IC were identified and quantified by comparing an immature state (postnatal day [P] 4) with a mature state (P60). Because hearing ability begins at about P12 in rats, the approach represents a proteomic comparison of a hearing system with a non-hearing system. Like in the above study, the cytosolic proteins were assessed using DIGE and MALDI-MS. However, the data for the IC was complemented by an analysis of the plasma membrane proteomes. Enriched plasma membrane fractions were obtained and characterized by the gel-free mass spectrometric iTRAQ-method (isobaric tags for relative and absolute quantification). The DIGE analysis yielded >800 proteins, of which 12% were differentially regulated. iTRAQ yielded 105 proteins, of which 57% were significantly up- or down-regulated. Our results show that the same functional pathways underlie temporal dynamics within the SOC and IC. Thus we conclude that very similar maturation processes occur in both centers. The identified ontogenetically relevant proteins reflect enzymes of energy metabolism as well as regulators and components of the cytoskeleton. Proteins of transmitter metabolism as well as transmitter transport were up-regulated with age. The findings were confirmed by immunohistochemistry, western blots, and metabolic assays.

Our study represents the first global proteomic analysis in the central mammalian auditory system and provides in-depth information about spatial and temporal differences between the protein profiles of three auditory centers. Profiles, region-typical proteins, and characteristic changes from the period of circuit refinement to the mature system will be addressed in detail during the talk.

# Monitoring activity-driven changes in the (phospho)proteome of plasma membrane proteins in the auditory brainstem

Jens Schindler[1], Maren Weber[1], Juanying Ye[2], Ole Nørregaard Jensen[2], Hans Gerd Nothwang[1]

[1]University of Oldenburg, Neurogenetics Group, Carl-von-Ossietzky-Str. 9-11, 26129 Oldenburg, Germany
[2]University of Southern Denmark, Protein Research Group, Campusvej 55, 5230 Odense M, Denmark

Processing of acoustic information in the central auditory pathway requires an exquisite set of proteins. In addition, the activity of these proteins has to be fine tuned to cope with the different demands in auditory neurons such as processing of low or high frequency acoustic information. This modulation has often to occur in a rapid and reversible manner to adjust to the ever-changing acoustic environment. These considerations point to phosphorylation/dephosphorylation as key events in the regulation of proteins in the auditory system. Yet, little is known about the phosphoproteome of auditory neurons and dynamic changes therein.

Plasma membrane proteins are key players for the transmission and processing of auditory information. To gain insight in their (dynamic) phosphorylation pattern in the auditory pathway, we first developed a strategy to identify phosphopeptides of plasma membrane proteins from small tissue samples. The strategy combines techniques for the efficient and comprehensive enrichment of plasma membranes and phosphopeptides as well as high-end mass spectrometric analyses. Starting with 50 mg of brain tissue, we were able to identify ~1000 different phosphorylation sites on ~ 500 different proteins. ~ 40% of these proteins were allocated to the plasma membrane. Subsequent analysis of the inferior colliculus and the superior olivary complex identified various proteins such as the hyperpolarization-activated cyclic nucleotide-gated channel HCN2 with 7 different phosphorylation sites, the metabotropic glutamate receptor mGluR5 with 3 different phosphorylation sites, and various voltage gated potassium channels, each with multiple phosphorylation sites.

Currently, we use this strategy in a differential proteomic approach to identify activity-induced changes in the (phospho)proteome. To this end, we perform a comparative analysis of auditory processing centers from wildtype mice and three different deafness models. This quantitative analysis will provide important information on activity-driven changes in the protein repertoire and its phosphorylation pattern in the auditory pathway.

# Symposium

## S15: Light sensors in new light: A comparative and integrative view on photoreceptors, their function, differentiation and degeneration

S15-1 PHOTORECEPTION AND PHOTORECEPTOR CELL BIOLOGY
*Uwe Wolfrum*

S15-2 Box jellyfish photoreception and photoreceptor evolution
*Zbynek Kozmik*

S15-3 The TRP ion channels of *Drosophila* photoreceptors
*Armin Huber*

S15-4 Comparative analysis of mammalian photoreceptor arrangements
*Leo Peichl*

S15-5 Differentiation of photoreceptor cells from retinal stem cells
*Yvan Arsenijevic*

S15-6 The making and breaking of the photoreceptor ribbon synapse
*Johann Helmut Brandstätter*

S15-7 Photoreceptor cell death mechanisms: Apoptosis, Necrosis, or What?
*François Paquet-Durand*

# PHOTORECEPTION AND PHOTORECEPTOR CELL BIOLOGY

Uwe Wolfrum[1]

[1]Johannes Gutenberg University of Mainz, Cell and Matrix Biology, Institute of Zoology, Mainz, Germany

No abstract available

# Box jellyfish photoreception and photoreceptor evolution

Zbynek Kozmik[1]

[1]Institute of Molecular Genetics, Department of Transcriptional Regulation, Videnska 1083, 14220 Praha 4, Czech Republic

Animal eyes can vary in complexity ranging from a single photoreceptor cell shaded by a pigment cell to elaborate arrays of these basic units, which allow image formation in compound eyes of insects or camera-type eyes of vertebrates. The evolution of the eye requires involvement of several distinct components – photoreceptors, screening pigment and genes orchestrating their proper temporal and spatial organization. Analysis of particular genetic and biochemical components shows that many evolutionary processes have participated in eye evolution. Cnidaria as the likely sister group to the Bilateria are the earliest branching phylum with a well-developed visual system. Remarkably, camera-type eyes of the Cubozoan jellyfish, *Tripedalia cystophora*, use genetic building blocks typical of vertebrate eyes, namely a ciliary-like phototransduction cascade and melanogenic pathway. Our findings indicative of parallelism provide a new insight into eye evolution. Combined, the available data favor the possibility that vertebrate and cubozoan eyes arose by independent recruitment of orthologous genes during evolution.

# The TRP ion channels of *Drosophila* photoreceptors

Armin Huber[1]

[1]University of Hohenheim, Department of Biosensorics, Garbenstr. 30, 70599 Stuttgart, Germany

The visual transduction cascade operating in *Drosophila* photoreceptor cells is a G-protein mediated signalling pathway that terminates in the opening of two cation channels, TRP (transient receptor potential) and TRPL (TRP-like). The *Drosophila* TRP channels are the founding members of a large family of ion channels that serve as sensors and transducers of environmental stimuli and also as regulators of ion homeostasis in neuronal and epithelial cells. More than 50 members of the TRP ion channel family have been isolated from *C. elegans*, *Drosophila*, mouse and men. In general, the functional characteristics of a neuron depend largely on the set of ion channels present in the plasma membrane. In fly photoreceptor cells the equipment of the photoreceptive membrane, that forms the rhabdomere, with TRP and TRPL changes when the flies are kept in different light conditions. This is because the TRPL channel translocates from the rhabdomere to the cell body when the flies are switched from a dark environment into the light. Therefore, in the dark TRPL and TRP are located in the rhabdomere and function in phototransduction whereas in the light TRPL is outside the rhabdomeres and TRP alone mediates the visual response.

I will present our studies on the mechanisms underlying light-dependent subcellular TRPL translocation. We used transgenic flies expressing eGFP-tagged TRPL and immunocytochemistry to determine the localization of the ion channel in intact eyes or in sections through the eyes of *Drosophila* wild type flies and mutants. We found that triggering the translocation of TRPL requires activation of the complete phototransduction cascade and $Ca^{2+}$-influx through TRP channels. Upon illumination TRPL is first transported to the base of the rhabdomeres and to the apical membrane adjacent to the rhabdomeres, and then is internalized into an intracellular storage compartment. The internalization of TRPL is mediated by a vesicular transport pathway which is also used for the internalization of rhodopsin and it requires specific Rab proteins. Massive rhodopsin internalization negatively affects TRPL internalization suggesting that there may be a competition between rhodopsin and TRPL for a limiting factor required for this process. Moreover, we have performed a genetic screen for novel genes involved in TRPL translocation using eGFP-tagged TRPL. First results of this screen will be presented. In conclusion, the TRP channels of *Drosophila* photoreceptors represent a powerful model system for studying the cell biological mechanisms of regulated, long-term alterations in the equipment of a neuron with specific ion channels.

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# Comparative analysis of mammalian photoreceptor arrangements

Leo Peichl[1]

[1]Max Planck Institute for Brain Research, Comparative Retinal Anatomy, Deutschordenstr. 46, 60528 Frankfurt/M., Germany

All mammalian retinae contain rod photoreceptors for lowlight (night) vision and cone photoreceptors for daylight and colour vision. Most mammals have 'dichromatic' colour vision, based on two spectral cone types containing a middle-to-longwave-sensitive (commonly green-sensitive) L opsin and a shortwave-sensitive (commonly blue-sensitive) S opsin, respectively. However, superimposed on this basic similarity there are remarkable quantitative and qualitative differences between species. Rod:cone ratios vary strongly and are loosely correlated with the activity pattern of the species, from ~1:100 in very nocturnal mammals to 1-3:10 in most diurnal mammals and ~10:1 in a few diurnal species (e.g., ground squirrels and tree shrew). Against expectation, subterranean mammals have comparatively low rod densities and high cone proportions (~1:10). The proportions of L and S cones and their topographies also vary considerably. They range from a conventional 10% proportion of S cones across the retina to a dominant or even exclusive expression of S cone opsin in the ventral retina of some species. In some mammals, e.g. some rodents, bats and insectivores, the S cones are UV- rather than blue-sensitive. Obviously most mammals, including nocturnal and subterranean ones, have the retinal prerequisites for colour vision, but their colour vision characteristics differ considerably. Whales and seals have no S cones at all, they are L cone monochromats and presumably colour-blind. It is widely assumed that the above variations represent adaptations to specific visual needs associated with particular habitats and lifestyles. However, in many cases we have not yet identified the adaptive value of a given photoreceptor arrangement (review: Peichl, Anat. Rec. Part A 287A: 1001-1012, 2005).

A particular "new light" was recently shed on rod properties: We found a unique 'inverted' nuclear architecture in the rods of nocturnal mammals, whereas the rods of diurnal mammals have the conventional nuclear architecture present in nearly all other eukariotic cells (Solovei et al., Cell 137: 356-368, 2009). In the 'inverted' rod nuclei, the inactive heterochromatin localizes in the nuclear centre, whereas the active euchromatin lines the nuclear border. This appears cell-biologically disadvantageous, so we suspected a role in vision. We could show that the inverted nuclei with the optically denser heterochromatin in their centre act as collecting lenses, and our computer simulations indicate that columns of such nuclei channel light effectively through the outer nuclear layer (ONL). The retinae of nocturnal mammals have a higher rod density for improved night vision and hence a thicker ONL, which on the other hand would lead to increased light scatter so that fewer of the precious photons reach the outer segments. Light-guiding by the inverted rod nuclei seems a suitable evolutionary solution to this dilemma. The retinae of diurnal mammals have a thinner ONL and operate at higher light levels, so their rods can have the conventional nuclear architecture.

# Differentiation of photoreceptor cells from retinal stem cells

Yvan Arsenijevic[1]

[1]Jules-Gonin Eye Hospital, University of Lausanne, Unit of Gene Therapy & Stem Cell Biology, 15, av. de France, 1004 Lausanne, Switzerland

Within the last few years, several reports have revealed that cell transplantation can be an effective way to replace lost neurons in the central nervous system (CNS) of patients affected with neurodegenerative diseases. Concerning the retina, the concept that newborn photoreceptors can integrate the retina and restore some visual functions was univocally demonstrated recently in the mouse eye (MacLaren et al. 2006) and remains to be achieved in human. These results pave the way to a standard approach in regenerative medicine aiming to replace lost photoreceptors. With the discovery of stem cells a great hope has appeared towards elaborating protocols to generate adequate cells to restore visual function in different retinal degeneration processes. Retinal stem cells (RSCs) and embryonic stem cells are good candidates to generate photoreceptors. To achieve a large production of photoreceptors, researchers aim to mimic crucial stage of eye field commitment, retina fate acquisition and photoreceptor differentiation. Knowledge about photoreceptor differentiation during retinogenesis will be discussed in view of the protocols applied to stem cells to generate retinal cells. A particular attention will be focused on the radial glia population which was poorly studied in the retina and on the importance of the Notch pathway to generate cones.

# The making and breaking of the photoreceptor ribbon synapse

Johann Helmut Brandstätter[1]

[1]University of Erlangen-Nuremberg, Department of Biology, Animal Physiology, Staudtstr. 5, 91058 Erlangen, Germany

The photoreceptor ribbon synapses, the first station in signal transfer in the visual system, belong to the most efficient and complex chemical synapses in the CNS. They are designed to transmit light signals over a dynamic range of several orders of magnitude in intensity and to dynamically regulate transmitter release in response to changes in stimulus intensity. The characteristic hallmark of photoreceptor ribbon synapses is an electron-dense presynaptic organelle that tethers arrays of synaptic vesicles at the site of transmitter release.

Functional defects at the photoreceptor ribbon synapses – so -called synaptopathies – can impair seeing ability up to total blindness. Despite their central role in visual signal transfer little is known about the molecular structure and function of the photoreceptor ribbon synapses. From our studies, e.g. on mouse mutants with loss-of-function mutations in key presynaptic proteins, we expect to gain a detailed molecular understanding of photoreceptor ribbon synaptic function – the basis for a better understanding of the physiology and pathophysiology of this unique synapse.

Work supported by grants from the DFG.

# Photoreceptor cell death mechanisms: Apoptosis, Necrosis, or What?

François Paquet-Durand[1]

[1]University of Tübingen, Institute for Ophthalmic Research, Cell Death Mechanisms Group, Röntgenweg 11, 72076 Tübingen, Germany[1]

Photoreceptor degeneration is the hallmark feature of a genetically heterogeneous group of inherited blinding diseases, collectively termed Retinitis Pigmentosa (RP). Even though the genetic causes are relatively well established, RP is at present untreatable, creating an urgent need for novel therapeutic approaches. A prerequisite for the development of a therapy is, however, a thorough understanding of the underlying neurodegenerative mechanisms. In the past, photoreceptor cell death has often been referred to as an apoptotic process, although the causal metabolic pathways are in fact still only poorly understood. More recently, it has become increasingly clear that in animal models for RP few, if any, of the processes characteristic for apoptosis are active. Instead, aberrant and excessive cGMP-signalling seems to play a preeminent role, leading to activation of a number of metabolic processes that eventually kill photoreceptors. This is particularly true for one of the most studied RP animal models, the human homologous rd1 mouse, which is characterized by mutation-induced dysfunction of phosphodiesterase-6 and consequent accumulation of cGMP in photoreceptors.

The analysis of cGMP-induced neurodegenerative processes necessitates methods that allow for cellular resolution, since in complex neuronal tissues, such as the retina, changes that affect only a small subset of cells at any given time are likely to go unnoticed if a tissue based analysis method is used. Using custom developed in situ biochemical assays and immunodetection methods, we could show that changes in enzymatic activity in individual cells were often dramatic while corresponding alterations in gene or protein expression were not noticeable.

The presentation will aim at providing a comprehensive mechanistic view of the neurodegenerative pathways governing photoreceptor cell death. Activation of cGMP-dependent protein kinase G (PKG) and cyclic nucleotide gated (CNG) channels – the two main cGMP targets in photoreceptors – are likely to be early events in cGMP-induced cell death. At later stages in the degenerative process, epigenetic processes such as enzymatic activity of histone deacetylase (HDAC) and poly-ADP-ribose polymerase (PARP) appear to play a major role. Interestingly, the two corresponding post-translational protein modifications – acetylation and poly-ADP-ribosylation – appear to be mutually exclusive, with healthy photoreceptors showing a high degree of protein acetylation and very low poly-ADP-ribosylation while degenerating photoreceptors show low acetylation but high poly-ADP-ribosylation. Eventually, PARP activity, together with calcium-activated calpain type proteases, seems to precipitate the execution of cell death.

Strikingly, none of these metabolic activities are normally associated with apoptosis, suggesting the presence and activity of one (or more) alternative, non-apoptotic cell death pathways in photoreceptors. Improved knowledge on these mechanisms may allow for the development of novel approaches for currently untreatable blinding diseases. Since the retina is an integral part of the central nervous system, many of the characteristics of photoreceptor cell death may be relevant for neurodegenerative diseases in general.

# Symposium

## S16: Barrel cortex function: From single cells to behaving animals

S16-1 Cell type-specificity of thalamic input to inhibitory interneurons in the mouse barrel cortex *in vitro*
*Jochen Staiger, Vivian Dambeck, Martin Möck*

S16-2 Dendritic target region specificity of excitatory synaptic connections from layer 4 to layer 6A in rat barrel cortex
*Dirk Feldmeyer, Guanxiao Qi*

S16-3 Functional imaging of neuronal populations in barrel cortex using a genetically-encoded calcium indicator
*Fritjof Helmchen, Henry Lütcke, Kristina Schulz, Maz Hasan, Sebastian Kügler, David Margolis*

S16-4 Cortical Processing during Behaviour
*James Poulet*

S16-5 Membrane potential dynamics of GABAergic neurons in the mouse barrel cortex during behaviour
*Carl Petersen*

S16-6 Sensorimotor cortex activity in rats related to whisking
*Cornelius Schwarz, Todor V Gerdjikov, Olga Rodriguez-Sierra, Waiblinger Christian, Haiss Florent*

# Cell type-specificity of thalamic input to inhibitory interneurons in the mouse barrel cortex *in vitro*

Jochen Staiger[1], Vivian Dambeck[1], Martin Möck[1]

[1]Center Anatomy, Department Neuroanatomy, Kreuzbergring 36, 37075 Göttingen, Germany

Inhibitory GABAergic interneurons of the neocortex offer a large diversity of cell types which can be differentiated on the basis of their molecular, electrophysiological and morphological properties. A consensus was reached that parvalbumin, somatostatin and vasoactive intestinal polypeptide (VIP) are suitable markers for three well-characterized and non- overlapping types, i.e. basket cells, Martinotti cells and bipolar -bitufted cells, respectively. We took advantage of the novel availability of a BAC-transgenic mouse where eGFP is expressed under the control of the VIP promoter. Double immunocytochemistry revealed that virtually all eGFP-expressing neurons are positive for VIP. We have observed that most of the bipolar-bitufted eGFP expressing cells are located in layers II/III, whereas a variety of GFP positive multipolar neurons are distributed across all layers of the barrel cortex. To test whether there is a layer- and/or cell type-specificity of thalamic inputs to the VIP neurons, we cut thalamocortical slices and applied electrical stimulation to the ventrobasal nucleus while performing whole cell recordings in the barrel cortex. We found three different responses: (i) no EPSPs in ca. 74%, (ii) monosynaptic and strong EPSPs in 10% and (iii) di - (or oligo -) synaptic and weak EPSPs in 16% of the recorded VIP neurons. In monosynaptically innervated VIP neurons short- term plasticity protocols showed depression for all studied frequencies. We conclude that only a specific subpopulation of VIP neurons is directly innervated by the lemniscal thalamic pathway and thus has the capability of imposing early feedforward inhibition on their still unknown target cells.

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# Dendritic target region specificity of excitatory synaptic connections from layer 4 to layer 6A in rat barrel cortex

Dirk Feldmeyer[1], Guanxiao Qi[1]

[1]RWTH Aachen University and Research Centre Jülich, Dept of Psychiatry and Psychotherapy, Function of Cortical Microcircuits Group, Pauwelstrasse 30, 52074 Aachen, Germany

In the barrel cortex, the major cortical input (thalamo-recipient) layers are layer 4 (L4) and to a lesser degree layers 5 and 6 (in particular sub-lamina 5B and 6A) which receive thalamo-cortical afferents from the ventroposterior (VPM) nucleus of the somatosensory thalamus. The somatosensory thalamus is in turn innervated by neocortical layer 5A and 6. Therefore, excitatory neurons in cortical layer 4 and 6A and the thalamus form a thalamo-cortico-thalamic feedback loop that may control thalamic activity.

The aim of our study was to investigate synaptic interactions between excitatory neurons in the main thalamo-recipient layers. In order to characterise the properties of both pre- and postsynaptic neurons dual whole-cell patch clamp recordings with simultaneous biocytin fillings were used.

We identified monosynaptic L4-to-L6A connections which were of an intermediate reliability and a relatively low efficacy. The EPSP amplitude was either depressing or weakly facilitating during repetitive presynaptic action potentials. We were able to identify three different types of excitatory synaptic connections: When the presynaptic neuron was a spiny stellate cell, the unitary EPSP rise time and latency was long (6.7±2.1 ms and 3.8±1.6 ms, respectively). Synaptic contacts established by these neurons were found only apical dendritic tufts of the postsynaptic L6A pyramidal neurons at an average somatic distance of 590±141 µm. Connections with presynaptic star pyramidal neurons in the centre of the barrel had markedly shorter rise times latency (1.5±0.9 ms and 1.7±0.2 ms, respectively) and synaptic contacts were on basal dendrites or proximal apical oblique dendrites located in layer 5B and 6A (mean somatic distance: 86±54 µm). A smaller subpopulation of presynaptic star pyramidal cells close to the border of a barrel column exhibited slower had also slow EPSPs of long latency (5.4±1.7 ms and 2.9±1.4 ms, respectively). This subpopulation of L4 neurons made also contacts on the apical dendrite with at a mean distance of 524±166 µm.

This data indicates that excitatory L4-to-L6A neuron connections show a subcellular target region specificity and did not connect to all target dendrites within the reach of their axon. Spiny stellate axons innervate only distal apical tufts of L6A pyramidal cells while the majority of star pyramid axons contact the proximal dendritic branches. This suggests that both L4 cell types perform different computational tasks in the barrel column microcircuitry.

# Functional imaging of neuronal populations in barrel cortex using a genetically-encoded calcium indicator

Fritjof Helmchen[1], Henry Lütcke[1], Kristina Schulz[1], Maz Hasan[2], Sebastian Kügler[3], David Margolis[1]

[1]Brain Research Institute, Dept. of Neurophysiology, Winterthurerstrasse 190, 8057 Zurich, Switzerland

[2]MPI for Medical Research, Heidelberg, Germany

[3]University of Göttingen Medical School, Göttingen, Germany

Recent progress in the expression and performance of genetically encoded calcium indicators (GECIs) now makes it feasible to functionally probe neuronal populations repeatedly and over long time periods. We have used viral expression of Yellow cameleon (YC) 3.60 in supragranular layers of mouse barrel cortex to examine neuronal population dynamics. Using a chronic glass window above the injected cortex area we investigated if and to what extent spontaneous and sensory-evoked neural dynamics in anesthetized mice remain stable over the period of days to weeks. We in addition performed whisker-trimming experiments to assess in how far the characteristics of local neural dynamics changes after such a deprivation protocol. Moreover, we compared neocortical neural dynamics during different behavioral states in awake mice adapted to head-fixation and assessed whether the observed differences are preserved in repeated imaging sessions over several days. In the majority of active L2/3 neurons we found a higher occurrence of calcium transients during non-whisking compared to whisking epochs; repeated imaging of the same neurons over several days showed stability of this effect. These types of experiments should help to further probe stable and variable aspects of network activity on an extended time scale.

# Cortical Processing during Behaviour

James Poulet[1]

[1]Max-Delbrück-Centrum für Molekulare Medizin (MDC), Depertment of Neuroscience, Robert-Rössle-Str. 10, 13092 Berlin, Germany

Internal brain states form key determinants for sensory perception, sensorimotor coordination and learning. A prominent reflection of different brain states in the mammalian central nervous system are the distinct patterns of cortical oscillations and synchrony, as revealed by extracellular recordings of the electroencephalogram, local field potential and action potentials. Such temporal correlations of cortical activity are thought to be fundamental mechanisms of neuronal computation.

In this talk I will present data using single and dual whole-cell recordings from primary somatosensory barrel cortex in mice performing whisker behaviour to examine the cellular correlates of cortical state change. Our recordings reveal that the membrane potential of nearby neurons undergo slow large amplitude fluctuations that are highly correlated during quiet wakefulness, but when the mouse is whisking, cortical neurons depolarise and undergo a state change that reduces the membrane potential fluctuations as well as the correlation between nearby neurons, resulting in a desynchronised local field potential and electroencephalogram.

I will go on to discuss recent experiments examining the network mechanisms underlying the change in cortical state during whisker movements. Cortical state change persists after cutting the primary sensory nerve from the whisker pad and therefore is generated internally, within the CNS. Juxtacellular recordings from thalamic neurons during whisker movements reveal an increase in thalamic spiking activity during whisker movements. Inactivation of thalamus, by thalamic injection of muscimol, increases cortical slow fluctuations during quiet periods. During active periods, when the mouse is whisking, cortical neurons become hyperpolarised during whisking, in contrast to the depolarised state during whisking under control conditions, and the slow fluctuations disappear. The thalamus is therefore responsible for one key aspect of the cortical state change - it appears to provide the tonic depolarising input during whisking.

# Membrane potential dynamics of GABAergic neurons in the mouse barrel cortex during behaviour

Carl Petersen[1]

[1]Ecole Polytechnique Federale de Lausanne (EPFL), Laboratory of Sensory Processing, SV-BMI-LSENS, Station 19, CH1015 Lausanne, Switzerland

The neocortex is composed of an intricate neuronal network of synaptically coupled excitatory glutamatergic neurons and inhibitory GABAergic neurons. Through whole-cell membrane potential recordings from different classes of layer 2/3 neurons in the barrel cortex of awake mice, we are beginning to understand their differential contributions to neocortical network function. We find that different types of GABAergic neurons are active during different behavioral states and they respond differentially to sensory stimulation.

# Sensorimotor cortex activity in rats related to whisking

Cornelius Schwarz[1], Todor V Gerdjikov[2], Olga Rodriguez-Sierra[3], Waiblinger Christian[1], Haiss Florent[4]

[1]Hertie Institute for Clinical Brain Research, Systems Neurophysiology, O. Müller Str. 27, 72076 Tübingen, Germany[2]4School of Psychology, University of Leicester, Leicester, UK[3]School for Neural and Behavioral Sciences, Eberhard Karls University Tübingen, Germany[4]Institute of Pharmacology & Toxicology, University of Zürich, Zürich, Switzerland

Perception is an active process as sensory signals are modulated by 'top -down' processes on all levels of neural processing. The model system we employ for this purpose is active whisking behavior in rats and the underlying sensorimotor signals in primary somatosensory ('barrel') and primary motor cortices. Similar to humans and other primates, which use rhythmic movements of fingertips, rats discriminate texture and form of objects by rhythmically sweeping their vibrissae over it.
Dissecting a sensorimotor behavior ('active touch') into its sensory ('passive touch') and motor parts ('movement alone') and recording multielectrode signals in barrel cortex we were able to show that a central, movement-dependent signal switches the characteristics of tactile processing in barrel cortex. We investigated whether the modulatory signals originate in a sub-region of the vibrissae motor cortex, which we called RW for rhythmic whisking. Continuous, high frequency stimulation (60 Hz) in this area evokes rhythmic vibrissa movements at 5-10 Hz that are virtually indistinguishable from the ones self-initiated by the individual. Spike recordings from RW show that most of these neurons do not contribute to movement initiation; rather they become active after the start of the whisker movement and are not modulated in a correlated way with detailed whisking kinematics. We currently test the effects of RW stimulation on sensory processing in the barrel cortex.

# Symposium

## S17: Neurobiology of complex social behaviour: from bonding to autism

S17-1 Evolutionary Convergence and Divergence in the Nonapeptide Mechanisms of Grouping and Monogamy
*James L. Goodson*

S17-2 Link between complex social behaviours and anxiety: Involvement of the pro-social neuropeptides oxytocin and vasopressin
*Inga D Neumann*

S17-3 The monogamous male brain - neurochemical regulation of social bonding
*Zuoxin Wang*

S17-4 Processing of social cues in the olfactory bulb by vasopressin
*Mike Ludwig*

S17-5 Effects of Oxytocin on the Social Brain in Asperger Autism
*Sabine C. Herpertz, Ekkehardt Kumbier, Gregor Domes*

S17-6 Genetic and systems-level mechanisms of social interactions in humans
*Andreas Meyer-Lindenberg*

# Evolutionary Convergence and Divergence in the Nonapeptide Mechanisms of Grouping and Monogamy

James L. Goodson[1]

[1]Indiana University, Department of Biology, 1001 East Third St., 47405 Bloomington, IN, USA

As evidenced by their phylogenetic distributions, components of social organization change vary rapidly over evolutionary time, and thus behavioral variables such as mating system and grouping ("sociality") are prone to repeated divergence and convergence. Given this, plus the complexity of relevant neural mechanisms, we cannot assume that convergence in social structure has been produced by convergent modifications to the same neural characters. However, using five estrildid finch species that differ selectively in their species-typical group sizes (all biparental and monogamous) we have demonstrated that neural motivational systems evolve in predictable ways in relation to sociality. These systems include nonapeptide circuits that encode social valence (positive-negative) and dopamine circuits that encode "incentive value" and drive appetitive social behaviors. Nonapeptide and dopamine systems exhibit functional and anatomical properties that are biased towards gregarious species, and experimental reductions of nonapeptide signaling by antisense oligonucleotides and receptor antagonism significantly decrease preferred group sizes in the gregarious zebra finch. Combined, these findings suggest that selection on species-typical group size may reliably target the same neural motivation systems when a given social structure evolves independently. Selection for monogamy also presents an important topic for mechanistic studies, given that the neural regulation of pair bonding has been outlined for only a single species, the prairie vole, in which endogenous vasopressin promotes bonding in males and endogenous oxytocin promotes pair bonding in females. In order to see whether similar mechanisms may have evolved in monogamous finches, we recently conducted two experiments in which zebra finches were observed twice daily for the first three days after colony formation while we twice daily infused either a vasopressin V1a antagonist (males only), an oxytocin receptor antagonist (both sexes), or vehicle into the lateral ventricle. Colony observations allow the scoring of bonding in a naturalistic group environment and the quantification of more than 20 other behaviors. Although males receiving a V1a antagonist showed no impairment in mate acquisition, birds of both sexes showed a dramatic and selective impairment in bonding when treated with the oxytocin antagonist. Thus, although nonapeptide mechanisms of bonding vary somewhat between voles and finches, the similarities are still quite striking and suggest that, as with grouping, evolution in mating system is reliably associated with functional modifications to nonapeptide sytems.

# Link between complex social behaviours and anxiety: Involvement of the pro-social neuropeptides oxytocin and vasopressin

Inga D Neumann[1]

University of Regensburg, Dept of Neurobiology, 93053 Regensburg, Germany

Brain neuropeptides represent viable novel research candidates for the development of effective treatment strategies of affective and stress -related disorders, which are often accompanied by social dys-functions. The neuropeptides oxytocin and vasopressin are significantly involved in the regulation of complex social behaviours such as maternal behaviour, mother-child bonding, pair-bonding, social recognition, but also of fine-tuned emotional and neuroendocrine stress responsiveness1. These diverse functions may explain the link between altered emotionality (anxiety, aggression) and differences in social behaviours/social dys-regulation often seen in psychiatric patients.

Here, I will present data demonstrating differences in social behaviours, such as maternal care and aggression, intermale aggression and social preference, of rats with extremes in innate anxiety, i.e. in high (HAB) and low (LAB) anxiety-related behaviour rats 1 . Altered gene activity, local release patterns within limbic or hypothalamic target regions or respective receptor binding of oxytocin and/or vasopressin may help to explain the behavioural differences. In support, early life stress (maternal separation) induces not only elevated levels of anxiety- and depression-related behaviours, but also changes in social behaviours such as intermale aggression2 or social recognition3 providing further evidence for the link between emotionality and social behaviours accompanied by significant alterations in release patterns of brain vasopressin..

With respect to the neuronal actions of oxytocin, we could recently show that its acute anxiolytic effects within the hypothalamic paraventricular nucleus are mediated via the extracellular signal -regulated kinase 1/2 (ERK1/2) cascade; local blockade of this signaling pathway prevented the local oxytocin-induced anxiolysis2. Our findings support the hypothesis that the capacity of oxytocin and vasopressin to modulate complex social behaviours is likely to be directly or indirectly linked to the regulation of fear, anxiety and hormonal stress responses.

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# The monogamous male brain - neurochemical regulation of social bonding

Zuoxin Wang[1]

[1]Florida State University, Psychology/Neuroscience, 1107 West Call Street, FL 32306 Tallahassee, USA

Prairie voles (Microtus ochrogaster) are socially monogamous rodents that form pair bonds after mating. In an early study in male prairie voles, we found that activation of dopamine (DA) D2 receptors (D2R), but not D1 receptors (D1R), in the nucleus accumbens (NAcc) facilitated pair bond formation. Pair-bonded males showed a significant up- regulation in NAcc D1R and displayed aggression selectively towards unfamiliar conspecifics. Furthermore, blockade of D1R in the NAcc abolished this aggression. These data suggest that NAcc DA regulates pair bonding in a receptor- and behavior-specific manner. In a recent study, we found that amphetamine (AMPH) is rewarding to prairie voles, as a 3- day AMPH conditioning paradigm induced conditioned place preference (CPP). This behavior was mediated by NAcc D1R. We also demonstrated that AMPH conditioning, at the doses effective to induce CPP, inhibited mating-induced pair bonding and enhanced D1R, but not D2R, in the NAcc. In addition, blockade of NAcc D1R rescued mating -induced pair bonding in AMPH -treated male voles. Together, our data indicate that NAcc DA mediates social bonding and AMPH reward in a receptor-specific manner, and repeated AMPH exposure results in the impairment of pair bonding via a D1R-mediated mechanism in the NAcc. (Supported by NIH grants DAR01-19627, DAK02-23048 and MHR01-58616).

# Processing of social cues in the olfactory bulb by vasopressin

Mike Ludwig[1]

[1]University of Edinburgh, Centre for Integrative Physiology, George Square, EH89XD Edinburgh, United Kingdom

The neuropeptide vasopressin is now known to be released in the brain where it plays important roles in social behaviours. We have shown that the rat olfactory bulb (OB) and the anterior olfactory nucleus (AON) contain large populations of interneurones which express and release vasopressin. In the OB, single cell recordings from mitral cells in vivo showed that vasopressin modulates the processing of information by olfactory bulb neurones. Blocking the actions of vasopressin in the OB impairs the social recognition abilities of rats. The treatments impaired habituation/dishabituation to juvenile cues, but not to volatile odours or object recognition, and did not affect locomotor activity or anxiety-related behaviours. Adult rats exposed to a conspecific juvenile showed increased Egr-1 expression in vasopressin neurones in multiple subdivisions of AON as compared to animals exposed to no odour or a non-social odour. These data suggest that vasopressin neurones in the AON may also play an important role in the coding of social odour information. The findings indicate that the vasopressin process olfactory signals relevant to social discrimination and vasopressin release may be involved in filtering out familiar signals.

# Effects of Oxytocin on the Social Brain in Asperger Autism

Sabine C. Herpertz[1], Ekkehardt Kumbier[2], Gregor Domes[3]

[1]University Hospital, Department of Psychiatry, Voss Str. 4, 69115 Heidelberg, Germany
2 Department of Psychiatry and Psychotherape; University of Rostock, Germany
3 Department of Biological and Differential Psychology; University of Freiburg, Germany

The neuropeptide oxytocin has recently been shown to enhance eye gaze and emotion recognition in healthy men. Autism spectrum disorder (ASD) has been associated with distinct impairments in eye gaze and facial emotion recognition, as well as with alterations of central nervous oxytocin. In 14 male patients with Asperger Syndrome, who had to process faces compared to objects in a face identity matching task, oxytocin upregulated the activity of the amygdalae together with the temporo-parietal junction (TPJ) while in healthy controls amygdalae activity decreased after intranasal application of oxytocin. The TPJ region was also one of the regions oxytocin exerted an enhancing effect on in an emotion recognition task in subjects with Asperger syndrome when inferring emotions from the eye but not the mouth region. Oxytocin-induced increased activity during the processing of eye stimuli was also found in the anterior insula, cuneus/precuneus and rostral anterior cingulate cortex and therefore in structures known to be involved in social cognition. On the cognitive level, oxytocin enhanced emotion recognition as indicated by the hit-rate specifically from the eye region of emotional faces. In conclusion, a model is introduced which claims that oxytocin is a prosocial hormone optimizing arousal in the social context and facilitating facial expression recognition selectively from the eye region, at least in Asperger-Syndrome. The cognitive effects of oxytocin might be mediated by specific alterations in regional brain activity in areas previously shown to be involved in attention and the processing of social stimuli.

# Genetic and systems-level mechanisms of social interactions in humans

Andreas Meyer-Lindenberg[1]

[1]Central Institute of Mental Health, Psychiatry and Psychotherapy, J 5, 68159 Mannheim, a.meyer-lindenberg@zi-mannheim.de

Well-being and survival in primates, including humans, depends critically on social interactions (1), and disturbed social behavior is a key component of diseases such as autism, schizophrenia, and anxiety disorders (2). Social cognitive neuroscience, a new research field, has started to delineate neural systems for social information processing (2, 3); however, little is known about specific neurobiological factors shaping the human social brain. Since many aspects of social function are highly heritable (4), we have adopted a genetic approach to identify molecular and systems-level mechanisms of social cognition in humans. We discuss work in the rare hypersocial genetic condition, Williams-Beuren-Syndrome, and common genetic variants linked to psychiatric disease that impact on regulatory circuitry of the extended limbic system by prefrontal cortex. We then turn to the prosocial neuropeptides, oxytocin and vasopressin, key effectors of social behavior. In acute administration, Oxytocin potently reduces amygdala activation and decreased coupling to brainstem regions implicated in autonomic and behavioral manifestations of fear (5). Vasopressin, given acutely, specifically reduces differential activation in the subgenual cingulate cortex (6). A previously evaluated circuit between amygdala, subgenual cingulate, and supragenual cingulate revealed altered effective connectivity between subgenual and supragenual cingulate under vasopressin. Genetic variants in the brain receptors for the neuropeptides have been linked to human social behaviors and traits, as well as to the severe disorder disrupting social function, autism. Two microsatellite polymorphisms, RS1 and RS3, near the promoter of *AVPR1A*, predict differential activation of amygdala in carriers of risk alleles (7). Furthermore, we show functional difference in human brain between short and long repeat lengths that mirror findings recently obtained in a corresponding variant in voles. In the oxytocin receptor gene OXTR, a common variant (rs53576) was linked to activation and interregional coupling of the amygdala during the processing of emotionally salient social and structural alterations in key oxytocinergic regions, particularly in the hypothalamus. These neural characteristics predicted lower levels of reward dependence, specifically in male risk allele carriers. Our results begin to delineate mechanisms of genetic risk on the neural systems level that can be mined for new treatments (8).

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# Symposium

## S18: ALS, Huntington's disease and Parkinson's disease: From molecular pathogenesis to target validation in aggregopathies

S18-1 Modification of alpha-synuclein oligomerization in living cells
*Tiago Fleming Outeiro*

S18-2 Mechanisms of alpha-synuclein mediated neurotoxicity
*Markus Zweckstetter*

S18-3 MICROGLIOSIS IN THE ANTERIOR OLFACTORY NUCLEUS OF PARKINSON AND ALZHEIMER PATIENTS
*Anne-Marie van Dam, A Goudriaan, C Blits-Huizinga, W van de Berg, P Hoogland, B Drukarch*

S18-4 BAG1 modulates detoxification of disease-specific proteins in neurodegeneration
*Pawel Kermer*

S18-5 SUMO wrestles with a-synuclein: An endogenous regulator of aggregation and toxicity
*Jochen Weishaupt*

# Modification of alpha-synuclein oligomerization in living cells

Tiago Fleming Outeiro[1,2]

[1]University of Gottingen, CMPB, Department of Neurodegeneration and Neurorestoration, Waldweg 33, 37073 Gottingen , Germany[2]Cell and Molecular Neuroscience Unit, Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisboa, Portugal

The accumulation of misfolded proteins is a common event in several neurodegenerative disorders (ND). These altered proteins enable aberrant protein-protein interactions, formation of protein aggregates, and the disruption of several essential cellular functions.
The misfolding and aggregation of alpha-synuclein (a-syn) is the pathological hallmark of both sporadic and familial Parkinson's disease (PD), the second most common age related ND. Recently, it has been postulated that the precursor oligomeric species of a-syn represents the toxic genus, rather than the complex aggregated forms of the protein.
A complete understanding of a-syn misfolding, protein-protein interactions and aggregation is essential for the development of novel therapeutic strategies in PD. We have been investigating the molecular mechanisms underlying the initial steps involved in a-syn oligomerization. Specifically, we established a system to monitor a-synuclein dimerization/oligomerization in living cells, using the novel bimolecular fluorescence complementation (BiFC) assay. Using this approach as a readout of a-syn dimerization/oligomerization, we started to identify and characterize genetic modifiers of a-syn oligomeric precursors, through an unbiased genome-wide lentiviral RNAi screen for human genes.
Among the hits we identified genes involved in intracellular transport/trafficking, kinases, phosphatases, chaperones, and genes involved in cell division.
The identification of genetic modifiers of a-syn oligomerization will enable the development of novel therapeutic strategies for PD and related diseases.

# Mechanisms of alpha-synuclein mediated neurotoxicity

Markus Zweckstetter[1]

[1]Max Planck Institute for Biophysical Chemistry, Department for NMR-based Structural Biology, Am Fassberg 11, 37077 Göttingen, Germany

The relation of alpha-synuclein (alphaS) aggregation to Parkinson's disease (PD) has long been recognized, but the mechanism of toxicity, the pathogenic species and its molecular properties are yet to be identified. To obtain insight into the function different aggregated alphaS species have in neurotoxicity in vivo, we generated alphaS variants by a structure-based rational design (1). Biophysical analysis revealed that the alphaS mutants have a reduced fibrillization propensity, but form increased amounts of soluble oligomers. To assess their biological response in vivo, we studied the effects of the biophysically defined pre-fibrillar alphaS mutants after expression in tissue culture cells, in mammalian neurons and in PD model organisms, such as Caenorhabditis elegans and Drosophila melanogaster. The results show a striking correlation between alphaS aggregates with impaired beta-structure, neuronal toxicity and behavioural defects, and they establish a tight link between the biophysical properties of multimeric alphaS species and their in vivo function (2). In addition, to correlate these findings with the native protein, we developed a biophysical method for production of on- pathway oligomers of wild-type alphaS at concentrations an order of magnitude higher than previously possible. Taking advantage of the achieved high concentrations, we showed that on-pathway oligomers of alphaS form ion channels with well-defined conductance states in a variety of membranes and their structure differs from that of amyloid fibrils of alphaS (3).

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# MICROGLIOSIS IN THE ANTERIOR OLFACTORY NUCLEUS OF PARKINSON AND ALZHEIMER PATIENTS

Anne-Marie van Dam[1], A Goudriaan[1], C Blits-Huizinga[1], W van de Berg[1], P Hoogland[1], B Drukarch[1]

VU University Medical Center, Department of Anatomy and Neurosciences, Van der Boechorststraat 7, 1081 BT Amsterdam, Netherlands[1]

Recently, the anterior olfactory nucleus (AON) in the olfactory bulb (OB) has emerged as an early affected region in Parkinson's disease (PD) and Alzheimer's disease (AD) patients. This is based on the early, local presence of disease-specific protein aggregates, and the loss of olfaction in an early (pre)clinical stage. Previous studies showed that microglial cells are activated in the, relatively late affected, nigro-striatal system, and found to be associated with local loss of dopaminergic neurons.

In the present study, we questioned whether microglial cells are activated in the AON of PD and AD patients and coincide with neuronal loss, and/or colocalize with pathological proteins as an indicator of phagocytic activity. By using human post-mortem material, we observed a significant increase in the number of activated microglial (CD68 positive) cells in the AON of both patient groups compared to control subjects. These activated microglial cells did not colocalize with ß-amyloid, hyperphosphorylated tau or a-synuclein, suggesting lack of phagocytosis of pathological proteins by microglia. Moreover, astrocytes neither colocalized with these pathological proteins and thus no obvious glial phagocytic redundancy occurred. Furthermore, although no clear neuronal loss (Nissl staining) was found, changes in the neuronal network as observed by Bodian staining were present, most clearly in the AON of AD patients.

We put forward that microgliosis in the olfactory bulb of PD and AD patients may be an indicator of neuronal dysfunction rather than just a reflection of neuronal cell death.

# BAG1 modulates detoxification of disease-specific proteins in neurodegeneration

Pawel Kermer[1]

[1]Universitätsmedizin Göttingen, Neurology, Robert-Koch-Str. 40, 37075 Göttingen, Germany

BAG1 (Bcl -2-associated athanogene -1) is a multifunctional protein being essential for neuronal survival and differentiation. Here, we present data indicating that BAG1 acts as a co-chaperone linking cellular detoxification mechanisms like the chaperone machinery and the ubiquitin-proteasome system (UPS) which both play a crucial role in the pathology of neurodegenerative diseases. Our results illustrate that BAG1 bears therapeutic potential for neurodegenerative diseases associated with protein misfolding like Huntington´s and Parkinson´s disease (PD). We show that BAG1 modulates toxicity, aggregation, degradation and subcellular distribution in vitro and in vivo of the disease-specific mutant huntingtin protein. These effects are dependent on the integrity of the C-terminal BAG domain. Furthermore, we present data regarding the DJ-1 protein which is responsible for an early-onset autosomal-recessively inherited form of PD. Our results show that BAG1 restores formation of functional DJ-1 L166P dimers and DJ-1 chaperone activity.

# SUMO wrestles with a-synuclein: An endogenous regulator of aggregation and toxicity

Jochen Weishaupt[1]

[1]University of Göttingen, Neurology, Robert-Koch-Str. 40, 37073 Göttingen, Germany

Posttranslational modification by SUMO proteins contributes to numerous cellular pathways. While sumoylation has been described to create and abolish protein binding interfaces, increasing evidence points to a role of SUMO in regulating protein solubility. Alpha-Synuclein plays a key role in the pathogenesis of Parkinson's disease (PD). This is underscored by gene duplications and three missense mutations in familiar forms of PD as well as genome-wide SNP association studies. Although the molecular mechanisms regulating its toxicity remain largely elusive, alpha-synuclein aggregation and subsequent enrichment in inclusion bodies is one of the hallmarks of several neurodegenerative diseases including PD. We characterized alpha-synuclein SUMOylation in vitro, in cell culture and in mouse brain, and show that SUMO conjugation blocks alpha-synuclein fibrillation. Even a small portion of SUMOylated alpha-synuclein is sufficient to significantly delay fibril formation. Conversely, impaired SUMOylation of alpha-synuclein leads to increased aggregation and toxicity in cell culture as well as in an in vivo model for Parkinson's disease. Our findings thus validate alpha-synuclein fibrillation as a critical step in alpha-synuclein toxicity. The data furthermore suggest a critical role for SUMO in keeping an aggregation prone protein soluble and implicate SUMOylation as a modulator of neurodegenerative disease pathology.

# Symposium

## S19: Neural cell adhesion molecule NCAM and its post-translational modifications at the crossroad of signalling pathways and neural functions

S19-1 Functional roles of the interaction between the neural cell adhesion molecule NCAM and calmodulin in NCAM signal transduction and in nuclear import of a transmembrane NCAM fragment
*Ralf Kleene, Mounir Mzoughi, Gunjan Joshi, Ina Kalus, Ulrich Bormann, Christian Schulze, Mei-Fang Xiao, Alexander Dityatev, Melitta Schachner*

S19-2 Fibroblast growth factor -regulated palmitoylation of the neural cell adhesion molecule and neuronal morphogenesis
*Evgeni G. Ponimaskin*

S19-3 NCAM function in the development of neural stem cells in vivo
*Simone Diestel*

S19-4 Pathological brain development of mice deficient in NCAM polysialylation
*Herbert Hildebrandt*

S19-5 NCAM-associated polysialic acid regulates synaptic plasticity and learning by restraining the signaling through GluN2B-containing NMDA receptors
*Alexander Dityatev*

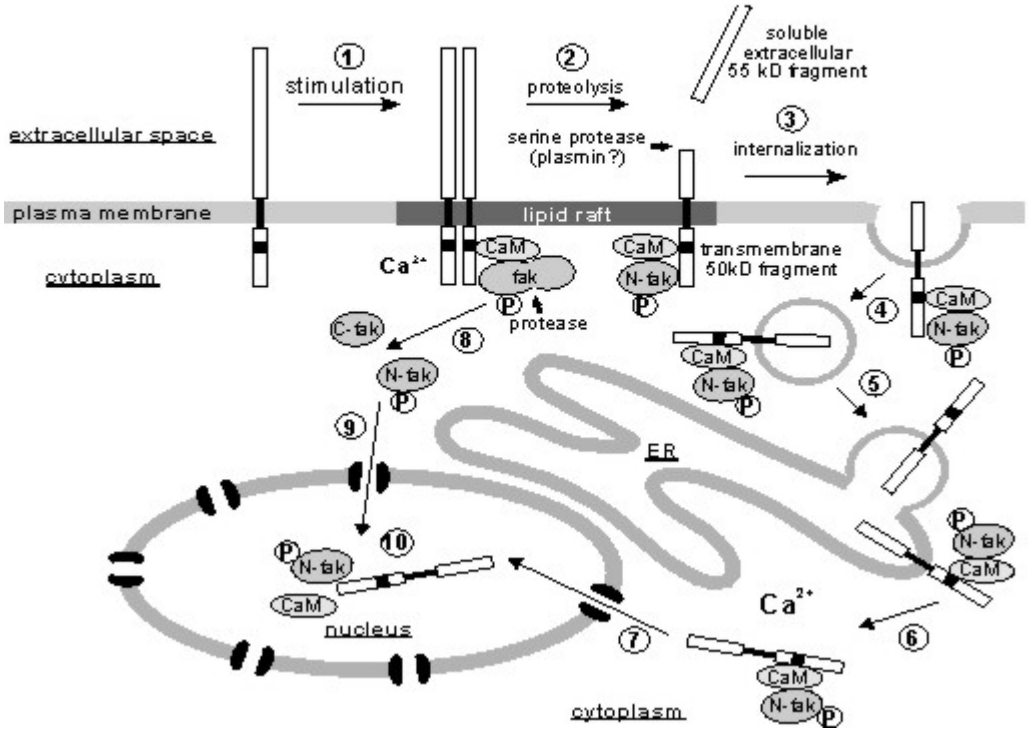
# Functional roles of the interaction between the neural cell adhesion molecule NCAM and calmodulin in NCAM signal transduction and in nuclear import of a transmembrane NCAM fragment

Ralf Kleene[1], Mounir Mzoughi[1], Gunjan Joshi[1], Ina Kalus[1], Ulrich Bormann[1], Christian Schulze[1], Mei-Fang Xiao[1], Alexander Dityatev[1], Melitta Schachner[1]

[1]Universitätsklinikum Hamburg-Eppendorf, Zentrum für Molekulare Neurobiologie Hamburg (ZMNH), Martinistrasse 52, 20246 Hamburg, Germany

The neural cell adhesion molecule NCAM plays important functional roles not only during nervous system development, but also in the adult after injury and in synaptic plasticity. Homophilic binding of NCAM triggers intracellular signaling events resulting in cellular responses such as neurite outgrowth that require NCAM palmitoylation-dependent raft localization, and activation of the non-receptor tyrosine kinases fyn and fak. We showed that stimulation of NCAM by a function-triggering NCAM antibody resulted in proteolytic processing of NCAM and fak. A 50 kDa C-terminal fragment of NCAM consisting of the intracellular domain, the transmembrane domain and a stub of the extracellular domain as well as a N-terminal fragment of fak are imported into the nucleus. NCAM-stimulated fak activation, generation and nuclear import of the NCAM fragment and the fak fragments as well as neurite outgrowth are abolished by mutation of an inverted 1-5-8-14 and an overlapping inverted 1-5-10 calmodulin binding motif in the intracellular domain of NCAM that is responsible for the calcium-dependent binding of calmodulin to NCAM. This mutation neither interferes with NCAM cell surface expression, palmitoylation and raft localization nor with fyn activation. The way by which the transmembrane NCAM fragment reaches the nucleus in a calmodulin- and calcium-dependent manner is by endocytotic transport via the endoplasmic reticulum and the cytoplasm. The generation and nuclear import of NCAM and phosphorylated fak fragments resulting from NCAM stimulation may represent a signal pathway activating cellular responses in parallel or in association with classical kinase- and phosphorylation-dependent signaling cascades.

We propose the following working model for the trafficking of NCAM and fak fragments to the nucleus. NCAM-stimulation leads to a recruitment of NCAM to lipid rafts, an increase in intracellular calcium levels, di-/olgomerization of NCAM at the cell surface, interaction with calmodulin and phosphorylation, activation and proteolytic cleavage of fak (step 1). Extracellular cleavage of NCAM by a serine protease, probably plasmin, results in the generation of a soluble 55 kD fragment comprising part of the extracellular domain and a membrane-bound 50 kD fragment containing the intracellular and transmembrane domains and part of the extracellular domain (step 2). The transmembrane 50 kD fragment, probably in association with calmodulin and/or the N-terminal fak fragment, is internalized by endocytosis (step 3) translocated to endosomes (step 4) and translocated to the ER (step 5). By unknown mechanisms the fragment is translocated from the ER membrane to the cytoplasm (step 6) and then imported through nuclear pores, probably in association with calmodulin, into the nucleus in a calcium and calmodulin-dependent manner (step 7). The proteolytic processing of fak generates a C-terminal fragment and phosphorylated N-terminal fragment (step 8) which is either transported into the nucleus through nuclear pores by an importin-dependent pathway (step 9) or in association with the NCAM fragment (step 7). In the nucleus, the NCAM fragment may associate with other nuclear proteins, such as transcription factors involved in gene regulation, possibly in association with the fak fragment (step 10).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protease
transmembrane
50kD fragment
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cytoplasm

# Fibroblast growth factor-regulated palmitoylation of the neural cell adhesion molecule and neuronal morphogenesis

Evgeni G. Ponimaskin[1]

[1]Medical School Hannover, Neurophysiology, Carl-Neuberg-Str. 1, 30627 Hannover, Germany

During development of the nervous system, short- and long-range signals cooperate to promote axonal growth, guidance, and target innervation. Particularly, a short-range signal transducer, the neural cell adhesion molecule (NCAM), stimulates neurite outgrowth via mechanisms that require posttranslational modification of NCAM and signaling via receptors to a long-range messenger, the fibroblast growth factor (FGF). In the present study we further characterized a mechanism which regulates the functional interplay between NCAM and FGF receptor(s). We show that activation of FGF receptor(s) by FGF2 leads to palmitoylation of the two major transmembrane NCAM isoforms, NCAM140 and NCAM180, translocation of NCAM to GM1 ganglioside-containing lipid rafts, and stimulation of neurite outgrowth of hippocampal neurons. Ablation of NCAM, mutation of NCAM140 or NCAM180 palmitoylation sites, or pharmacological suppression of NCAM signaling inhibited FGF2-stimulated neurite outgrowth. Of the 23 members of the aspartate-histidine-histidine-cysteine (DHHC) domain containing proteins, DHHC -7 most strongly stimulated palmitoylation of NCAM, and enzyme activity was enhanced by FGF2. Thus, our study uncovers a molecular mechanism by which a growth factor regulates neuronal morphogenesis via activation of palmitoylation, which in turn modifies subcellular location and thus signaling via an adhesion molecule.

# NCAM function in the development of neural stem cells in vivo

Simone Diestel[1]

[1]University of Bonn, Institute of Animal Sciences, Department of Biochemistry, Katzenburgweg 9a, 53115 Bonn, Germany

The neural cell adhesion molecule NCAM has important functions during neural development and in the adult brain, which in many cases have been ascribed to its unique polysialic acid (PSA) modification. We developed a novel approach of in vivo electroporation of early postnatal mouse brain. Using this method we show that NCAM expression interferes in vivo with the maintenance of forebrain neuronal stem cells. We further determined the fate of cells generated from NCAM -overexpressing stem cells in postnatal mouse brain and elucidated the functional domains of NCAM mediating this effect. Ectopic expression of the NCAM140 isoform in radial glia and type C cells induces an increase in cell proliferation and consequently the presence of additional neuronal type A cells in the rostral migratory stream. A mutant NCAM protein comprising only fibronectin type III repeats and immunoglobulin-like domain 5 was sufficient to induce this effect. Furthermore, the neurogenic effect is independent of PSA, as transgenic NCAM is not polysialylated in radial glia and type C cells. These results suggest that heterophilic interactions of NCAM with other components of the cell membrane must be involved.

# Pathological brain development of mice deficient in NCAM polysialylation

Herbert Hildebrandt[1]

[1]Hannover Medical School, Cellular Chemistry (OE 4330), Carl-Neuberg-Str. 1, 30625 Hannover, Germany

The neural cell adhesion molecule NCAM is modified with the unique carbohydrate polysialic acid, which is added to NCAM by two polysialyltransferase enzymes. Abnormal levels of NCAM or polySia as well as polymorphisms in NCAM and one of the polysialyltransferase genes have been related to schizophrenia. The postnatally lethal phenotype of mice lacking both polysialyltransferases reveals the vital role of this sugar modification. In my presentation, I will highlight several aspects of defective brain development in these mice, such as hypoplasia of major axon tracts and altered densities of GABAergic neuron populations. The striking parallels to structural brain pathology in schizophrenia will be discussed.

# NCAM-associated polysialic acid regulates synaptic plasticity and learning by restraining the signaling through GluN2B-containing NMDA receptors

Alexander Dityatev[1]

[1]Fondazione Istituto Italiano di Tecnologia, Department of Neuroscience and Brain Technologies, via Morego 30, 16163 Genova, Italy

Neurotransmitter receptors as well as cell adhesion molecules are important for maintenance and plasticity of synaptic function. The ways by which they may cooperate with each other have remained largely elusive. The neural cell adhesion molecule NCAM and its unusual carbohydrate, alpha2,8 linked polysialic acid (PSA) play important functional roles in formation of synapses during ontogenesis and regulate synaptic efficacy in the adult. Abnormalities in PSA and NCAM expression are associated with schizophrenia in humans and cause deficits in hippocampal synaptic plasticity and contextual fear conditioning in mice. Our data revealed that PSA inhibits opening of native NR2B -containing NMDA receptors at low concentrations of glutamate. PSA also inhibits recombinant NMDA receptors (NRs) composed of NR1/NR2B or NR1/NR2A/NR2B subunits. In hippocampal slices, deficits in NCAM/PSA increase NR2B -mediated transmission and Ca2+ transients at extrasynaptic sites, and impair long-term potentiation (LTP) in the CA3-CA1 synapses. LTP in NCAM or PSA deficient slices is rescued by suppressing the activity of NR2B -NRs with Ro25 -6981 and chelation of extrasynaptic glutamate concentration with a glutamate scavenger, glutamic-pyruvic transaminase. Intrahippocampal injection of Ro25-6981 before fear conditioning rescues contextual fear memories in NCAM deficient mice. Ablation of Ras-GRF1, a mediator of NR2B signaling to p38 MAPK, or inhibition of hyperactive p38 MAPK also restore impaired LTP in PSA deficient slices (Kochlamazashvili et al., J. Neuroscience, 2010). These findings for the first time implicate carbohydrates carried by adhesion molecules in modulating extrasynaptic signaling in the brain and demonstrate reversibility of cognitive deficits associated with ablation of a schizophrenia-related cell adhesion molecule.

# Symposium

## S20: Cellular actions of neuropeptides and biogenic amines in invertebrates

S20-1 Insect neuropeptides and their receptors – a comparative genomics approach
*Frank Hauser, Cornelis J. P. Grimmelikhuijzen*

S20-2 The neurohormone serotonin regulates plasma membrane V-ATPase activity in the blowfly
*Otto Baumann, Martin Voss, Julia Rein, Claudia Röser, Kristoffer Heindorff, Wolfgang Blenau, Bernd Walz*

S20-3 Cellular polarity of peptidergic neurons and possible implications for the organisation of peptidergic signalling networks
*Christian Wegener, Gergely Karsai, Mareike Selcho, Ronja Hensgen, László Molnár, Edit Pollák*

S20-4 Exploring peptide signaling involved in honey bee foraging behavior
*Axel Brockmann*

S20-5 Monoamines and neuropeptides interact to inhibit nociceptive behavior in *Caenorhabditis elegans*
*Richard Walter Komuniecki*

S20-6 Neurochemical control of the decision to fight or flee in crickets
*Paul Anthony Stevenson*

# Insect neuropeptides and their receptors – a comparative genomics approach

Frank Hauser[1], Cornelis J. P. Grimmelikhuijzen[1]

[1]University Copenhagen, Biology, Universitetsparken 15, 2100 Copenhagen, Denmark

Using bioinformatics tools, we screened various sequenced insect genomes for the presence of neuropeptide and protein hormones genes. Using this approach, we recently identified 30 neuropeptide or protein hormone precursor genes in the parasitic wasp *Nasonia vitripennis*. Compared to *Nasonia*, there are more neuropeptide genes present in *Drosophila melanogaster*, *Aedes aegypti* (both Diptera), *Bombyx mori* (Lepidoptera), *Tribolium castaneum* (Coeloptera), *Apis mellifera* (another Hymenoptera) and *Acyrthosiphon pisum* (Hemiptera). The sets of neuropeptide precursor genes are remarkably different in all these insects. They can be subdivided into a basal set of 20 precursor genes that have orthologues in all these species, and a variable set of genes that are only present in some insects, but can readily get lost in others. One of the identified *Nasonia* precursor genes was completely novel, encoding neuropeptides containing the C-terminal sequence RYamide. Interestingly, we could find orthologues of this RYamide gene in nearly all arthropods with sequenced genomes, and its expression could be confirmed by mass spectrometry in the terminal ganglion of mosquitoes. In parallel to this neuropeptide identification, we also annotated the putative G protein-coupled receptors for neuropeptides and biogenic amines in various insect genomes. The common ligand/receptor co-evolution will be discussed and an update of deorphanized receptors will be presented.

# The neurohormone serotonin regulates plasma membrane V-ATPase activity in the blowfly

Otto Baumann[1], Martin Voss[1], Julia Rein[1], Claudia Röser[1], Kristoffer Heindorff[1], Wolfgang Blenau[1], Bernd Walz[1]

[1]Universität Potsdam, Institut für Biochemie und Biologie, Karl-Liebknecht-Str. 24/25, 14476 Potsdam, Germany[1]

Vacuolar-type proton pumps (V-ATPases) are multisubunit heteromeric complexes composed of a membrane-bound, $H^+$-translocating $V_0$ domain and a cytosolic, ATP-hydrolyzing $V_1$ domain. V-ATPases are located in endomembrane systems and in the plasma membrane of eukaryotic cells. V-ATPases fulfill a variety of functions, such as intracellular pH homeostasis, extracellular acidification, activation of acid hydrolases in acidic organelles, and loading of synaptic vesicles with neurotransmitter molecules. Since V-ATPases may consume a considerable amount of energy in some cell types, their activity can be adjusted to the temporary needs and to extracellular signals. Using salivary glands of the blowfly *Calliphora vicina* as a model, we examine the poorly characterized intracellular signalling cascade(s) linking serotonin receptors to V-ATPase activation.

Salivation in blowflies is regulated by serotonin (5-hydroxytryptamine, 5-HT) released by the thoracic ganglion and acting as a neurohormone. 5-HT binding to its receptors on the secretory activates the $InsP_3/Ca^{2+}$ and the cAMP/PKA signalling pathways. We have identified the latter pathway to be absolutely required for 5-HT-dependent regulation of V-ATPase. PKA activity leads to phosphorylation of V-ATPase subunit C, and this event may serve as a trigger for the reversible assembly of inactive $V_O$ and $V_1$ domains to functional V-ATPase holoenzymes. Our recent studies demonstrate further that the 5-HT-induced intracellular $Ca^{2+}$ increase modulates the cAMP/PKA signalling pathway and, thus, V-ATPase activity. We suggest that crosstalk between both signalling pathways occurs at the level of the adenylyl cyclase.

# Cellular polarity of peptidergic neurons and possible implications for the organisation of peptidergic signalling networks

Christian Wegener[1], Gergely Karsai[2], Mareike Selcho[1], Ronja Hensgen[1], László Molnár[2], Edit Pollák[2]

[1]Philipps-Universität Marburg, FB Biologie, Tierphysiologie-Neurobiologie, Karl-von-Frisch-Strasse 8, 35032 Marburg, Germany
[2]Department of General Zoology, University of Pécs, Hungary

Determination of neuronal polarity - i.e. the identification of in- and output regions- represents a considerable difficulty in the morphological analysis of modulatory neurons and networks. This difficulty is especially relevant for peptidergic interneurons of invertebrates. What may seem a specialist problem is in fact relevant to the current activities in morphological dissection of neuronal circuitry on the identified single cell level in various insect models.

We here report on our systematic efforts to spatially map peptidergic neurons and to characterise their in- and output sites on the light- and electron microscopic level in the fruitfly *Drosophila*. Molecular tools available for the fruitfly allow to express fluorescent pre- and postsynaptic marker proteins, and enable us to immunostain peptide vesicles in large populations of peptidergic neuron by ectopic expression of marker peptides. Our study focusses on *dimmed*-positive LEAP neurons (Large cells that display episodic release of amidated peptides) and CCAP neurons involved in control of ecdysis behaviour. Our results suggest that peptidergic neurons can be classified into different

# Exploring peptide signaling involved in honey bee foraging behavior

Axel Brockmann[1]

[1]Department of Entomology, University of Illinois at Urbana-Champaign, USA

The honey bee genome predicts approximately 100 peptides from 36 different prohormones, but the physiological and behavioral functions of most of these peptides are unknown (Hummon et al., 2006). To explore the influence of neuropeptides in behavioral maturation and social foraging, we adapted a quantitative peptidomics approach using differential isotope labeling for small brains (Brockmann et al., 2009). In our first study, we compared brain peptide abundances between arriving and departing nectar or pollen foragers. There were 6 peptides from 6 different prohormones [tachykinin-related peptides (TKRP-3), sNPF, PBAN (IYLPLFASRLamide), allatostatin C, FMRFamide-related peptides (extended FMRFa-1) and MVP-containing peptide] showing robust and dynamic regulation in the context of foraging. Some of these peptides appear to be associated with the decision whether to forage for pollen or nectar [e.g. sNPF (SPSLRLRFamide), TKRP-3], some appear to be associated with the direct regulation of food collection [e.g. TKRP-3, PBAN (IYLPLFASRLamide)]. In our second study, we analyzed bees engaged in dance language communication. Comparing peptide abundances in dancing and non-dancing returning foragers, we found 4 peptides from 3 prohormones [TKRP-3, TKRP-6, allatostatin (AST-5), and ITG] showing significant differences. To be able to efficiently study the effects of selected neuropeptides on behavior, we are developing a quantitative sugar-elicited search behavior in the laboratory with automated movement analysis. We intend to use a combination of quantitative peptidomics and manipulative behavioral experiments under laboratory and natural conditions to learn more about neuropeptide regulation of bee behavior.

Brockmann A, Annangudi SP, Richmond TA, Ament SA, Xie F, Southey BR, Rodriguez-Zas SR, Robinson GE, and Sweedler JV (2009) Quantitative peptidomics reveal brain peptide signatures of behavior. Proc Natl Acad Sci U S A. 2009 106:2383-8.

Hummon AB, Richmond TA, Verleyen P, Baggerman G, Huybrechts J, Ewing MA, Vierstraete E, Rodriguez-Zas SL, Schoofs L, Robinson GE, Sweedler JV (2006) From the genome to the proteome: uncovering peptides in the Apis brain. Science 314:647–649.

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# Monoamines and neuropeptides interact to inhibit nociceptive behavior in *Caenorhabditis elegans*

Richard Walter Komuniecki[1]

[1]University of Toledo, Biological Sciences, 2801 West Bancroft St., 43606-3390 Toledo, Ohio, USA

The modulation of pain is complex, but noradrenergic signaling promotes anti-nociception, with a2-adrenergic agonists used clinically. To better understand the adrenergic/peptidergic modulation of nociception, we examined the octopaminergic inhibition of aversive behavior initiated by the *C. elegans* nociceptive ASH sensory neurons. Octopamine (OA), the structurally–related invertebrate counterpart of noradrenaline, modulates sensory-mediated reversal through three a-adrenergic-like OA receptors. OCTR-1 and SER-3 antagonistically modulate ASH signaling directly, with OCTR-1 signaling mediated by Gao. In contrast, SER-6 inhibits aversive responses by stimulating the Gas -dependent release of an array of "inhibitory" neuropeptides from additional neurons that stimulate receptors on sensory neurons mediating attraction, suggesting that peptidergic signaling integrates both attractive and repulsive inputs to modulate locomotory transitions. These studies highlight the complexity of octopaminergic/peptidergic interactions and the similarities of this modulatory network to the noradrenergic inhibition of nociception in mammals, where norepinephrine suppresses chronic pain through inhibitory a2 adrenoreceptors on afferent nociceptors and stimulatory a1 receptors on inhibitory peptidergic interneurons.

# Neurochemical control of the decision to fight or flee in crickets

Paul Anthony Stevenson[1]

[1]University of Leipzig, Institute for Biology, Talstr. 33, 04103 Leipzig, Germany

Aggression is a behavioral strategy adapted to secure some limited resource at minimal cost. Accordingly, potential costs and benefits must be evaluated in order for the contestants to decide whether it is more opportune to fight or flee. Our experiments are revealing the neuronal mechanisms underlying this fundamental decision-making process in crickets. The decision to fight is promoted by numerous experiences, including in crickets, flying, winning a contest, contact to females and occupancy of a shelter. Pharmacological manipulations, including semi-selective amine depletion and treatments with amine -receptor agonists and antagonists, demonstrate that experience dependent enhancement of aggressive motivation is mediated by the biogenic amine octopamine. For the decision to flee, contestants are thought to assess agonistic signals exchanged during fighting. By manipulating agonistic signaling, we confirmed the cumulative assessment hypothesis for crickets, according to which animals assess only the opponent's actions, and flee when the accumulated sum exceeds some critical level. Since crickets persist longer in fighting when treated with drugs that block nitric oxide (NO) signaling, we speculate that the accumulation of sensory signals from the opponent activates the NO/cGMP pathway, which suppresses aggression and or promotes retreat. In a first step towards revealing the cellular mechanisms underlying the control of aggression, we characterized the population of fast-conducting giant descending interneurones (gDINs) that respond to mechanical stimulation of the antenna as it occurs to initiate - and during fighting. Two gDINs with contralateral descending axons were identified that receive direct supra -threshold inputs from antennal mechanoreceptors. Their morphologies are strikingly similar, except in the 3rd thoracic ganglion where one one has unilateral- and the other bilateral terminal projections. Interestingly, octopamine agonists dramatically enhance the responsiveness of the one gDIN to mechanical antennal stimulation, while diminishing that of the other. This system has potentially decision making properties. We speculate that differential modulation of gDINs may influence whether a cricket turns towards or away from a conspecific. Our data thus shows that the decision to fight or flee in crickets is at least partially controlled by the octopaminergic and NO/cGMP signaling pathways which mediate the influences of social and other experiences on aggression. Considering also work of others implicating additional amines (serotonin) and some peptides (opiates) in the decision to fight or flee, the neurochemical control of aggression in insects seems to have many parallels to that in mammals. *The contributions of colleagues and coauthors who performed much of the original work, and recent support by the DFG (FOR 1363, STE 714/4-1) is gratefully acknowledged.*

# Symposium

## S21: Optogenetics in neuroscience: From basic principles to applications

S21-1 Molecular properties and new developments of channelrhodopsins as optogenetic tools
*Ernst Bamberg, S. Kleinlogel, K. Feldbauer, P. Wood, U. Terpitz, R. Dempski, C. Bamann, M. Müller, W. Kühlbrandt*

S21-2 Optical control architecture for optogenetic neural stimulation
*Patrick Degenaar*

S21-3 Modifying neuronal connections with light
*Thomas G. Oertner*

S21-4 Optogenetic screen for synaptic vesicle recycling mutants and analysis of synaptic ultrastructure after optical hyperstimulation in *Caenorhabditis elegans*
*Alexander Gottschalk, Jan Hegermann, Martin Brauner, Jana Liewald, Christian Schultheis, Sebastian Wabnig, Jeffrey Stirman, Hang Lu, Stefan Eimer*

S21-5 An Optogenetic Toolbox Designed for Primates
*Ilka Diester, Matthew T Kaufman, Werapong Goo, Murtaza Mogri, Ramin Pashai, Ofer Yizhar, Charu Ramakrishnan, Karl A Deisseroth, Krishna V Shenoy*

S21-6 Channelrhodopsin-2 mediated optical stimulation of the Cochlea
*Victor Hugo Hernandez Gonzalez, Gerhard Hoch, Nicola Strenzke, Zhizi Jing1, Hideki Takago, Gerhard Vogt, Carolyn Garnham, Ernst Bamberg, Tobias Moser, George Augustine*

# Molecular properties and new developments of channelrhodopsins as optogenetic tools

Ernst Bamberg[1], S. Kleinlogel[1], K. Feldbauer[1], P. Wood[1], U. Terpitz[1], R. Dempski[2], C. Bamann[1], M. Müller[1], W. Kühlbrandt[1]

[1]Max Planck Institute of Biophysics, Max von Laue Str. 3, D-60438 Frankfurt am Main, Germany

[2]Life Sciences & Bioengineering Center – Worcester Polytechnic Institute, 100 Institute Road, Worcester, MA 01609-2280, USA

Channelrhodopsin-2 (ChR2) from *Chlamydomonas reinhardtii* with the seven transmembrane helix motif act as light-gated cation channel. ChR2 is now widely used as an optogenetic tool to control membrane excitability. The functional and structural description of ChR2 is given by electrophysiology, noise analysis for the determination of the unit conductance of the channel, and flash photolysis for the description of the photocycle, and 2D cryo electron microscopy.

Although the optogenetic toolbox is expanding continuously, several enticing possibilities still remain. Amongst them are microbial rhodopsin variants with 1) an intrinsic "bidirectional optical switch", to provide precise localized control of excitation and inhibition 2) expanded bandwidths of excitation and/or inhibition over the whole visible spectrum, 3) altered kinetics and 4) an increased ion permeability for $Ca^{2+}$. Despite being kinetically and spectrally almost identical to the wildtype, this variant exhibits an increased light-sensitivity by a factor of 70 and elicits potent depolarization in neurons. The mechanism of this effect is given on the basis of the enhanced Ca++ permeability.

# Optical control architecture for optogenetic neural stimulation

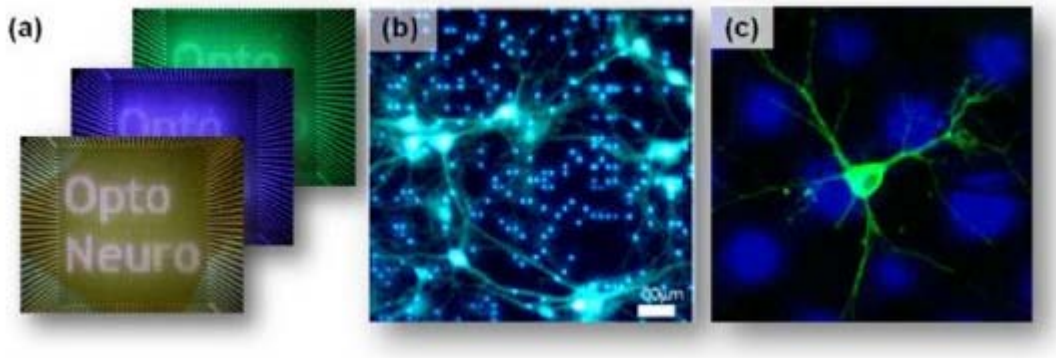
Patrick Degenaar[1]

[1]Newcastle University, School of Electrical, Electronic and Computer Engineering, Merz Court, , NE1 7RU Newcastle upon Tyne, United Kingdom

The recent emergence of optogenetic neural stimulation techniques have been revolutionizing the fields of electrophysiology and prosthetics. In this method, neurons are genetically modified to express light sensitive ion channels such as Channelrhodopsin -2 (ChR2). This allows a previously unattainable level of precise spatiotemporal control over neural activity. Significantly, it is possible to use the technique to inhibit as well as stimulate, and different neural cells and circuits can be genetically targeted to express ion channels of different chromatic sensitivity. This level of control allows for investigation of neurons and neural networks in ways not previously possible, and opens up possibilities for novel clinical applications such as retinal prosthesis. ChR2 is a light-sensitive cation channel with an action-spectrum peak at around ~460nm. It has been successfully expressed in numerous neuron types and its operation has been shown both in vitro and in vivo, including primates.

The main engineering challenge with optogenetic neural stimulation lies with its relatively high light requirement for stimulation. Typically 1mW/mm2 is required in instantaneous pulsed irradiance. This is much higher than can be provided by most standard spatial light modulation technologies such as liquid crystal displays. There is therefore great scope for the co -development of complementary optoelectronic stimulator technology. My Neurobionics group and collaboratorys first demonstrated the use of Gallium Nitride microLED arrays for stimulation of optically sensitized neurons in 2008. Since then, we have been further refining the technology towards our long term aim for its use in optogenetic retinal prosthesis.

This talk will cover the main issues surround engineering technologies for optogenetic neural stimulation. I will focus on our use of a micro-Light Emitting Diode Arrays as a powerful tool for complex spatiotemporal control of photosensitized neurons. The array can generate arbitrary, 2D, excitation patterns with millisecond and micrometer resolution. In particular, we describe an active matrix control address system to allow simultaneous control of 256 individual micro light emitting diodes. I will present the how the system can be optically integrated into a microscope environment and patch clamp electrophysiology. This talk will also discuss long term translation to prosthetic vision.



# Modifying neuronal connections with light

Thomas G. Oertner[1]

[1]Friedrich Miescher Institute for Biomedical Research, Maulbeerstr. 66, 4058 Basel, Switzerland

Long-term plasticity of synaptic connections is often referred to as a mechanism for memory storage in the brain. The time scales of memory, however, are orders of magnitude longer than the duration of electrophysiological recordings. It would be desirable to follow the fate and assess the function of individual synapses over several days in order to investigate the long-term stability of LTP and LTD.

We have combined optogenetic stimulation with two-photon imaging of genetically encoded calcium indicators in slice cultures of the hippocampus. This combination enables non-invasive optical recording from individual synapses, and we are now able to measure the amplitude and frequency of synaptically evoked spine calcium transients over several days. In my presentation, I will introduce improved Channelrhodopsin variants that are particularly suitable for optical plasticity experiments and explain strengths and limitations of current optogenetic technology.

# Optogenetic screen for synaptic vesicle recycling mutants and analysis of synaptic ultrastructure after optical hyperstimulation in *Caenorhabditis elegans*

Alexander Gottschalk[1,2], Jan Hegermann[3], Martin Brauner[1], Jana Liewald[1,2], Christian Schultheis[1,2], Sebastian Wabnig[1,2], Jeffrey Stirman[4], Hang Lu[4], Stefan Eimer[3]

[1]Goethe University Frankfurt, Department of Biochemistry, Chemistry and Pharmacy; Biocenter, Max von Laue Strasse 9 , D-60438 Frankfurt, Germany

[2]Frankfurt Institute for Molecular Life Sciences (FMLS), Goethe University, Max von Laue Strasse 15, D-60438 Frankfurt, Germany

[3]European Neuroscience Institute, Göttingen, Germany

[4]Georgia Institute of Technology, Atlanta, USA

Synaptic transmission relies on the rapid release of synaptic vesicles (SVs) at active zones of chemical synapses. Following release, SVs are retrieved trough endocytosis within presynaptic terminals. This local recycling allows fast refilling of SV pools, and further ensures maintenance of synaptic transmission without the need for de-novo SV synthesis and transport. Efficient SV recycling is thus most important during prolonged neuronal activity and at high rates of release.

In most systems, SV recycling occurs through clathrin-mediated endocytosis. After fusion with endosomes and protein sorting, mature SVs bud from these structures. Yet, at steady-state, endosomes are hardly detectable at presynaptic terminals, likely due to low SV release rates at steady-state conditions.

To investigate how *C. elegans* synapses operate under high-activity conditions, we used Channelrhodopsin-2-(ChR2-) mediated optical stimulation, combined with behavioural, electrophysiological and electronmicroscopic (EM) analyses. Animals in which cholinergic neurons are continuously (> minutes) photo-stimulated exhibit a constant body contraction, showing that synapses are able to constantly release and recycle SVs. Mini-analyses show that the release rate is initially high, then falls down to a plateau release rate during the stimulus. To probe clathrin-mediated endocytosis during sustained release, we targeted factors such as adapter proteins AP180 (UNC-11) and synaptotagmin (SNT-1), or proteins involved in clathrin uncoating, like endophilin (UNC-57) and synaptojanin (UNC-26). The respective mutants display phenotypes that clearly indicate reduced ability to recycle SVs during sustained activity.

To analyze synaptic ultra-structure and SV pools after long-term photo-stimulation of cholinergic neurons, we used high-pressure freeze (HPF) EM. In wild type animals, photo-stimulation induces depletion of (30 nm) SVs, and appearance of large (100-250 nm) endosomal structures, which are only transiently present and disappear within 10 sec after the light stimulus ended, and this was largely altered in SV recycling mutants. The recovery period as evidenced by EM corresponds well to post-stimulation electrophysiological and behavioural analyses: Subsequent photo-stimuli evoked maximal post-synaptic currents only after ca. 10-20 sec, and animals were uncoordinated for about 30-60 sec, suggesting that SV pools can be refilled faster than the active zone regains full release capability.

In order to uncover additional factors involved in SV recycling, we use RNA interference and a behavioural readout for synaptic efficacy, namely light-induced body contraction in animals expressing ChR2 in cholinergic neurons. To facilitate analysis on a genome-wide scale, we developed microfluidic devices in which animals are confined in up to 24 parallel channels, to easily combine photostimulation and imaging.

# An Optogenetic Toolbox Designed for Primates

Ilka Diester[1,2], Matthew T Kaufman[2], Werapong Goo[1,2], Murtaza Mogri[1], Ramin Pashai[1,5], Ofer Yizhar[1], Charu Ramakrishnan[1], Karl A Deisseroth[1,3,4], Krishna V Shenoy[2]

[1]Stanford University, Bioengineering, 318 Campus Drive, W080 Clark Center, 94305 Stanford, USA
[2]Stanford University, Electrcial Engineering, W100-A Clark Center, 318 Campus Drive West, 94305 Stanford, USA
[3]Stanford University, Department of Psychiatry and Behavioral Sciences, Stanford, USA
[4]Stanford University, Howard Hughes Medical Institute, Stanford, USA
[5]Department of Electrical Engineering and Computer Science, University of Wisconsin-Milwaukee, Milwaukee, WI, USA

Optogenetics is a technique for controlling subpopulations of neurons with light in the intact brain, which complements classical electrophysiology and may enhance both basic systems physiology research as well as inform the mechanisms and treatment of brain disease. Before launching large-scale primate studies, the method needs to be further characterized and adapted for use in the primate brain. We report here the analysis of an optogenetic toolbox designed for primates, including two optogenetic viral vector systems based on lentiviruses and adeno-associated viruses (AAV), combined with the human promoters hSyn and hThy-1 in rhesus macaques. We also assess the use of three different mammalian codon -optimized opsins in the primate brain: channelrhodopsin-2 (ChR2), enhanced Natronomonas pharaonis halorhodopsin eNpHR2.0, and a step-function opsin (SFO), which we characterize electrophysiologically, histologically, and behaviorally. We show that expression of these opsins is strong under control of the human promoters hSyn and hThy-1, and that low light levels are sufficient to activate or deactivate those opsins as measured on a single cell and local field potential level. We also introduce a new device for measuring in vivo fluorescence over time without sacrificing animal subjects, allowing the assessment of construct expression levels in the intact brain. Together, we present a set of optogenetic tools designed for, and available for use in, optogenetic experiments in the non-human primate brain.

# Channelrhodopsin-2 mediated optical stimulation of the Cochlea

Victor Hugo Hernandez Gonzalez[1,2], Gerhard Hoch[1,2], Nicola Strenzke[1,2], Zhizi Jing1[1], Hideki Takago[1, 2], Gerhard Vogt[3], Carolyn Garnham[3], Ernst Bamberg[4], Tobias Moser[1,2], George Augustine[5]

[1]University of Goettingen, InnerEarLab, Department of Otolaryngology, Robert-Kochstr. 40, D-37075 Göttingen Germany
[2]Bernstein Focus for Neurotechnology Goettingen
[3]MED-EL Company
[4]MPI for Biophysics Frankfurt
[5]Duke University

Electrical auditory prostheses are among the most advanced neuroprostheses. Providing auditory input to the auditory pathway, cochlear implants, the most commonly used prostheses, enable open speech comprehension in the majority of the implanted deaf subjects. Still, sound encoding driven by the current cochlear implants is limited. For example, cochlear implants make limited use of the tonotopically ordered projections to the brain employing only 12-22 separate channels in order to avoid electrode cross-talk. While the auditory performance of successful cochlear implant users highlights the incredible capabilities of the CNS to extract information from the limited sensory input, it remains an important task to improve the frequency resolution of cochlear implants. Here, we explored the use of channelrhodopsin-2 (ChR2) expression in spiral ganglion neurons for optical stimulation of the auditory pathway. Coupling blue light (emitted by LED or laser) into a cochleaostomy of transgenic mice expressing ChR2 (Arenkiel et al., 2007) in the first auditory neurons caused large compound potentials in scalp recordings. These potentials were present also after acute deafening but were blocked when action potential generation was inhibited by application of tetrodotoxin and lidocaine. The dependence of response amplitude on stimulus duration, rate and light power was systematically explored. Neural responses to sound could be masked by optical stimulation. Single auditory neuron responses to optical stimulation of the cochlea are currently being studied in the cochlear nucleus and the inferior colliculus. In summary, ChR2-mediated optical stimulation of cochlea seems feasible in animal experiments.

# Symposium

## S22: Unravelling the activity-dependent mechanisms of network formation in the neonatal cortex

S22-1 Circuits that control cortical development and plasticity, subplate neurons and beyond
*Patrick O Kanold*

S22-2 Synaptic circuit formation and plasticity in the developing visual system
*Colin Jon Akerman*

S22-3 Induction of GABAergic activity patterns during early neocortical development
*Werner Kilb*

S22-4 Plasticity, seizures and chloride regulation in neonatal neurons
*Peter Blaesse*

S22-5 Maturation of prefrontal-subcortical neuronal networks as result of early synchronized activity patterns
*Ileana Livia Hanganu-Opatz*

S22-6 At immature mossy-fiber-CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA.
*Sudhir Sivakumaran*

# Circuits that control cortical development and plasticity, subplate neurons and beyond

Patrick O Kanold[1]

[1]University of Maryland, Dept. of Biology, Institute for Systems Research, 1205 Biosciences Bldg, 20742, MD College Park, USA

One of the hallmarks of the young brain is it ability to be sculpted by experience especially during critical periods in development. However, the mechanisms underlying this early learning process and how they relate to learning processes in adult are unknown.
The young brain is structurally different from the adult brain and contains additional circuits that are formed by subplate neurons (SPNs). These neurons reside in the white matter and most of them disappear during development. After the critical period ends – when SPNs are no longer present - only limited plasticity is present. Thus SPNs might participate in types of synaptic plasticity that occur only during the critical period.
We describe functionally the changing circuits present in the developing neocortex. In particular we describe the circuits SPNs are associated with and how these circuits are engaged by thalamic activity. We then show how the presence of SPN circuits can aid neocortical development. We then show how disruption of these circuits in early development can lead to altered cortical function and cause symptoms of neurological disorders.
We then describe how these developmental mechanisms of controlling plasticity compare to mechanism controlling plasticity in the adult.

# Synaptic circuit formation and plasticity in the developing visual system

Colin Jon Akerman[1]

[1]University of Oxford, Department of Pharmacology, Mansfield Road, OX1 3QT Oxford, United Kingdom

The development of neural circuits involves a diverse array of endogenous and exogenous signals, including both spontaneous and environmentally driven neural activity. A fundamental idea is that information carried in early activity patterns can guide specific changes to the functional and structural properties of developing circuits. More than simply permitting changes, the spatiotemporal statistics of spiking activity are reflected in the circuit changes that occur. Some of the first experimental demonstrations that neural activity plays an instructive role in development involved imposing high levels of correlation between neurons' spiking activity and showing that this disrupted the development of receptive field properties in the visual system. Later work showed that imposing temporal sequences of pre- and post-synaptic spiking activity is able to modify receptive fields via processes such as spike-timing dependent plasticity. However, very little is known about the mechanisms by which a developing system might be equipped to translate information in the environment in to specific changes in its functional connectivity. We have explored this by recording from neurons in the developing visual system of Xenopus laevis. We show that neurons of the optic tectum can be "trained" by repeatedly presenting a visual stimulus and that the resulting changes induced in their receptive fields reflect the spatiotemporal properties of the training stimulus. As in other systems, tectal neurons at these stages receive glutamatergic and GABAergic synaptic inputs. We have focussed on the contribution of local GABAergic circuits in enabling young tectal neurons to interpret the spatiotemporal statistics of environmentally driven activity. Intriguingly, we find that when GABAergic transmission in the tectum is disrupted, the instructive effects of training are eliminated. This loss of instructive learning is related to changes in spike-timing patterns because when GABAergic inputs are blocked, there is a substantial increase in the spike -timing correlations between tectal cells and greater potential for tectal-tectal synaptic plasticity. In support of this, we find that instructive learning is eliminated when spike-time correlations between tectal neurons are increased by other manipulations. Furthermore, recordings of synaptic inputs during visual stimulation reveal that features of the early GABAergic system mean that it is ideally placed to influence spike-timing at these stages. Rather than decreasing the variance in spike -timing, as they do in many adults systems, early GABAergic circuits in the optic tectum enhance spatiotemporal differences in spiking patterns and minimize correlations that may be introduced via recurrent excitation. This may provide a mechanism to ensure that receptive field changes are primarily instructed by the statistics of the visual environment.

# Induction of GABAergic activity patterns during early neocortical development

Werner Kilb[1]

[1]University Medical Center Mainz, Institute of Physiology and Pathophysiology, Duesbergweg 6, 55128 Mainz, Germany

GABA is a main inhibitory neurotransmitter in the adult nervous system and mediates its action via distinct interneurons and mainly synaptic processes. In the immature nervous system activation of $GABA_A$ receptors generates depolarizing membrane responses that can promote excitation and both nonsynaptic and synaptic processes contribute to GABAergic synaptic transmission. However, the neuronal elements and activity patterns by which the GABAergic system influences the cortical activity in the early postnatal cerebral cortex are only marginally understood.

Our studies in the early postnatal cerebral cortex of rodents revealed that in subplate neurons only a fraction of spontaneous postsynaptic currents (PSCs) were GABAergic, while in pyramidal neuron of the cortical plate the majority of PSPs and in Cajal-Retzius cells virtually all PSCs were mediated by $GABA_A$ receptors. Tonic activation of $GABA_A$ and glycine receptors by taurine induced a significant increase in the frequency of GABAergic PSCs. The taurine-induced GABAergic PSCs were blocked by TTX and could also be observed in minislice preparations without any subcortical structures, suggesting that the tonic activation of $GABA_A$ and glycine receptors specifically activates GABAergic interneurons within the cortex. A global enhancement of the neuronal activity by bath-application of the cholinergic agonist Carbachol led to a massive increase in the PSPs frequency. This Carbachol-induced synaptic activity consist in pyramidal neuron of the cortical plate mainly of glutamatergic PSCs, while in subplate neurons about 50% of the PSCs and in Cajal-Retzius cells all PSCs are mediated by $GABA_A$ receptors. The GABAergic PSCs in Cajal-Retzius cells were suppressed in TTX and could also be observed in minislices, in which all subcortical structures including the subplate are missing. These findings suggest that Carbachol leads to the activation of GABAergic interneurons within the cortex, which project to Cajal-Retzius cells. Perforated-patch recordings revealed that this Carbachol-induced GABAergic PSCs were able to trigger action potentials, but that such episodes of high frequency GABAergic activity significantly reduce the intracellular $Cl^-$ concentration and the excitatory potential of GABAergic inputs.

In summary, these findings indicate that already in the early postnatal cerebral cortex synaptic GABAergic connections contribute to the neuronal activity in a highly stimulus and projection specific manner. The depolarizing GABAergic responses can clearly promote excitation in the investigated circuits, but the activity-dependent dynamic $Cl^-$ changes suggest a considerable context dependency of GABAergic responses.

# Plasticity, seizures and chloride regulation in neonatal neurons

Peter Blaesse[1]

[1]University of Helsinki, Laboratory of Neurobiology, Viikinkaari 1, 00014 Helsinki, Finland

Fast inhibitory postsynaptic transmission is mediated by $GABA_A$ receptor channels, which are selectively permeable to $Cl^-$ and to a lesser extent to $HCO_3^-$. Whether $GABA_A$ receptor -mediated currents are hyperpolarizing or depolarizing (and sometimes even excitatory) depends on their reversal potential, which is set by the distribution of $Cl^-$ and $HCO_3^-$ across the plasma membrane of the target neuron. The Na-K-2Cl cotransporter NKCC1 and the neuron-specific K-Cl cotransporter KCC2, which belong to the family of cation-chloride cotransporters (CCCs), are key molecules in neuronal chloride homeostasis and, thereby, have a major impact on GABAergic signaling (for review, see Blaesse et al. 2009, Neuron 61: 820-38).

A ubiquitous feature of neurons is a developmental shift in the intracellular $Cl^-$ concentration ($[Cl^-]_i$) from relatively high levels in immature neurons towards lower levels in mature neurons. This shift has been attributed to a concerted change in the actions of NKCC1 and KCC2. In immature neurons, NKCC1 uses the inward-directed $Na^+$ gradient to transport Cl- into the cell, thereby increasing the $[Cl^-]_i$ to a level higher than what is expected on the basis of a passive $Cl^-$ distribution. In contrast, KCC2 uses the outward-directed $K^+$ gradient to lower the $[Cl^-]_i$ in mature neurons. Elucidating the mechanisms underlying the functional development of NKCC1 and KCC2 is of much importance since it is generally thought that CCCs, by modulating GABAergic transmission, contribute to neuronal circuit formation in the hippocampus and other cortical structures.

A striking example of the regulation of CCCs by neuronal activity is the activation of KCC2 by a neonatal seizure episode (Khirug et al., 2010, JNeurosci 30: 12028 35). The KCC2 protein level is low and functionally insignificant in the early postnatal rodent hippocampus. However, a single neonatal seizure episode induced by kainate injection during postnatal day 5-7 leads to a dramatic increase in the plasmalemmal KCC2 pool and results in a rapid functional activation of the transporter. In addition, seizure-like activity induced by kainate application in vitro results in a similar increase in neuronal $Cl^-$ extrusion and in the surface expression of KCC2. Both effects are blocked by the kinase inhibitor K252a. The activity-dependent increase in KCC2 functionality may act as an intrinsic anticonvulsant mechanism in the neonate hippocampus.

Powerful homeostatic regulation of neuronal activity in the developing hippocampal circuitry is seen when the depolarizing action of GABA is absent in the immature hippocampus due to a genetic or pharmacologically-induced loss of NKCC1 function (Sipilä et al., 2009, JNeurosci: 29: 6982-88). Surprisingly, slices from NKCC1 knockout mice generate endogenous network events similar to "Giant Depolarizing Potentials" (GDPs). Unlike in slices from wildtype mice, the GDPs are not blocked by the NKCC1 inhibitor bumetanide and not facilitated by the $GABA_A$ agonist isoguvacine. While the lack of depolarizing GABAergic transmission has no effect on the developmental upregulation of KCC2, it results in a fast and dramatic compensatory increase in the intrinsic excitability of glutamatergic neurons.

The significance of the activity-dependent regulation of KCC2 function and its effect on GABAergic transmission and the putative mechanisms and the implications of the homeostatic regulation of neuronal activity by CCCs will be discussed.

# Maturation of prefrontal-subcortical neuronal networks as result of early synchronized activity patterns

Ileana Livia Hanganu-Opatz[1]

[1]Center for Molecular Neurobiology, UKE, Dev. Neurophysiology, Falkenried 94, 20251 Hamburg, Germany

Binding of neuronal assemblies by synchronizing their activity patterns in oscillatory rhythms enables higher brain abilities such as sensory perception, attention and memory. The ability to generate oscillatory rhythms is not a hallmark of the adult brain, but is present already during early development. Synchronized patterns of oscillatory activity are present in sensory (visual, somatosensory) cortical areas of both premature infants and neonatal rodents and are triggered by endogenous activation of sensory periphery and intracortical activation. They appear to act as a template facilitating the establishment of cortical maps requested for sensory processing. Whether such early oscillatory activity interferes with the maturation of neuronal networks underlying the cognitive performance remains unknown. Combining in vivo electrophysiology and pharmacology with immunohistochemistry and behavioral testing we identified and characterized for the first time the patterns of oscillatory activity in the developing rat prefrontal cortex (PFC) and unraveled their mechanisms of generation within a prefrontal-hippocampal-subcortical circuit. Discontinuous oscillatory patterns with characteristic temporal and spatial organization synchronize in theta-gamma frequency band the neonatal PFC. They result from the activation of local prefrontal networks driven by the theta bursts of intermediate and ventral hippocampus and by the inputs from the entorhinal cortex. With ongoing maturation, the PFC switches to continuous theta-gamma oscillatory rhythms, although their precision of coupling decreases in juvenile pups. At this age mutual interactions between prefrontal and hippocampal rhythms shape the refinement of connectivity. During the neonatal and juvenile maturation, the early prefrontal-hippocampal network is subject of intense subcortical modulation, especially by the cholinergic input from the basal forebrain. Selective impairment of subcortical cholinergic neurons led to abnormal patterns of oscillatory activity and to behavioral deficits (ultrasound vocalization, short-term memory). Whereas muscarinic, but not nicotinic receptors mediate the cholinergic modulation of activity patterns in the neonatal PFC, both receptors types contribute to acetylcholine-induced effects on the continuous theta-gamma rhythms of juvenile pups. Early miswiring within this prefrontal-hippocampal-subcortical circuit due to abnormal patterns of early activity and synchronization may account for specific impairment of cognitive abilities in several neurodevelopmental disorders.

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# At immature mossy-fiber-CA3 synapses, correlated presynaptic and postsynaptic activity persistently enhances GABA release and network excitability via BDNF and cAMP-dependent PKA.

Sudhir Sivakumaran[1]

[1]SISSA/ISAS, Neurobiology, Lab 551/554, 5th Floor, Via Bonomea 265, 34136 Trieste, Italy[1]

In the adult rat hippocampus, the axons of granule cells in the dentate gyrus, the mossy fibers (MF), form excitatory glutamatergic synapses with CA3 principal cells. In neonates, MF release into their targets mainly GABA, which at this developmental stage is depolarizing. Here we tested the hypothesis that, at immature MF-CA3 synapses, correlated presynaptic [single fiber-evoked GABAA-mediated postsynaptic potentials (GPSPs)] and postsynaptic activity (back propagating action potentials) exert a critical control on synaptic efficacy. This form of plasticity, called spike-timing-dependent plasticity (STDP), is a Hebbian type form of learning extensively studied at the level of glutamatergic synapses. Depending on the relative timing, pairing postsynaptic spiking and single MF-GPSPs induces bidirectional changes in synaptic efficacy (long-term potentiation, LTP or long-term depression, LTD). In case of positive pairing (pre before post), LTP was associated with a persistent increase in GPSP slope and in the probability of cell firing. The potentiating effect required a rise in postsynaptic calcium via voltage-dependent calcium channels. In addition, spike-timing dependent LTP needed the combined activity of cAMP-dependent PKA (protein kinase A) and brain-derived neurotrophic factor (BDNF). The release of BDNF from the postsynaptic neuron activated presynaptic TrkB receptors, leading to a persistent increase in GABA release. In 'presynaptically' silent neurons, pairing-induced enhancement of GABA release produced synapse unsilencing. Shifting EGABA from the depolarizing to the hyperpolarizing direction with bumetanide, a specific inhibitor of Na-K-Cl-Cotransporter isoform 1 (NKCC1), failed to modify synaptic strength. Thus, spike-timing dependent LTP of GPSPs provides a reliable way to convey information from granule cells to the CA3 associative network at a time when glutamatergic synapses are still poorly developed.

# Symposium

## S23: The social brain - in health and disease

S23-1 Juvenile mouse ultrasonic vocalizations emitted during a dyadic encounter respond to the distinct past experiences of one individual
*Garet Paul Lahvis, Vanessa R Jimenez, Jules B Panksepp*

S23-2 Rodent ultrasonic communication - Brain mechanisms underlying social approach behavior
*Markus Wöhr*

S23-3 Rodent ultrasonic vocalizations as a valuable readout in animal models of psychiatric diseases
*Kurt Hammerschmidt*

S23-4 Gene-environment interaction and response to ambient CO2: an animal model for panic disorder
*Francesca R D'Amato*

S23-5 Is stress always bad for the brain?
*Eberhard Fuchs*

S23-6 Fear is only in our minds: A novel animal model for claustrophobia
*Ahmed El-Kordi, Konstantin Radyushkin, Hauke Werner, Klaus-Armin Nave, Hannelore Ehrenreich*

# Juvenile mouse ultrasonic vocalizations emitted during a dyadic encounter respond to the distinct past experiences of one individual

Garet Paul Lahvis[1], Vanessa R Jimenez[1], Jules B Panksepp[1]

[1]Oregon Health and Science University, Department of Behavioral Neuroscience, L-470, 3181 SW Sam Jackson Park Road, 97239 Portland, OR, USA

Various species within the order Rodentia emit vocalizations that communicate referential and emotional information. Among mice and rats, many of these vocalizations are emitted at frequencies that exceed human hearing abilities. Understanding the function of mouse ultrasonic vocalizations (USVs) could help us employ mouse models to elucidate how various drugs and genetic manipulations modulate affective experience. While vocalizations emitted under distress conditions can elicit fear in the mice that hear these calls, it remains unknown whether the highly variable calls emitted under more affiliative conditions are responsive to changes in environment. In this study, we recorded the vocalizations of juvenile (PD 30) C57Bl/6J mixed-sex dyads that were reunited after 15 minutes of social separation. During separation, the female mouse was transferred to either a large cage that was 'enriched' by a variety of novel objects or was transferred to a large cage that contained only bedding. When the mice were subsequently reunited, females that were exposed to the 'enriched' environments elicited higher levels of social approach than females from standard cage environments. There were also substantial differences between USVs emitted during dyadic encounters when females returned from the distinct environments. USV quantity, duration and patterns of frequency modulation were reproducibly distinct between treatments. The observation that vocalizations among mice can sensitive to differences in the experience of one member of a dyad suggests that USVs play a role in mouse communication.

# Rodent ultrasonic communication - Brain mechanisms underlying social approach behavior

Markus Wöhr[1]

[1]Philipps-University of Marburg, Experimental and Physiological Psychology, Gutenbergstraße 18, 35032 Marburg, Germany

Mice and rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals. In the rat, three USV types are known: 1) 40-kHz-USVs, emitted by pups when isolated from mother and littermates; 2) 22-kHz-USVs, emitted by juvenile and adult rats in aversive situations such as fear-conditioning or social defeat; and 3) 50-kHz-USVs, emitted by juvenile and adult rats in appetitive situations such as rough-and-tumble play, mating or when tickled playfully. In the mouse, similar USV types exist – with the exception of 22-kHz-USVs that are not present in mice. Currently, USVs predominantly serve as measures for the rodent's affective state as it is widely believed that the distinct USV types reflect positive and negative affective states, respectively.

In behavioral, pharmacological and neuronal studies, evidence was provided that USVs serve communicative purposes. It is known for a long time that 40- kHz-USVs elicit maternal search and retrieval behavior. More recently, however, it was demonstrated that also 22-kHz-USVs and 50-kHz-USVs induce call-specific behavioral responses in the receiver. While 22-kHz-USVs induce freezing behavior, indicating an alarming function, 50-kHz-USVs induce social approach behavior, supporting the notion that they serve as social contact calls. The opposite behavioral responses are paralleled by distinct patterns of brain activation. While 22-kHz-USVs induce activation in amygdala and periaqueductal gray, 50-kHz-USVs are followed by activation in the nucleus accumbens, an area where opioids exert their effects on social behavior. As shown in pharmacological studies, social approach behavior in response to 50- kHz-USVs is regulated by the endogenous opioid system. Thus, enhanced social approach behavior was found in morphine treated rats, whereas naloxone treatment caused its reduction. Social approach behavior in response to 50- kHz USVs further depends on social interactions during adolescence as no preference towards 50- kHz-USVs was found in rats exposed to long -term post -weaning social isolation, highlighting the importance of social experience during adolescence for affiliative behavior.

Measuring USV production and the behavioral responses to USVs provides therefore an unique tool to study communication in rodents. This is especially relevant when applying rodent models to elucidate genetic, biochemical and neuroanatomical factors underlying neuropsychiatric disorders characterized by social and communication deficits such as depression, schizophrenia and autism. In a series of experiments, the BTBR T+tf/J mouse model of autism was found to display an unusual pattern of USV categories as pups and an untypical lack of USVs in adulthood. Thus, adult male BTBR T+tf/J mice displayed not only low scent marking behavior but also minimal USV responses to female urine obtained from both C57BL/6J and BTBR T+tf/J females. This indicates that the BTBR T+tf/J mouse model of autism incorporates phenotypes relevant to the second diagnostic symptom of autism, communication deficits, along with its strong behavioral phenotypes relevant to the first and third diagnostic symptoms, impairments in social interactions and high levels of repetitive behavior. Together, these data support the relevance of USVs for rodent models of neuropsychiatric disorders characterized by social deficits.

# Rodent ultrasonic vocalizations as a valuable readout in animal models of psychiatric diseases

Kurt Hammerschmidt[1]

German Primate Center, Cognitive Ethology Lab, Kellnerweg 4, 37077 Göttingen, Germany

The last years have shown an increased interest in mouse ultrasonic vocalizations (USV) as readouts in animal models of psychiatric disease. However, until now the research has mainly focused on male mice courtship songs and many studies restricted themselves to counting the number of calls. Recent studies have shown that it can be an important improvement to include the structural features of USVs. Further it could be shown that it is possible to use the resident-intruder paradigm to elicit USVs also from female mice. We modified the classic resident-intruder design in the way that we placed an anaesthetized intruder in the resident 'home' cage. In this way we were able to circumvent the major disadvantage of rodent USVs that it is not possible to determine the caller by call accompanying behavior (e.g. open mouth) or a technical solution, like phase differences. The results showed that female mice produce similar call sequences during resident-intruder encounters as male mice produce during courtship encounters, implicating that male mice courtship songs may be not as unique as thought. In addition both, females and males, produced comparable USVs in response to anaesthetized intruders, which makes this design an important alternative in using USVs as behavioral readouts. In this talk I present results showing that a detailed acoustic analysis together with the described design improvements is helpful to characterize disease specific behavior.

# Gene-environment interaction and response to ambient CO2: an animal model for panic disorder

Francesca R D'Amato[1]

[1]CNR, Neuroscience Institute, Behavioral Neuroscience, Via del Fosso di Fiorano 64, 00143 Roma, Italy

It is now clear that mental disorders are multifactorial conditions, caused by many elements of genetic and environmental origin that act independently or in interplay. Panic disorder/agoraphobia (PD) -and its precursor in childhood: separation anxiety disorder (SAD) - constitute common (5% -6% of prevalence) and debilitating illnesses. Decisive contributions has been provided showing that some specific responses (hyperventilation, acute anxiety) to stimulation with CO2 -enriched air mixtures, observed in people at heightened risk for PD/SAD, constitute a valuable tool that can be transferred to the laboratory to dissect the nature of these disorders. On the basis of this endophenotype we developed an animal model of PD in mice, based on the disturbance of mother-infant bond during development.

After having spent the first 24 hours after birth with their biological mother, outbred NMRI mice were cross-fostered to adoptive mothers for the following 4 post -natal days. They were successively compared to normally-reared individuals for: number of ultrasonic vocalizations during isolation, respiratory physiology responses to normal air (20% O2), hypercapnia (6% CO2 -enriched air), hypoxia (10%O2 air ), and avoidance towards CO2-enriched environments. Cross -fostered pups showed significantly more ultrasonic vocalizations during isolation, more pronounced hyperventilatory responses (larger tidal volume and minute volume increments) during hypercapnia, and heightened aversion towards CO2-enriched environments than normally-reared individuals. Enhanced tidal volume increment during hypercapnia was present at 16- 20, and 75- 90 postnatal days, implying trait's stability. Quantitative genetic analyses of unrelated individuals, sibs and half-sibs, showed that the genetic variance for tidal volume increment during hypercapnia was significantly higher (Bartlett $\chi$=8.3, p=0.004) among the cross-fostered than the normally-reared individuals, yielding heritability of 0.37 and 0.21 respectively. Maternal grooming/licking behaviour, and corticosterone basal levels did not differ between cross-fostered and normally-reared individuals.

The use of an endophenotype in this animal model of PD allows to further investigate the causal mechanisms that connect environmental adversities occurring in sensitive periods of development to health status in childhood and early adulthood.

# Is stress always bad for the brain?

Eberhard Fuchs[1]

[1]German Primate Center, Clinical Neurobiology, Kellnerweg 4, 37077 Göttingen, Germany

When Hans Selye formulated his stress theory about 70 years ago, stress was thought to have a merely endocrine character, and noxious stimuli of physical or chemical nature were the primary stressors discussed in those days. Subsequent research, however, demonstrated that psychological stimuli are also strong activators of the endocrine system and there was increasing evidence that 'stress hormones' such as adrenocorticotropin and adrenocortical steroids profoundly influence brain excitability. Since than, great progress has been made to understand the biology of stress reactions as well as the physiological and behavioral alterations that occur as a consequence of exposure to challenging events in the environment. Today the stress response is regarded as an alarm system which is initiated whenever there is a discrepancy between what an organism is expecting and what really exists. Uncertainty, lack of information or loss of control induce alarm reactions whereas the presence of social support, information and control reduce such alarm reactions. Therefore, the stress response per se is not harmful or pathological in itself. Only when demanding, prolonged and sustained, or when an individual's predisposition hinders an adaptive stress response, body and brain homeostasis will be threatened, and health may be endangered. Since this is a consistent finding across species including man, it is important to understand the relationship between stressors and diseases.

The brain is the key organ to guide stress responses because in that it 'decides' what is stressful and subsequently initiates the physiological and behavioral responses. Within the brain, the stress response is mediated through in-concert activity of many areas and there is experimental evidence that chronic stress in rats induces structural changes in neural networks, as e.g. detected in hippocampus, prefrontal cortex and amygdala. Within the hippocampal formation, stress exposure remodels dendrites of the CA3 pyramidal neurons and reduces numbers of synapses on these neurons. Furthermore, stress inhibits adult neurogenesis in the dentate gyrus and modulates the GABAergic system. In the prefrontal cortex, chronic stress causes pronounced dendritic remodeling of pyramidal cells and suppresses gliogenesis whereas in the amygdala, stress can elicit dendritic hypertrophy. These microscopically detectable changes in cell structures indicate a reorganization of neural networks. Moreover, molecular studies demonstrate that stress modulates expression of genes involved in neuronal differentiation and/or structural remodeling.

Since a wealth of data documents the adverse effects of stress on emotions and cognition, these alterations are commonly interpreted as the deleterious effect of (chronic) stress on the central nervous system. However, it is also possible that at least part of these changes reflect adaptive responses, as the network system rearranges its connections in order to cope with the changing requirements from the internal or external environment. Future studies should clarify which of the today known stress-induced central nervous events constitute adaptive processes and which are pathological.

# Fear is only in our minds: A novel animal model for claustrophobia

Ahmed El-Kordi[1], Konstantin Radyushkin[1], Hauke Werner[2], Klaus-Armin Nave[2], Hannelore Ehrenreich[1]

[1]Max Planck Institute of Experimental Medicine, Division of Clinical Neuroscience, Göttingen, Germany, [2]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Göttingen, Germany

Anxiety disorders are among the most common mental health conditions and cause a substantial functional and social impairment. Phobias (specific anxiety disorders) inflict significant social stress and burden. Their genetic and neurobiological roots are as yet poorly studied. Here we show for the first time a genetic animal model, leading to a behavioural phenotype reminiscent of claustrophobia in humans. A novel strain of mutant mice, created by gene targeting, exhibit overall normal sensory capabilities and basic behaviour. However, they demonstrate an abnormal pattern of avoiding and escaping closed spaces. Interestingly, this abnormal behaviour is remediated after 'exposure therapy', i.e. repeated confrontation with closed space. Taken together, the here presented animal model is the first showing potential genetic contribution to claustrophobia. This knowledge may open new approaches to treating anxiety disorders and will help to understand how behavioural therapy affects brain functions.

# Symposium

## S24: How do neurodegenerative diseases develop and how to cure them: What can we learn from diverse animal models?

S24-PR Investigations into molecular role of Leucine-rich repeat kinase 2 in disease models
*Wright Jacob, Rafael Klosowski, Stephanie Putz, Bernhard Hovemann, Rolf Heumann*

S24-1 A WORM MODEL OF TAUOPATHY
*Polychronis Fatouros, Jeelani Pir Ghulam, Eckhard Mandelkow, Eva-Maria Mandelkow, Jacek Biernat, Ralf Baumeister, Enrico Schmidt*

S24-2 *Drosophila Glued*, a genetic model for the identification of new genes involved in axonal transport and neurodegenerative processes.
*Patrick Callaerts, Els Janssens*

S24-3 Exploring Neurodegeneration in transgenic Zebrafish
*Dominik Paquet, Ratan Bhat, Eva-Maria Mandelkow, Reinhard Köster, Bettina Schmid, Christian Haass*

S24-4 FROM MICE TO MEN: TARGETING AMYLOID PATHOLOGY WITH ABETA IMMUNOTHERAPY
*Roger M. Nitsch*

S24-5 Mouse models of demyelinating diseases
*Klaus-Armin Nave*

# Investigations into molecular role of Leucine-rich repeat kinase 2 in disease models

Wright Jacob[1], Rafael Klosowski[2], Stephanie Putz[2], Bernhard Hovemann[2], Rolf Heumann[1]

Molecular Neurobiochemistry[1] and Molecular Cell Biochemistry[2], Faculty of Chemistry and Biochemistry, Ruhr-University Bochum, Germany, D-44801

Leucine-Rich Repeat Kinase 2 (LRRK2) is a multifunctional protein carrying domains with homologies to Ras and MAPKKK. Mutations in the LRRK2 gene, are the most prevalent genetic cause of Parkinsonism. The reduction in viability of neuroblastoma SH-SY5Y cells and of cortical neurons by disease related LRRK2 mutants (R1441C and G2019S) was strongly attenuated, through constitutively active Val12 Ha-Ras expression. Furthermore, expression of activated Ha-Ras in cortical neurons completely rescued the adverse effects caused by mutant LRRK2 expression on primary neurite length and branching points. Application of effector-region specific mutants of Val12 Ha-Ras (Y40C, T35S) suggests that, the PI-3 kinase but not the Ras/MAPK is involved in the rescue pathway. Endogenous neuronal Ras activity is not affected by mutant LRRK2 indicating that the disease causing mechanism is not related to Ras signaling.

The protein-protein interactions and pathways involved in LRRK2-mediated signalling remain elusive. Utilizing yeast-two hybrid, biochemical techniques, mass spectrometry and disease model this study identified and described the association between LRRK2 and protein phosphatase 2A(PP2A). The Roc or GTPase-like domain of LRRK2 is sufficient and essential for association with PP2A. This interaction occurs in a guanine nucleotide dependent manner, suggesting that PP2A may be an effector of the LRRK2 GTPase domain. The LRRK2 pathogenic mutant retains interaction with PP2A, suggesting that disruption of this interaction is not likely the mechanism whereby mutation leads to disease. Okadaic-acid (OA) and RNAi mediated inhibition of PP2A augmented LRRK2-induced cell death. Mass-spectrometry analysis of LRRK2 revealed rearrangement of phosphosites on PP2A inhibition. Several confirmed and putative LRRK2 interactions were identified using Parkinson`s disease models. These models may provide useful for understanding the role of LRRK2 in human physiology and disease.

A *Drosophila* model for LRRK2-linked parkinsonism was created using GAL4/UAS system expressing either wild-type human LRRK2 or LRRK2-R1441C. Expression of either wild-type LRRK2 or of LRRK2-R1441C in dopaminergic neurons caused their significant loss. Expression of mutant human LRRK2-R1441C caused a more severe parkinsonism-like phenotype than wild-type LRRK2 expression. In conclusion, our data suggest that Ras is a novel therapeutic target for attenuating disease related mutant LRRK2-induced neurodegenerative effects and *Drosophila* model recapitulated LRRK2-linked pathogenesis.

# A WORM MODEL OF TAUOPATHY

Polychronis Fatouros[1], Jeelani Pir Ghulam[2], Eckhard Mandelkow[2], Eva-Maria Mandelkow[2], Jacek Biernat[2], Ralf Baumeister[1], Enrico Schmidt[1]

[1]Institute of Biology III, ZBSA, Albert Ludwig University of Freiburg, Germany
[2]Max Planck Unit for Structural Molecular Biology, 22607 Hamburg, Germany

The Microtubule-Associated-Protein Tau is found mutated in several neurodegenerative diseases. Under pathological conditions, Tau gets hyper-phosphorylated and forms intracellular aggregates. Deletion of K280 was found by the Mandelkow group to enhance the aggregation propensity of Tau and is a mutation present in patients with FTDP-17. In contrast, two Isoleucine-to-Proline substitutions in the hexapeptide motifs of Tau confer anti-aggregation properties. In this study we established a *C. elegans* model of Tauopathy by expressing pro- or anti-aggregating Tau fragments, along with full length human Tau, from a pan-neuronal promoter. This resulted in accelerated Tau pathology in the pro-aggregation transgenic lines, manifested by progressively impaired motility in young adult animals, neuronal defects such as axonal gaps and varicosities, as well as less pre-synaptic termini along the dorsal cord. In addition, preliminary data show that axonal transport of mitochondria is impaired in the pro-aggregation lines as well. The control lines expressing the anti-aggregation variants of Tau shows some mild defects only at older age. In collaboration with the MEMOSAD partner Senexis, we have tested a novel compound which can dissolve aggregated Tau in vitro and we observed a significant improvement of the movement defect in our model. In order to identify new modulators of the Tau pathology, we carried out a genome-wide RNAi screen for suppressors of the motility defect. We uncovered some novel potentially interesting genes that when knocked-down alleviate Tau pathology in our model. Further validation of these candidates is under way and a cross-test in a mammalian system is in progress.

# *Drosophila Glued*, a genetic model for the identification of new genes involved in axonal transport and neurodegenerative processes.

Patrick Callaerts[1,2], Els Janssens[1,2]

[1]Laboratory of Behavioral and Developmental Genetics, Flanders Institute for Biotechnology (VIB)
[2]Laboratory of Behavioral and Developmental Genetics, Department of Human Genetics, University of Leuven, 3000 Leuven, Belgium

Motorneurodegenerative diseases are all characterized by the degeneration of specialized motorneurons, which results in progressive weakness and wasting of muscles. Recently, pathogenic mutations were found in genes important for axonal transport, thereby identifying the axonal transport machinery as a potentially important mediator in neurodegenerative disorders. We used the *Drosophila dynactin1 (DCTN1)* homolog, *Glued*, to characterize a possible role of *Glued*-interacting genes in motorneurodegeneration. We found that the dominant mutation $Glued^1$ can serve as a mechanistic model for axonal transport defects. $Glued^1$ mutants show progressive decline in motor activity and characterization of these mutants revealed morphological and neurodegenerative hallmarks. One known *Glued*-interacting gene is *DLis1*, the *Drosophila* homolog of LIS1. Mutations in human LIS1 are associated with classical Lissencephaly, a congenital brain disorder, but no association has yet been found between LIS1 and motorneurodegeneration. We found that reduced levels of Lis1 delay onset of the progressive motorphenotype of $Glued^1$ mutants. Our results suggest that a stoichiometric balance between *dynactin* and *Lis1* is important for proper function of axonal transport and that disturbing this balance might accelerate degenerative phenotypes.
A genetic modifier screen identified several interacting genes. All genes seem to play a role in pathways that have previously been linked to neurodegeneration, i.e. RNA processing, transport and degradation processes. In this study we show that, by using the $Glued^1$ mutant as a model, it is possible to identify new modifier genes with a possible role in axonal transport and/or (motor)neurodegeneration. These novel insights also identify possible new avenues towards treatment.

# Exploring Neurodegeneration in transgenic Zebrafish

Dominik Paquet[1], Ratan Bhat[2], Eva-Maria Mandelkow[3], Reinhard Köster[4], Bettina Schmid[1], Christian Haass[1]

[1]DZNE - German Center for Neurodegenerative Diseases, Munich & Adolf-Butenandt-Institute, Ludwig-Maximilians-University, Munich, Germany

[2]AstraZeneca R&D, Södertälje, Sweden

[3]Max-Planck-Unit for Structural Molecular Biology, Hamburg, Germany

[4]HelmholtzZentrum Munich, Institute of Developmental Genetics, Neuherberg-Munich, Germany

Our ageing society is confronted with a dramatic increase of patients suffering from Tauopathies, which include Alzheimer's disease and certain types of Frontotemporal dementia. These disorders are characterized by typical neuropathological lesions including hyperphosphorylation and subsequent aggregation of Tau protein and neuronal cell death. Currently, no mechanism-based cures are available. Genetically modified animals are invaluable models to understand the molecular mechanisms of pathology and screen for disease-modifying compounds. In an approach to model Tauopathies in a non- murine vertebrate animal model, we have generated the first Tau-transgenic zebrafish. Zebrafish larvae are ideally suited for both *in vivo* imaging and drug development due to their optical transparency and small size. Therefore, Tau-transgenic fish could be important tools to better understand the pathology of Tauopathies and develop treatment approaches.

The fish rapidly recapitulate key pathological features of Tauopathies including phosphorylation and conformational changes of human Tau protein, tangle formation, as well as neuronal and behavioral disturbances and cell death. We could already demonstrate for the first time that tau-induced neuronal cell death can be imaged by time-lapse microscopy *in vivo*. Furthermore, we used our fish- model to identify new compounds targeting the Tau-Kinase GSK3ß, since phosphorylation of Tau is believed to be a trigger for disease progression. We identified a novel highly active GSK3ß inhibitor, which was developed by rational drug design and validated for in vivo activity in the transgenic fish model. Currently, we are establishing assays to study early disease associated processes in zebrafish neurons, such as axonal transport deficits, by *in vivo* imaging in the zebrafish CNS.

# FROM MICE TO MEN: TARGETING AMYLOID PATHOLOGY WITH ABETA IMMUNOTHERAPY

Roger M. Nitsch[1]

[1]Zürich, Switzerland

No abstract available

# Mouse models of demyelinating diseases

Klaus-Armin Nave[1]

[1]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Goettingen, Germany

In virtually all nervous systems, long axons engage with glia cells. However, the mechanisms by which neurons and glia communicate troughout life are not well understood. In the CNS, there is increasing evidence that oligodendrocytes support long-term axon function and survival, which is noz only relevant for human myelin diseases, such as multiple sclerosis (MS), but also other neurodegenerative disorders. Patients with MS suffer from slowly progressive loss of axons in inflammatory demyelinating lesions. This raises the question whether inflammation, demyelination, oligodendrocyte dysfunction, or any combination thereof is the underlying cause of axonal failure. Acute inflammation, such as in EAE mice, perturbs the energy balance of demyelinated axons. However, genetic observations suggest that oligodendrocytes themselves maintain long -term axonal integrity, independent of myelination and inflammation. Moreover, genetic defects of oligodendrocytes not only cause axon loss, but can trigger a secondary inflammation that includes the invasion of activated CD8+ T cells into white matter. The molecular mechanism of how oligodendrocytes support axon function are not well understood. The phenotype of axonal swellings and Wallerian degeneration in mouse models resembles those in patients with mitochondrial defects. To study possible contributions of oligodendrocytes in maintaining the axonal energy balance in myelinated fibers, we are generating and studying novel mouse models lacking specific organell functions in myelinating glial cells.

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# Satellite Symposia

Sat 2nd Schram Foundation Symposium: From Synapse to Neurological Disease

# Satellite Symposium

# Sat: 2nd Schram Foundation Symposium: From Synapse to Neurological Disease

Sat-1 Melodies of developing cortical networks
*Heiko J. Luhmann*

Sat-2 Optogenetic analysis of small neuronal networks in *Caenorhabditis elegans*
*Alexander Gottschalk*

Sat-3 SENSORY MAP FORMATION IN THE DROSOPHILA BRAIN
*Thomas Hummel, Özkan Aydemir, Anna Brochtrup, Britta Kuhlmann*

Sat-4 Sending signals from the synapse to the nucleus
*Michael R. Kreutz, Anna Karpova, Marina Mikhaylova*

Sat-5 The role of chromatin plasticity in neuropsychiatric diseases
*Andre Fischer*

Sat-6 Stonin2-dependent endocytic sorting of synaptotagmin 1 provides evidence for loss of synaptic vesicle identity during exo-endocytosis
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Sat-7 Shaping membranes – Roles in neuromorphogenesis, vesicle formation and neuronal network activity
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Sat-8 DEFINING THE NEURONAL CIRCUITRY OF FEAR
*Andreas Lüthi*

# Melodies of developing cortical networks

Heiko J. Luhmann[1]

[1]Institute of Physiology and Pathophysiology, University Medical Center of the Johannes Gutenberg University Mainz, Duesbergweg 6, D-55128 Mainz

During early stages of development, immature neuronal networks in different brain regions (retina, hippocampus, cerebral cortex etc.) show distinct patterns of spontaneous and evoked electrical activity (Khazipov and Luhmann, 2006). In conventional in vitro brain slice preparations of the developing cerebral cortex, these patterns have been termed "cortical songs" (Ikegaya et al., 2004). We provide evidence that distinct spontaneous and stimulus-evoked network patterns can be also observed under highly artificial conditions in developing networks of dissociated neurons (Sun et al., 2010), as well as in the cerebral cortex of immature rats in vivo (Yang et al., 2009). The molecular and cellular mechanisms underlying these early activity patterns (Dupont et al., 2006) and the role of certain neurons acting as "hub stations" will be discussed (Kanold and Luhmann, 2010).

Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ (2006) Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature 439:79-83.

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Sun JJ, Kilb W, Luhmann HJ (2010) Self-organization of repetitive spike patterns in developing neuronal networks in-vitro. Eur J Neurosci in press.

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# Optogenetic analysis of small neuronal networks in *Caenorhabditis elegans*

Alexander Gottschalk[1,2]

[1]Goethe Universität, Frankfurt, Germany

Understanding how neuronal networks generate behaviour is of key interest in the neurosciences. We analyze simple neuronal circuits that generate behaviour in the nematode *C. elegans*, which has only 302 neurons with precisely known physical connectivity. To analyze individual neurons within circuits, we use optogenetic methods. Neurons are genetically modified to express light-gated ion channels or pumps (Channelrhodopsin-2, Halorhodopsin, Bacteriorhodopsin), allowing depolarization or hyperpolarization of the respective neuron by illuminating it with light of different colours. Since *C. elegans* is transparent, this is done completely non-invasively, in freely behaving animals.

To achieve cell-specific expression, we use combinations of promoters with expression patterns overlapping in the cell of interest only, by *Cre* or FLP recombinases. In cases where this is not possible, we use structured illumination, in freely moving animals, to activate just the neuron of interest. Neuronal signalling in the circuit is also analyzed by electrophysiology, coupled with photo-stimulation of the neurons of interest. However, the latter requires dissection of the animals, so observations here can only indirectly be correlated to behaviour.

Circuits under study involve sensory neurons (mechanosensitive and nociceptive), as well as the downstream command interneurons that integrate the sensory signals to evoke escape responses, in turn executed by motor neurons. We could recapitulate signalling in the sensory-interneuron network in freely moving animals optogenetically, i.e. optically activating sensory or command neurons, but also optically blocking escape responses induced by optical stimulation of mechanosensors. We find that mechanosensors for gentle stimuli show pronounced habituation to repeated photo-stimulation, just as for their natural mechanosensitive responses. However, neither chemo- nor mechano-nociceptive neurons show appreciable habituation. Interestingly, also the interneurons that integrate several sensory inputs show no or only very little habituation, yet, cross-modal habituation appears to occur: repeated photo-stimulation of chemo-nociceptive neurons causes a (partial) habituation also of the mechanical response in mechanosensory neurons.

Lastly, we study the circuitry downstream of the command neurons. These cells innervate the motor neurons, which in turn cause coordinated locomotion by contralateral excitation and inhibition of muscle cells. Repeated units of motorneurons are found along the worm's body, and these may represent local pattern generators. The motor neuron units are all innervated by the same command interneuron, thus interneuron activity triggers activity in the motor units simultaneously (and not sequentially). Therefore it is not clear how activity between adjacent motor units is coordinated. We find that photoactivation of command neurons evokes activity patterns, sometimes even rhythmic, in the motor neurons, as evidenced by electrophysiology. Our findings speak for several independent pattern generators, as opposed to a central pattern generator; coordination of motor units may be achieved by mechanical feedback from the neighbouring body segment.

# SENSORY MAP FORMATION IN THE DROSOPHILA BRAIN

Thomas Hummel[1], Özkan Aydemir[1], Anna Brochtrup[1], Britta Kuhlmann [1]

[1]Westfälische Wilhelms-Universität, Institut für Neurobiologie, Badestr. 9, 48149 Münster, Germany

In the olfactory system, sensory neurons are characterized by the expression of a single type of odorant receptor (OR) and axonal connections to a single synaptic glomerulus, thereby providing the anatomical basis for odor recognition in the brain. In contrast to the mouse olfactory system, the Drosophila odorant receptors are dispensable for the development of sensory and synaptic specificity, raising the question of how these two differentiation programs are coordinated.
In a mosaic screen for genes that control Drosophila olfactory receptor neuron (ORN) differentiation we identified several transcription factors that regulate distinct aspects of ORN differentiation. Here we describe the functional characterization of Psc and Sequoia in the specification of three lineage-related ORN classes. In Psc mutants these three ORN classes show a mixing of their axonal projection inside their synaptic glomeruli and a switch in OR expression. Interestingly, the level of Psc in ORN precursors determines OR class identity. In contrast to the specific switch in OR expression in Psc mutants, loss of Sequoia leads to the co-expression of two ORs in a single sensory neuron. Double mutant analysis suggests that Psc and Sequoia function in a sequential manner in ORN development, in which Psc determines the OR ground state and Seq subsequently restrict the expression to a single OR type. Interestingly, in the Drosophila visual system, Psc and Sequoia control photoreceptor neuron differentiation in a mechanism distinct from the one identified in ORNs, providing new insights into how genes establish region-specific sensory maps in the brain.

# Sending signals from the synapse to the nucleus

Michael R. Kreutz[1], Anna Karpova[1], Marina Mikhaylova[1]

[1]PG Neuroplasticity, Leibniz Institute for Neurobiology, Brenneckestr.6, 39118 Magdeburg, Germany

The activation of N-Methyl-D-aspartate (NMDA)-receptors at synaptic and extrasynaptic sites impacts opposite occurrences. While synaptic NMDA-receptors provide plasticity and cell survival signals, recent studies have provided strong evidence that their extrasynaptic counterparts trigger neurodegeneration in various brain disease states. This fundamental dichotomy in terms of cell signaling is reflected by a fundamentally different gene expression. At present, however, it is essentially unclear how plasticity and survival as compared to degenerative signals are transduced to the nucleus. We now show that Jacob is a protein messenger that enters the nucleus following activation of both types of receptors and induces either cell death or survival depending on the phosphorylation of a serine at position 180 by the MAP-kinase ERK. In both cases the nuclear accumulation of Jacob requires signaling of NR2B-containing NMDA-receptors. However only synaptic NR2B-receptors induce the phosphorylation of Jacob at this crucial position and the trafficking of Jacob from synaptic and distal dendritic sites after activation of synaptic but not extrasynaptic NR2B-receptors depends upon ERK-phosphorylation. The nuclear overexpression of a phospho-mimicking mutant of Jacob prevents cell death after extrasynaptic NMDA-receptor activation and induces the expression of plasticity-related genes. The Janus face of Jacob is based on MAP-kinase activity and ERK-dependent phosphorylation decides whether Jacob is a messenger of cell survival or cell death after activation of synaptic or extrasynaptic NR2B-containing NMDA-receptors.

# The role of chromatin plasticity in neuropsychiatric diseases

Andre Fischer[1]

[1]Laboratory for Aging and Cognitive diseases, European Neuroscience Institute Göttingen, Grisebachstr. 5, 37077 Göttingen

The remarkable capability of the nervous system of animals to adapt their behavioral responses to ever-changing environmental conditions is thought to rely on the plasticity of neuronal circuits and synaptic connections. Cognitive function also critically depends on differential gene -expression that is required to shape neuronal circuitries. The availability of genes for transcription involves chromatin remodeling via epigenetic mechanisms. Especially, epigenetic mechanisms such as DNA methylation and histone acetylation have been shown to be important for memory processes in the adult brain. There is now accumulating evidence that altered chromatin plasticity and histone acetylation are also involved in cognitive funtion, age -associated memory loss, neurodegeneration and neuropsychiatric diseases. Histone deacetylase (HDAC) inhibitors exhibit neuroprotective and neuroregenerative properties in animal models of various brain diseases. As such, targeting of HDACs seems to be a promising therapeutic strategy. In this presentation, we will present novel data on the specific roles of individual HDAC proteins and the possible function of distinct histone modifications in the adult brain. As such, a specific emphasis will be on the role of selective epigenetic processes during memory function and brain diseases.

# Stonin2-dependent endocytic sorting of synaptotagmin 1 provides evidence for loss of synaptic vesicle identity during exo-endocytosis

Natalia L. Kononenko[1,2], Tanja Maritzen[2], Seong Joo Koo[2], M. Kasim Diril[2], Nadja Jung[2], Volker Haucke[1,2]

[1]Institute of Chemistry and Biochemistry, Department of Membrane Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany

[2]Charite University Medicine Berlin, Neurocure, Germany

Synaptic transmission depends on the regulated exo -endocytosis of presynaptic vesicles (SVs), specialized organelles that store and secrete non-peptide neurotransmitters. How precisely SV identity is maintained over multiple rounds of vesicle cycling has been debated controversially. Whereas some studies have suggested that SVs maintain their identity during exo-endocytosis others have suggested intermixing of newly exocytosed SV proteins with a surface-stranded pool on the presynaptic plasmalemma. Moreover, endocytic mutants analyzed so far have displayed generalized defects in the endocytic retrieval of SV proteins, suggesting that recycling may involve clustered SV proteins, in line with data obtained by STED microscopy. We report here on the phenotypic characterization of knockout mice, lacking the synaptotagmin-specific endocytic sorting adaptor stonin 2. Stonin2 KO mice, although viable and fertile, display signs of progressive degeneration including an abnormal dilatation of the lateral ventricle and a partial loss of the lateral septum. These defects appear to be caused by the partial and selective loss of the stonin 2 binding partners AP2 and synaptotagmin 1 from the CA3 region of the hippocampus of KO animals. These alterations correlate with a strong accumulation of plasma membrane-stranded synaptotagmin 1 in brain slices of stonin2 KO mice and impaired endocytic retrieval of synaptotagmin-pHluorin but not synaptobrevin -pHluorin from mutant terminals of postnatal hippocampal neurons in culture. We will discuss the implications of these and other data for the mechanism of SV retrieval at central synapses in the mammalian brain.

# Shaping membranes – Roles in neuromorphogenesis, vesicle formation and neuronal network activity

Britta Qualmann[1], D. Koch[1], I. Spiwoks-Becker[2], T. Dugladze[3], V. Sabanov[4], A. Stellmacher[1], R. Ahuja[1], J. Grimm[1], S. Schüler[1], A. Müller[1], F. Augenstein[5], T. Ahmed[4], A. Diesler[6], M. Moser[7], S. tom Dieck[8], R. Spessert[6], T. M. Boeckers[9], R. Faessler[7], D. Balschun[4], T. Gloveli[3], M. M. Kessels[1]

[1]Institute for Biochemistry I, University Hospital Jena - Friedrich Schiller University of Jena, 07743 Jena, Germany
[2]Institute for Microanatomy and Neurobiology, Johannes Gutenberg-University of Mainz, 55128 Mainz, Germany
[3]Cellular and Network Physiology Group, Institute of Neurophysiology, Charité Universitätsmedizin Berlin, 10117 Berlin, Germany
[4]Laboratory of Biological Psychology, Department of Psychology, Katholieke Universiteit Leuven, Leuven, 3000, Belgium
[5]Non-invasive brain imaging, Leibniz Institute for Neurobiology, 39118 Magdeburg, Germany
[6]Institute for Clinical and Functional Anatomy, Johannes Gutenberg-University of Mainz, 55128 Mainz, Germany
[7]Department of Molecular Medicine, Max Planck Institute of Biochemistry, 82152 Martinsried, Germany
[8]Max Planck Institute for Brain Research, 60528 Frankfurt, Germany
[9]Institute for Anatomy and Cellbiology, University of Ulm, 89081 Ulm, Germany

Synaptic transmission relies on effective and accurate compensatory endocytosis. F-BAR proteins are predestined to serve as membrane curvature sensors and/or inducers thereby supporting membrane remodeling processes - yet, these functions remained to be tested in vivo. To analyze F-BAR domain functions and their requirement in vesicle formation processes in vivo we have set out to generate a knockout mouse of the brain specific F-BAR domain containing protein syndapin I.

Our data demonstrate that the F-BAR protein syndapin I is crucial for proper brain function, as knockout mice suffer from altered hippocampal network activity manifesting in epileptic seizures. Loss of syndapin I causes defects in presynaptic membrane trafficking processes evident by loss of synaptic vesicle size control and accumulation of endocytic intermediates most striking upon high-capacity retrieval. Our detailed molecular and mechanistic analyses furthermore show that syndapin I acts as pivotal membrane anchoring factor for dynamins in the regeneration of synaptic vesicles. Syndapin I thus plays a crucial role in the correct and timely recruitment of the vesicle fission machinery particularly during high-capacity retrieval indispensable for proper function of the mammalian brain.

# DEFINING THE NEURONAL CIRCUITRY OF FEAR

Andreas Lüthi[1]

[1]Basel (Switzerland)

No abstract available

# Poster Topics

T1 Stem cells, Neurogenesis and Gliogenesis

T2 Axon and Dendrite Development, Synaptogenesis

T3 Developmental Cell Death, Regeneration and Transplantation

T4 Neurotransmitters, Retrograde messengers and Cytokines

T5 G Protein-linked and other Receptors

T6 Ligand-gated, Voltage-dependent Ion Channels, and Transporters

T7 Synaptic Transmission, Pre- and Postsynaptic organization

T8 Synaptic Plasticity, LTP, LTD

T9 Glia, Glia-Neuron Interactions

T10 Aging and Developmental Disorders

T11 Alzheimer's, Parkinson's and other Neurodegenerative Diseases

T12 Neuroimmunology, Inflammation, and Neuroprotection

T13 Cognitive, Emotional, Behavioral State Disorders and Addiction

T14 Vision: Invertebrates

T15 Vision: Retina and Subcortical Pathways

T16 Vision: Striate and Extrastriate Cortex, Eye Movement and Visuomotor Processing

T17 Auditory Mechanoreceptors, Vestibular, Cochlea, Lateral Line and Active Sensing

T18 Auditory System: Subcortical and Cortical Processing

T19 Chemical Senses: Olfaction, Taste, Others

T20 Somatosensation: Touch, Temperature, Proprioception, Nociception

T21 Motor Systems

T22 Homeostatic and Neuroendocrine Systems, Stress Response

T23 Neural Networks and Rhythm Generators

T24 Attention, Motivation, Emotion and Cognition

T25 Learning and Memory

T26 Computational Neuroscience

T27 Techniques and Demonstrations

# Poster Topic

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T1-1A Altered densities of defined GABAergic interneuron populations in polysialic acid-deficient mice
*Tim Kröcher, Iris Röckle, Birgit Weinhold, Hannelore Burkhardt, Herbert Hildebrandt*

T1-2A Brain regeneration potential is directly linked to adult neurogenesis in zebrafish
*Jan Kaslin, Volker Kroehne, Francesca Benato, Francesco Argenton, Michael Brand*

T1-3A Characterization of the role of FoxG1 in Tgfß-dependent neuronal differentiation
*Riccardo Vezzali, Kerstin Krieglstein, Tanja Vogel*

T1-4A Chromatin Remodeling Mediated by BAF170 - Pax6 Interaction Controls Direct -versus-Indirect Cortical Neurogenesis
*Tran Cong Tuoc, Mara-Elena Pitulescu, Anastassia Stoykova*

T1-5A Co-culture of human neural progenitors with rat hippocampal brain slices: influence of neural environment on differentiation
*Peter Morgan, Andrea Liedmann, Arndt Rolfs, Moritz Frech*

T1-6A Contrary results with different commercially available CD133 antibodies in brain tumors: How can results be interpreted?
*Christina Pfister, Heike Pfrommer, Susan Noell, Jens Schittenhelm, Antje Bornemann, Florian Roser*

T1-7A Differentiation and Survival of Human Neural Progenitor Cells in self-assembling peptide hydrogel 3D Scaffolds
*Andrea Liedmann, Peter Morgan, Arndt Rolfs, Moritz J. Frech*

T1-8A Effects of neuronal noise and population heterogeneity on population coding in electrosensory systems
*Jan Grewe, Henriette Walz, Jan Benda*

T1-9A Ephrin-B3 reverse signaling regulates the tangential migration of cortical interneurons in the basal telencephalon
*Judith Rudolph, André Steinecke, Geraldine Zimmer, Jürgen Bolz*

T1-10A FGF-2 deficiency causes defects in adult hippocampal neurogenesis, which are not rescued by exogenous FGF-2
*Sandra Werner, Klaus Unsicker, Oliver von Bohlen und Halbach*

T1-11A Functional Characterization of Satb1 in Neocortical Development
*Swathi Srivatsa, Olga Britanova, Paraskevi Sgourdou, Victor Tarabykin*

T1-12A Glial cells and the development of the central complex in the embryonic grasshopper *Schistocerca*

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*Michael Hans Loser, Yu Liu, George Boyan*

T1-1B Glycinergic signaling during postnatal neurogenesis in the SVZ
*Jean-Claude Platel, Severine Stamboulian, Frank Zufall*

T1-2B Hox genes in the pre-specification of sympathetic and parasympathetic ciliary neuron progenitors
*Leslie Huber, Jutta Stubbusch, Marius Ferdin, Hermann Rohrer*

T1-3B Impaired neuronal differentiation caused by 2,4 Dicholorophenol in NTera2/D1 cells correlates to reduced expression of Cx43 and functional gap junction coupling
*Bernhard Reuss*

T1-4B Impairment of adult hippocampal neurogenesis alters hippocampus-dependent tasks but not learning
*Paulina Jedynak, Leszek Kaczmarek, Robert K. Filipkowski*

T1-5B In vitro tests for developmental neurotoxicity
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T1-6B In vivo evidence for purinergic control of adult neurogenesis
*Herbert Zimmermann, Kristine Gampe, Anne Schänzer, Karl-Heinz Plate, Simon C. Robson*

T1-7B miR-128: a pleiotropic regulator of neuronal translation
*Eleonora Franzoni, Heiko Fuchs, Srinivas Parthasarathy, Victor Tarabykin, F.Gregory Wulczyn*

T1-8B Neurogenesis from androgenetic and biparental mouse ES cells.
*Wanja Wolber, Soon Won Choi, Sigrid Eckardt, John K. McLaughlin, Christian Geis, Manfred Heckmann, Albrecht Müller, Anna-Leena Sirén*

T1-9B Novel regulatory mechanisms of Schwann cell maturation
*André Heinen, Nevena Tzekova, Hans-Peter Hartung, Patrick Küry*

T1-10B Ontogeny of hippocampal neurogenesis and spatial learning in cyclin D2KO mice
*Anne Ansorg, Otto W. Witte, Anja Urbach*

T1-11B Overexpression of the Chondroitinsulfotransferases Chst 3, Chst 7 and Ust in Cortical Neural Stem Cells
*Denise Harrach, Alexander von Holst*

T1-12B A role for Reelin and Notch1 cooperation during hippocampal development and in the adult
*Mirjam Sibbe, Onur Basak, Ute Häussler, Carola Haas, Verdon Taylor, Michael Frotscher*

T1-1C POSITIVE CORRELATION BETWEEN CLIC1 FUNCTIONAL EXPRESSION AND HUMAN GLIOMA AGGRESSIVENESS
*Nicoletta Savalli, Matteo Setti, Marina Angelini, Daniela Osti, Giuliana Pelicci, Michele Mazzanti*

T1-2C Purinergic receptor-mediated $Ca^{2+}$ signaling in the olfactory bulb and the neurogenic area of the lateral ventricles
*Thomas Hassenklöver, Philipp Schulz, Anna Peters, Peter Schwartz, Detlev Schild, Ivan Manzini*

T1-3C Regulation of astrocyte maturation by the extracellular matrix molecule Tenascin C
*Michael Karus, Stefan Wiese, Andreas Faissner*

T1-4C Regulation of neurotrophin secretion in hippocampal neurons by CAPS1
*Robert Eckenstaler, Thomas Munsch, Volkmar Lessmann, Tanja Brigadski*

T1-5C Regulators of miRNA biogenesis control neural differentiation
*Anna Maria Rohde, Duong Nguyen, Elisa Cuevas, Heiko Fuchs, Agnieska Rybak, F. Gregory Wulczyn*

T1-6C Serotonin Transporter Knock-out and Chronic Mild Stress: Impact on Adult Neurogenesis in the Hippocampus
*Margherita Maria Lee, Sandy Popp, Eva Gerten, Antonia Post, Marion Winnig, Klaus Peter Lesch, Angelika G. Schmitt*

T1-7C THE ROLE OF CORTICAL FEEDBACK SIGNALS IN REGULATING PROGENITOR CELL-FATE SWITCH DURING NEOCORTICOGENESIS
*Srinivas Parthasarathy, Anjana Nityanandam, Victor Tarabykin*

T1-8C The role of orphan nuclear receptor Nurr1 (nr4a2) in layer specification of the cerebral cortex
*Dmitriy Lanshakov, Victor Tarabykin*

T1-9C The STAR Family Proteins Sam68, Slm -1 and Slm -2 Differentially Regulate Proliferation and Differentiation of Cortical Neural Stem/Progenitor Cells
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T1-10C The transcription factors AP-2ß and AP-2a are required for survival of sympathetic progenitors and differentiated sympathetic neurons
*Mirko Schmidt, Leslie Huber, Afsaneh Majdazari, Günther Schütz, Trevor Williams, Hermann Rohrer*

T1-11C The zinc-finger homeodomain factor Teashirt1 (Tshz1) controls
the layering and differentiation of olfactory bulb granule cells
*Alistair Neil Garratt, Elena Rocca, Carmen Birchmeier*

T1-12C Unravelling effects of Tgfß mediated target genes on forebrain neuronal progenitor cells of different developmental stages
*Shalaka Dhanraj Wahane, Kerstin Krieglstein, Tanja Vogel*

T1-13C Mechanism of the reversal of neurobehavioral teratogenicity in mice with neural stem cells
*Joseph Yanai, Adi Pinkas*

# Altered densities of defined GABAergic interneuron populations in polysialic acid-deficient mice

Tim Kröcher[1], Iris Röckle[1], Birgit Weinhold[1], Hannelore Burkhardt[1], Herbert Hildebrandt[1,2]

[1]Hannover Medical School, Institute of Cellular Chemistry, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Center for Systems Neuroscience (ZSN) Hannover

The neural cell adhesion molecule NCAM and its posttranslational modification with polysialic acid (polySia) are major determinants of cellular interactions during brain development and plasticity. Polysialylation of NCAM is implemented by the two polysialyltransferases ST8SiaII and ST8SiaIV. In humans, variations in the genes for NCAM and ST8SiaII have been linked to schizophrenia. Genetic ablation of ST8SiaII and ST8SiaIV generates polySia-negative but NCAM-positive mice that are characterized by severe defects of major brain fiber tracts. In addition, polySia deficiency impairs migration of subventricular zone-derived interneuron precursors towards the olfactory bulb and of undefined progenitors during neocortex development. In the current study, we analyzed how loss of polySia affects selected interneuron populations in brain regions relevant to the pathophysiology of schizophrenia. Densities of cells immunoreactive for major interneuron markers were comparatively analyzed in the forebrain of *St8sia2* and *St8sia4* single and double knockout mice. Pronounced but distinct alterations of different GABAergic interneuron subtypes were detected in the prefrontal cortex, hippocampus and olfactory bulb of these mouse lines with different degrees of polySia-deficiency during brain development. The findings reveal that compromised polysialic acid synthesis causes aberrations of defined interneuron subtypes, which are reminiscent to neuropathological findings in schizophrenia.

# Brain regeneration potential is directly linked to adult neurogenesis in zebrafish

Jan Kaslin[1], Volker Kroehne[1], Francesca Benato[2], Francesco Argenton[2], Michael Brand[1]

[1]Biotechnology center/Center for Regenerative Therapies Dresden, TUD, Tatzberg 47, 01307 Dresden, Germany

[2]Dipatimento di Biologia dell'Università degli Studi di Padova, Via U. Bassi 58b, 35131 Padova, Italy

It is well established that the mammalian brain has very limited regeneration capacity. In contrast to mammals, bony fish can regenerate parts of their CNS, such as spinal cord and retina, but it is unclear if fish can regenerate different parts of the brain. Fish display widespread life-long neurogenesis. However, it is not known if all brain progenitor cell types persist throughout the adulthood and if all cell types are produced.
To address this we used the zebrafish cerebellum and cerebellar lesions as a model to study the regeneration capacity of zebrafish CNS and to understand if endogenous progenitors participate in the regenerative process. By using transgenic zebrafish and molecular markers we show that endogenous progenitor availability and potential are the major determining factors for CNS regeneration outcome in zebrafish. We found that rhombic lip derived progenitors remain active throughout life while ventricular zone derived progenitors show a significant loss of activity and potential during juvenile stages. Importantly, neither are all cerebellar cell types are produced nor do all cerebellar progenitor types remain active throughout life. Surprisingly, after cerebellar lesion only the cell types that are normally produced in the adult cerebellum were regenerated. This shows that the cerebellar progenitors remain committed to produce fixed lineages of cells even after injury. There is no evidence for de-differentiation of progenitors during regeneration. This is in contrast to other regenerating tissues in zebrafish were de-differentiation or epimorphic regeneration of lost cell types has been reported. These results illustrate the importance of understanding neural stem cell heterogeneity and the potential and limits of endogenous neural progenitors in CNS regeneration. Furthermore, it shows that CNS regeneration potential is closely linked to the presence of adult neurogenesis.

# Characterization of the role of FoxG1 in Tgfß-dependent neuronal differentiation

Riccardo Vezzali[1], Kerstin Krieglstein[2,3], Tanja Vogel[1]

[1]Department of Neuroanatomy, Centre for Anatomy, University Medical Center Göttingen, Germany
[2]Department of Molecular Embryology, Institute for Anatomy and Cell biology, Albert-Ludwigs University, Freiburg, Germany
[3]FRIAS, Albert-Ludwigs University, Freiburg, Germany

Transforming Growth Factor ß (Tgfß) is able to induce differentiation in cortical progenitor cells during embryonal neurogenesis by promoting the expression of p21Cip1, a gene which prevents transition from G1 to S phase, thus blocking cell cycle progression. In order to do so, Tgfß acts by activating Smad proteins, which in turn form a transcriptional complex with FoxO proteins and activate the expression of target genes. FoxG1, another member of Forkhead box family, can interact directly with the Smad/FoxO transcriptional complex, inhibiting p21Cip1 expression as well as cortical progenitor cells' differentiation. Thus FoxG1 is antagonising Tgfß signalling.
Tgfß not only exerts anti-mitotic effects through regulation of p21Cip1 expression, but also induces neuronal differentiation in cortical cells derived from E16.5 mouse brains. However, Tgfß-induced neurogenesis is inhibited in cortical cells isolated from E13.5 and in adult stem cells. In order to investigate possible antagonistic effects of FoxG1 and Tgfß, we studied Tgfß-mediated neuronal differentiation in FoxG1-deficient cortical cells. These data indicated that loss of FoxG1 at E13.5 mediates the competence to conduct neuronal differentiation upon Tgfß-signalling. To analyse for genes involved in neuronal differentiation in a Tgfß-/FoxG1-dependent manner, we performed microarray and real time RT-PCR analyses of cortical cells derived from E13.5 FoxG1-knockout mice and control animals. Among many regulated genes, our data showed a significant increase in p21Cip1 levels in mutant cells. However this increase in p21Cip1 expression was related to the loss of FoxG1 expression rather than to Tgfß treatment. Gene expression analyses on cells from FoxG1 knockout mice have also revealed that Tgfß treatment leads to regulation of many genes implicated in angiogenesis, neuronal differentiation, Wnt signalling pathway and other important processes. Interestingly, we also found regulation of genes which are expressed in developing hindbrain and midbrain. Genes with regulations also observable in a conditional FoxG1-cre/TgfßRII mouse mutant are chosen for further characterisation in vivo using immunohistochemistry and in situ hybridization.

# Chromatin Remodeling Mediated by BAF170 - Pax6 Interaction Controls Direct-versus-Indirect Cortical Neurogenesis

Tran Cong Tuoc[1], Mara-Elena Pitulescu[1, 2], Anastassia Stoykova[1, 3]

[1]Max-Planck-Institute for biophysical Chemistry, Research group: Molecular Developmental Neurobiology, Am Fassberg 11, 37077 Göttingen, Germany

[2]Current address: Max-Planck-Institute for Molecular Biomedicine, Münster, Germany

[3]Corresponding author: Anastassia Stoykova
Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany, E-mail: astoyko@gwdg.de

Radial glial progenitors (RGPs) produce the majority of cortical neurons, including intermediate progenitors (IPs), through both direct and indirect neurogenesis. The mechanisms that determine which of these two modes are engaged are unknown. Here, we show that Smarcc2, encoding the BAF170 subunit of the mSWI/SNF chromatin remodeling complex, is a Pax6-interacting protein that is transiently expressed in dividing apical RGPs during the period of massive IP generation. Loss of BAF170 function results in an initial decrease in generation of lower layer neuronal subtypes followed by an excessive generation of IPs to compensate for this decrease. Moreover, BAF170 elimination causes overproduction of neurons with upper layer fates. Mechanistically, BAF170 suppresses Pax6- dependent transcription and promotes activity of Pax6 target genes involved in proliferation/specification of IPs. Our findings reveal a molecular mechanism mediated by the SWI/SNF remodeling complex that is essential in controlling direct versus indirect neurogenesis decisions, and thereby shapes the cortical architecture.

# Co-culture of human neural progenitors with rat hippocampal brain slices: influence of neural environment on differentiation

Peter Morgan[1], Andrea Liedmann[1], Arndt Rolfs[1], Moritz Frech[1]

[1]University of Rostock, Albrecht-Kossel-Institute for Neuroregeneration, Gehlsheimer Strasse 20, D-18147 Rostock, Germany

A fundamental question for cell replacement therapies is how to promote the development of neural progenitor cells (NPCs) when transplanted into an environment lacking the appropriate developmental cues. With in vitro cell cultures it is possible to investigate the differentiation of NPCs under well controlled conditions. However, when transplanted into the brain progenitors are exposed to a very different environment, with a complex extracellular milieu and mature, active neural networks. How this affects their development is unclear. To examine this we have characterised the development of human fetal progenitor cells co-cultured with organotypical rat hippocampal brain slices. This system provides a complex neural environment, while at the same time allowing much greater control over conditions than would be possible in vivo.

Upon transplantation NPCs formed a distinctive pattern along the region of the hippocampal fissure or stratum-moleculare/lacunosum-moleculare. Differentiation into neurons could be detected, demonstrated by immunocytochemistry for betaIII-tubulin and the expression of Nav and Kv currents. However, in contrast to cell cultures very few cells expressed GFAP. Spontaneous postsynaptic currents could be detected in some cells, and pharmacologically induced by application of kainate, indicating that NPCs have the ability to form functional synapses. In contrast, NPCs growing on the membrane away from the slice failed to differentiate and express Nav or Kv currents, suggesting that it is the slice environment which is inducing differentiation.

These results highlight the potential of interactions with the host environment to regulate the development of transplanted NPCs, a greater understanding of which will be important if guided differentiation of NPCs in vivo is to be achieved.

# Contrary results with different commercially available CD133 antibodies in brain tumors: How can results be interpreted?

Christina Pfister[1], Heike Pfrommer[1], Susan Noell[1], Jens Schittenhelm[2], Antje Bornemann[2], Florian Roser[1]

[1]Department of Neurosurgery, University of Tübingen, Germany [2]Institute of Brain Research, University of Tübingen, Germany

Background:
CD133 (Prominin 1) is a five transmembrane domain glycoprotein, which is widely used for selection of brain tumor stem cells, although the biological function of CD133 is not well unterstood. It is also used as prognostic factor in brain tumors. CD133 expression in brain tumor tissue is correlated with poor prognosis. The most used CD133 antibodies target glycosylated epitopes, which are poorly characterized. Also higher expression of CD133 mRNA indicates the presence of CD133 protein despite the absence of glycosylated epitopes upon cell differentiation. Here we investigated CD133 antibodies for their applicability for reliable detection of CD133 as prognostic factor in brain tumors.
Materials and Methods:
Six different CD133 antibodies raised against epitopes mapping within an extracellular domain (both against glycosylated epitopes and unmodified epitopes) and intracellular domain were used for immunohistochemical staining and Western Blot analysis in different human brain tumours (meningiomas and gliomas). As positive control Y79 cell line was used. A human retinoblastoma cell line positive for several stem cell markers including CD133. As negative control HeLa (Human epithelial carcinoma cell line) was used, which is described as lacking CD133 expression in literature.
Results:
Immunohistochemical staining of CD133 displayed a wide range of results from strongly positive stainings to no stainings in the same tissue (meningioma, glioma, mamma -carcinoma and normal kidney tissue) using serial paraffin sections. One antibody mapping the C-terminus of CD133 showed no staining in 11 of 36 (31%) meningiomas, in 9 (25%) meningiomas sporadic cells were stained, in 8 (22%) meningiomas up to 10% of the cells were stained and in 8 (22%) meningiomas more than 10% of the cells were stained. In contrast one antibody mapping within the extracellular domain displayed no staining in 20 of 36 (56%) meningiomas and in 15 (42%) meningiomas sporadic cells were stained. Only one meningioma showed stainings in up to 10% of the cells. In Western Blot analysis five of six antibodies displayed bands at 97 kDa for positive control Y79 cell lysate and kidney. One widely used CD133 antibody showed false positive staining for HeLa cell line. Five of six antibodies showed no stainings for an malignant meningioma cell line, only the CD133 antibody, that showed false positive staining for HeLa cell line also displayed a specific band for CD133.
Conclusion:
All six analysed antibodies detected CD133 highly inconsistent independent of their extra- or intracellular epitope localisation. Antibodies displayed both false positive and false negative CD133 expression in distinct controls in both immunohistochemical stainings and Western Blot. These findings suggest that commercially available antibodies have a big influence in the obtained results. Therefore conclusions based on only one CD133 antibody should be examined carefully and verified with confirmed negative and positive controls.

# Differentiation and Survival of Human Neural Progenitor Cells in self-assembling peptide hydrogel 3D Scaffolds

Andrea Liedmann[1], Peter Morgan[1], Arndt Rolfs[1], Moritz J. Frech[1]

[1]University of Rostock, Albrecht-Kossel-Institute for Neuroregeneration (AKos), Gehlsheimer Strasse 20, D-18147 Rostock, Germany

The influence of 3 dimensional (3D) scaffolds on growth, proliferation and finally neuronal differentiation is of great interest in order to find new methods for cell-based and standardized therapies in neurological disorders, like stroke, spinal cord injury or neurodegenerative diseases. 3D structures are expected to provide an environment much closer to in vivo condition rather than 2D cultures.
In this project we used a human neural progenitor cell line, whereat the cells were embedded in the synthetic self-assembling peptide hydrogel RADA16. Different protocols of culture conditions (2D versus 3D) were compared to optimize growth and differentiation conditions of the cells. After differentiation, cells were stained for neuronal markers by standard immunocytochemical methods and total number of positive cells was analyzed by FACS. Highest amount of betaIII-tub positive cells was observed after 4 days of differentiation (3±0.45%) in 2D culture and after 7 days of differentiation (7±0.76%) in the 3D scaffolds. As the number of neuronal cells decreased over time in both systems, we additionally performed a quantification of the survival of the cells within the 2D system as well as in the 3D scaffolds whereat the amount of apoptotic cells was determined. Quantification revealed a significantly higher proportion (up to a 2.7 fold change after 7 days) of cells in the late apoptosis in the 2D system compared to the 3D scaffold system. The number of cells in early apoptosis, was not significantly different between cells cultured in the 2D system and 3D scaffolds. First results indicate that neuronal cells undergo apoptotic cell death. Further studies will elucidate if this accounts for the higher amount of neuronal cells in the 3D system or if an increased differentiation is responsible for the differences in 2D cell cultures and 3D scaffolds.

# Effects of neuronal noise and population heterogeneity on population coding in electrosensory systems

Jan Grewe[1], Henriette Walz[1], Jan Benda[1]

[1]Ludwig-Maximilians-Universität München, Department Biologie II, Großhadener Strasse 2, 82152 Planegg- Martinsried, Germany

The role of noise in neural information processing is still a controversial topic. The notion that intrinsic noise inevitably degrades information transmission still dominates the debate. However, in a population of spiking neurons noise can reduce redundancy and/or control the level of synchrony in response to certain stimulus features. Target neurons may selectively read out synchronous spikes only (coincidence detector) or unspecifically integrate over all input spikes, thus extract different aspects of the signal that stimulated the population of input neurons. In this view the amount of noise in a neural system might be optimally tuned to achieve the required signal processing properties.

Weakly electric fish are an ideal model system for tackling this question experimentally. They have two parallel electrosensory systems that share a similar architecture and process similar stimuli but exhibit different response characteristics/variability: the active electrosensory system receiving input from tuberous receptors and the passive one receiving input from ampullary receptors. Interestingly, the baseline discharge of P-units, the majority of the tuberous receptors, show a much larger variability of their interspike intervals than the one of the ampullary receptors. Thus, the target neurons of the respective systems receive input from populations of receptor neurons that discharge more (ampullary receptors) or less (tuberous receptors) regular.
Furthermore, we observe that both receptor populations show different degrees of heterogeneity. The receptors of the active electrosensory system vary considerably stronger with respect to their baseline-firing rate and response variability.

The two subsystems of the electrosensory organ are thus very well suited to allow for a comparative study on the role of noise and population heterogeneity. We here present, as a first step, recordings from the receptor neurons of the wave-type fish *Apteronotus leptorhynchus* and simulations of both systems. We compare basic response properties of both receptors and address by random amplitude modulations the tuning of both receptor types to dynamical stimuli and the influence of noise and heterogeneity on coding performance.

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# Ephrin-B3 reverse signaling regulates the tangential migration of cortical interneurons in the basal telencephalon

Judith Rudolph[1], André Steinecke[1], Geraldine Zimmer[1], Jürgen Bolz[1]

[1]Friedrich-Schiller-Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstr. 1, 07743 Jena, Germany

During cortical development most GABAergic interneurons are generated in the medial and caudal ganglionic eminences of the basal telencephalon and migrate towards the cortex along defined tangential routes, avoiding the striatum. Early in mouse development interneurons migrate ventrally of the striatum in the intermediate zone (IMZ), whereas at later stages they preferentially take a dorsal route in the subventricular zone (SVZ) of the basal telencephalon. This process is regulated by several guidance molecules on the migratory pathway or in flanking regions, for example by semaphorins and slits which act as repulsive cues for cortical interneurons. Another class of signaling molecules that is expressed in the basal telencephalon during interneuron migration are Eph receptor tyrosine kinases and their ligands, the ephrins. As EphB1 is expressed in the developing striatum during the period of migration, we examined its potential role in repulsion of cortical interneurons from the striatum via reverse signaling. We first performed grafting experiments, where explants of the medial ganglionic eminence (MGE) from EGFP -expressing transgenic mice were homotopically grafted into host slices from wild-type littermate embryos. After blocking ligands or receptors of the B -system, many interneurons invaded the striatal anlage. Moreover, experiments using the stripe assay revealed that MGE-derived interneurons avoid the EphB1 substrate. Thus EphB1 repels migrating neurons from the striatum via reverse signaling, i.e. the signaling cascade is triggered by a cell-bound ligand. To examine which ligand mediates this repulsion, we used siRNA transfection to selectively downregulate ephrin-B ligands, indicating that the EphB1 response is mediated by ephrin-B3 reverse signaling. Moreover, our data suggest that Src is required to mediate the repulsive effect of EphB1 on GABAergic interneurons of the IMZ. Inhibition of Src with PP2 switches the repulsive effect of EphB1 into attraction. In contrast Src inhibition has no effect on the growth of interneurons of the VZ/SVZ.
Together, these results suggest that ephrin-B3 reverse signaling mediates the repulsive activity of EphB1 that restricts cortical interneurons from entering the developing striatum and thus contributes to define the migratory route of cortical interneurons.

# FGF-2 deficiency causes defects in adult hippocampal neurogenesis, which are not rescued by exogenous FGF-2

Sandra Werner[1], Klaus Unsicker[2], Oliver von Bohlen und Halbach[3]

[1]Institute of Anatomy and Cellbiology, Department of Molecular Embryology, University of Freiburg, Albertstr. 17, 79104 Freiburg, Germany

[2]Institute of Anatomy and Cellbiology, Department of Molecular Embryology,University of Freiburg, Albertstr. 17, 79104 Freiburg, Germany

[3]Institute of Anatomy and Cell Biology, Ernst Moritz Arndt University of Greifswald, Friedrich Loeffler Strasse 23c, 17487 Greifswald, Germany

Neurogenesis within the adult brain is restricted to selected areas, one of which is the dentate gyrus (DG). Several growth factors have been reported to affect neurogenesis in the adult DG. However, a role of fibroblast growth factor–2 (FGF -2) in adult hippocampal neurogenesis has not been firmly established. We have analyzed neurogenesis in the DG using in vivo and in vitro approaches, including FGF-2 deficient mice and hippocampal organotypic slices. FGF -2-/- mice revealed no alterations in the number of phosphohistone H3 positive proliferating cells, but a significant decrease in the numbers of newly generated neurons identified by NeuroD, doublecortin and calretinin immunoreactivities. Results obtained with hippocampal slice cultures from FGF-2-/- mice supported the notion that loss of FGF-2 did not affect cell proliferation, but interfered with differentiation of new neurons. FGF-2 added to FGF-2-/- slices did not rescue the phenotype suggesting that FGF-2 knockout mice may be deficient in other factor(s), which may cooperate with FGF-2 in adult neurogenesis, or may have a reduced pool of stem cells in the adult DG. Although an increase in cell death of neurogenic cells in the FGF-2 deficient DG could not be specifically demonstrated, there was a massive increase in global cell death in FGF -2-/- hippocampal slice cultures compared to slices from wt mice. Cell death could not be prevented by addition of FGF-2 suggesting again that FGF -2 cooperated with additional factors in the maintenance of hippocampal cells. Neutralization of endogenous FGF-2 in hippocampal slices using specific antibodies did not interfere with neurogenesis in a short-term (12-day) paradigm. Together, our data suggest that FGF-2 is essentially required for maturation of new neurons in adult hippocampal neurogenesis, but is likely to operate synergistically in combination with other mechanisms/growth factors.

# Functional Characterization of Satb1 in Neocortical Development

Swathi Srivatsa[1], Olga Britanova[1], Paraskevi Sgourdou[1], Victor Tarabykin[1,2]

[1]Max Planck Institute for Experimental Medicine, Cortical Development Group, Hermann-Rein-Str 3, 37075 Göttingen, Germany
[2]Institute of Cell biology and Neurobiology,Charité-Universitätsmedizin Berlin

The mammillian neocortex is the most evolved structure in the central nervous system made up distinct neuronal subtypes. Cell-type specific gene regulation at the level of chromatin organization is an important aspect of such a cellular differentiation. Satb1 and Satb2, sharing 61% amino-acid homology, constitute a family of nuclear proteins that bind to the matrix attachment regions (MARs) in the DNA, dictating the organisation and structure of chromatin domains and thereby modulating the transcription potential of multiple genes.

We have previously characterized the expression and function of Satb2 in the developing neocortex. Satb2 is expressed in callosally projecting neurons of the cortex. In the absence of Satb2 these upper layer neurons lose their identity and activate a deep layer specific program. These neurons do not contribute to the corpus callosum but to the corticospinal track instead. In the absence of Satb2, Ctip2 (a gene required for the formation of cortico-spinal tract) is ectopically expressed in the upper layers.

The role of Satb1 has primarily been studied in the context of T-cell differentiation, where it has been shown that Satb1 acts as a global regulator, controlling the spatial and temporal expression of multiple genes and hence ensuring the proper development of this lineage. We now show that Satb1 is expressed in a subset of neurons in the neocortex. The expression of Satb1 is turned on at E16.5 bordering the cortical plate and by early postnatal stages it is expressed in the mature postmitotic neurons of mainly the deep layers (IV to VI). Our studies on the Satb1 knock-in mice, where Satb1 is incorporated into the Satb2 locus so as to deactivate Satb2 completely and replace its expression with Satb1, has revealed that all the Satb2 loss of function phenotype can be rescued. We are now interested in elucidating the role of Satb1 in nocortical development basing our studies on Satb1 conditional knock-out mice where its expression is preferentially deleted in postmitotic cortical neurons.

# Glial cells and the development of the central complex in the embryonic grasshopper *Schistocerca gregaria*

Michael Hans Loser[1], Yu Liu[1], George Boyan[1]

[1]Biozentrum LMU, Developmental Neurobiology AG Boyan, Großhadernerstr. 2, 82152 Martinsried, Germany

The neuroarchitecture of the central complex of the grasshopper *Schistocerca gregaria* has been extensively investigated in the adult and is being increasingly well described at both cellular and molecular levels in the embryo. Surprisingly little is known about the contribution of glia cells to the developmental program generating this structure, and this aspect is the focus of the present study. The axonal organization of the early embryonic midbrain of the grasshopper resembles that of the ventral nervous system (VNC), but at midembryogenesis a process known as fascicle switching initiates the chiasmal neuroarchitecture characteristic of the mature central body. At about this time, histological sections, and DAPI staining, reveal cells located at stereotypic locations in, and around, the neuropilar regions of the developing central complex where neurons are not conventionally found. Double staining with DAPI and HRP reveal that some of these cells are not neurons, and so could represent glia. These non-neuronal cells could subsequently be shown via double immunolabelling involving glia-specific markers (anti -repo, anti- glutamine synthetase) and HRP to in fact represent glia cells. We are currently investigating the distribution of these cells with respect to the various neuropils of the embryonic central complex. For example, glia cells putatively migrate to their future sites in the central complex from the pars intercerebralis and we are currently mapping their distribution and examining their ontogeny using BrdU incorporation and anti-phosphohistone 3 immunohistochemistry.

# Glycinergic signaling during postnatal neurogenesis in the SVZ

Jean-Claude Platel[1], Severine Stamboulian[1], Frank Zufall[1]

[1]Saarland University, Physiology department, Kirrberger Strasse, Bldg. 45.2, 66421 Homburg, Germany

Neurogenesis persists in the brain after birth in two main regions, the hippocampal dentate gyrus and the subventricular zone (SVZ). The SVZ contains the largest pool of dividing neural progenitors in the adult mammalian brain. These Neural progenitors give birth to immature neurons (called neuroblasts). Neuroblasts leave the SVZ, migrate along the rostral migratory stream (RMS) while being ensheathed by the process of SVZ astrocytes and reach the olfactory bulb where they differentiate into GABAergic interneurons. We have shown recently that expression and activation of specific neurotransmitter receptors, i.e. glutamate receptors, is critically involved in migration, proliferation and survival of migrating neuroblasts (Platel et al. 2008; Platel et al. 2010). Likewise, activation of GABA receptors has been shown to control many aspects of neurogenesis (Bordey 2007; Ge et al. 2007). These effects of GABA are due to the fact that the chloride gradient differs in immature versus mature neurons, causing GABA receptor activation to depolarize the membrane potential early in development. During embryonic development, a second chloride-permeable receptor, the glycine receptor, has also been shown to cause membrane depolarizations because of this chloride gradient (Flint et al. 1998). We are investigating the role of glycine receptors in different process of the postnatal neurogenesis: proliferation, migration, survival and integration of newborn neurons.

Bordey, A. (2007).

# Hox genes in the pre-specification of sympathetic and parasympathetic ciliary neuron progenitors

Leslie Huber[1], Jutta Stubbusch[1], Marius Ferdin[1], Hermann Rohrer[1]

[1]Max-Planck-Institute for Brain Research, RG Developmental Neurobiology, Deutschordenstr. 46, 60528 Frankfurt am Main, Germany

Autonomic neuron development is initiated by BMPs acting on neural crest cells in the primordia of both, sympathetic and parasympathetic ganglia. However, different patterns of transcription factors are induced by BMPs in sympathetic and parasympathetic ciliary ganglia, which indicates intrinsic differences between BMP-responsive progenitors in different autonomic ganglia (Müller and Rohrer, 2002).
Here, we have addressed the molecular basis of this pre-specification towards sympathetic and parasympathetic neuron fate. Anterior-posterior patterning of neural crest by *Hox* genes is a likely explanation, as *Hox* genes are expressed in the trunk but not in the mesencephalic region where ciliary ganglion progenitors originate. Screening for *Hox* genes that are expressed in chick sympathetic ganglia by RT-PCR and in situ hybridization identified a number of *Hox* genes observed in sympathetic neuron progenitors at E4 (HH23). Interestingly, expression was maintained in differentiated sympathetic neurons at least up to E8. To test whether the identity of parasympathetic ciliary progenitors could be altered by *Hox* genes present in trunk sympathetic ganglia, *HoxB8*, *HoxC8* and *HoxB1* were ectopically expressed in ciliary progenitors, using RCAS vectors. We used these *Hox* genes as examples for a *Hox* gene expressed (*HoxB8*) or not expressed (*HoxC8*) in sympathetic ganglia or expressed in the anterior region of the embryo (*HoxB1*).
During normal ciliary neuron development *Hand2* is virtually absent and *TH* and *DBH* are expressed only transiently, whereas *Hand2*, *TH* and *DBH* are continuously expressed in sympathetic neurons. Interestingly, the forced expression of all three *Hox* genes in ciliary progenitors leads to a strong induction of the bHLH transcription factor *Hand2*. However, only *HoxB8* is able to induce the noradrenergic marker genes *TH* and *DBH* from E5 at least up to E8.
Our findings that parasympathetic progenitors can be re-specified by *HoxB8* to noradrenergic, sympathetic neuron differentiation imply suggest that *Hox* genes expressed in trunk neural crest are involved in the pre-specification and/or noradrenergic differentiation of sympathetic progenitors.

# Impaired neuronal differentiation caused by 2,4 Dicholorophenol in NTera2/D1 cells correlates to reduced expression of Cx43 and functional gap junction coupling

Bernhard Reuss[1]

[1]University of Göttingen, Center for Anatomy - Neuroanatomy, Kreuzbergring 36, 37075 Göttingen, Germany

2,4 Dichlorophenol (2,4DCP) is the major soil degradation product of 2, 4 Dichlorophenoxy acetic acid (2,4D), a systemic herbicide widely used to control broadleaf weeds. 2,4DCP is known to affect neuronal differentiation and survival by yet not fully understood mechanisms. A system known to control early neuronal differentiation is intercellular communication by gap junctions (GJ), which is diminished during retinoic acid dependent neuronal differentiation of human derived NTera2/D1 cells. These cells were used here to clarify, whether 2,4D or 2,4DCP is able to modulate expression of the GJ protein Connexin(Cx)43 and functional GJ coupling. Cells were treated 2, 4, 6 and 8 days with up to 10 µmol/l of either 2,4D or 2,4DCP. Detection of Cx43 protein by immunocytochemistry and Western blot analysis revealed a significant time and concentration dependent downregulation of Cx43 immunoreactivity by 10µmol/l 2,4DCP but not 2,4D. Likewise, scrape loading with Lucifer yellow, revealed a significant reduction in GJ coupling by 10 µmol/l 2,4DCP, but not 2,4D. These results demonstrate a significant effect of 2,4DCP on amount and functionality of GJs in NTera2/D1 cells, suggesting a role of GJ communication in effects of this substance on neuronal differentiation. This project was supported by the medical faculty of the University of Göttingen (UMG).

# Impairment of adult hippocampal neurogenesis alters hippocampus-dependent tasks but not learning

Paulina Jedynak[1], Leszek Kaczmarek[1], Robert K. Filipkowski[2]

[1]Nencki Institute of Experimental Biology, Lab. of Molecular Neurobiology, Warsaw, Poland
[2]University of Finance and Management, Dept. of Cognitive Psychology, Warsaw, Poland

The functional role of adult brain neurogenesis remains elusive, although it has been suggested to play a role in learning processes and memory formation. Cyclin D2 gene knock-out (D2 KO) mice show 90 % reduction of newborn neurons when compared to WT animals. Furthermore, they have slight morphological abnormalities of the brain, including the hippocampal formation (Kowalczyk et al., J Cell Biol, 2004). Results from D2 KO mice suggest new hippocampal neurons are not obligatory for memory formation (Jaholkowski et al., Learn Mem, 2009). In the present study, the animals were subjected to behavioral tests previously shown to be involving intact hippocampal formation, but concerning none or little learning component. D2 KO mice showed significant impairment in digging, marble burying, and nest building. In particular, D2 KO mice were digging less robustly and burying less marbles when compared to WTs. Moreover, D2 KO animals were building no or poorer nests than WTs. In addition, D2 KO mice showed decreased than WTs explorative behavior in IntelliCage automated system as well as neophobia while exposed to novel conditions. These data support previous results from lesioned mice. On the other hand, locomotor functions of D2 KO mice were not impaired, and they showed normal anxiety levels. Presented results suggest that either anatomical abnormalities of the hippocampal formation or adult brain neurogenesis impairment (or both) alter hippocampal-dependant behaviors without influencing learning abilities.

# In vitro tests for developmental neurotoxicity using a human neuronal precursor cell line

Michael Stern[1], Andrea Gierse[1], Saime Tan[1], Gerd Bicker[1]

[1]University of Veterinary Medicine Hannover, Cell Biology, Bischofsholer Damm 15/102, 30173 Hannover, Germany

Current in vivo test methods for developmental neurotoxicity are laborious, costly and necessitate the use of high numbers of laboratory animals. The aim of this study was the development of standardized predictive cell-based in vitro assays as a possible replacement for in vivo tests. We employ clone D1 of the human teratocarcinoma cell line NTera-2, which has properties of a neuronal precursor line and can be induced to differentiate into terminally differentiated neurons within one month (Podrygajlo et al., Cell Tissue Res 2009, 336:439–452).

To assess developmental neurotoxicity, we established molecular and mechanistic endpoints for proliferation, differentiation, and migration. These endpoints were tested in the presence of compounds with known developmentally toxic potential (positive test compounds): methyl mercury, lead, arsenic, valproic acid (VPA), methylazoxy methanol acetate (MAM), and chlorpyrifos. As control substances with acute neurotoxic but not developmentally neurotoxic potential (negative test compounds), we used parathion, paracetamol, and glutamate. We focus our efforts onto applicability of the tests for high throughput screening in a 96-well format.

Proliferation of NT-2 precursor cells was measured with BrdU-incorporation assays and resazurin conversion assays after different substance exposure times. In both assays, lead, methyl mercury, chlorpyrifos, and MAM inhibit proliferation, whereas negative test compounds do not.

To assess differentiation into neurons, we examined the expression of the neuron specific marker ß-tubulin type III under treatment with test substances. Using quantitative immunofluorescence, we automatically evaluated expression using a fluorescence plate reader, confirmed the results by manual counting and compared the result to acute cytotoxicity determined by a viability assay. Chlorpyrifos, VPA, and MAM specifically inhibited neuronal differentiation, whereas methyl mercury and the negative control substances did not.

Cell migration was assayed by placing aggregates of differentiating NT2-cells, which form during culture under non-adherent conditions, onto an adherent substrate in the presence of test compounds (Tegenge and Bicker, 2009, J Neurochem. 110:1828-1841). Cell migration out of these aggregates was determined microscopically and compared to acute toxicity. We now employ a commercial cell invasion assay (Oris) to adapt the migration protocol to a 96-well format for high throughput screening and semi-automated analysis. In both assays, positive test substances specifically reduce migration whereas negative test compounds affect viability at the same concentrations that reduce cell migration.

In summary, we could perform in vitro tests for developmental endpoints (proliferation, differentiation, and migration) which are predictive for most of the effects of known developmental neurotoxins. Thus, the NTera-2 cell line offers a promising perspective towards establishing in vitro assays as replacement for in vivo developmental neurotoxicity tests in a high throughput screening scenario.

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# In vivo evidence for purinergic control of adult neurogenesis

Herbert Zimmermann[1], Kristine Gampe[1], Anne Schänzer[2], Karl-Heinz Plate[2], Simon C. Robson[3]

[1]Goethe-University Frankfurt, Institute of Cell Biology and Neuroscience, Max-von-Laue-Str. 9, 60438 Frankfurt am Main, Germany
[2]Goethe-University Frankfurt, Edinger Institute, Heinrich-Hofmann-Str. 7, 60528 Frankfurt am Main, Germany
[3]Harvard Medical School, Department of Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Ave. CLS 612, Boston, MA 02215,USA

In the adult mammalian CNS the subgranular layer of the hippocampus (SGL) and the subependymal zone (SEZ) at the lateral ventricles harbour progenitors that continuously provide new neurons for the granule cell layer of the hippocampus and the olfactory bulb, respectively. Adult neurogenesis involves a set of events including proliferation, cell fate determination, migration, differentiation, integration and survival of young neurons.
In the CNS nucleotides and their derivatives - once released from cells to the extracellular milieu - represent key mediators of cellular communication. They can act on a multiplicity of receptors (G protein-coupled P2Y and ionotropic P2X) that differ regarding agonist specificities. Nucleotide signalling can be terminated or modulated by cell surface-located nucleotide-hydrolyzing enzymes belonging to several enzyme families.
We have previously shown that the ectonucleoside triphosphate diphosphohydrolase 2 (NTPDase2), an enzyme that hydrolyzes nucleoside triphosphates, is specifically expressed by neural progenitors of the two neurogenic regions and that functional P2Y receptors are expressed in primary culture of adult neural stem cells (1,2,3). Furthermore nucleotides and the growth factor EGF stimulated in vitro progenitor cell proliferation and induced converging intracellular signalling pathways, implicating a possible role for nucleotides in adult neurogenesis (3,4).

Deletion of NTPDase2 should increase the extracellular concentrations of the P2 receptor agonists ATP or UTP in the vicinity of the NTPDase2-depleted cells and thus enhance any nucleotide-mediated effect on neurogenesis. Using NTPDase2 knockout mice and time-controlled protocols of BrdU application we investigated short-term progenitor cell proliferation and young neuron survival. As compared to wild type controls, progenitor cell proliferation was increased approx. twofold in both the SEZ and the dentate gyrus whereas young neuron survival in the olfactory bulb and the hippocampus was not significantly altered. Similarly, in vitro progenitor cell proliferation was enhanced in neurospheres derived from NTPDase2 knockout mice. Accordingly, cell proliferation was reduced in neurospheres derived from P2Y1 or P2Y2 receptor knockout mice. Young neuron survival was increased in the hippocampus of mice lacking the P2Y2 receptor.
These data provide first in vivo evidence for a contribution of purinergic signalling to the control of adult neurogenesis.
1. Braun et al. 2003, Eur J Neurosci, 17, 1355-1364; 2. Shukla et al. 2005, J Neurosci Res 80, 600-610; 3. Mishra et al. 2006, Development 133, 675-684; 4. Grimm et al. 2009, J Cell Sci 122, 2524-2533

# miR-128: a pleiotropic regulator of neuronal translation

Eleonora Franzoni[1], Heiko Fuchs[2], Srinivas Parthasarathy[1], Victor Tarabykin[1], F.Gregory Wulczyn[1]

[1]Charitè Universitätsmedizin Berlin, Centrum für Anatomie- Institutefür Zell- und Neurobiologie, Phillipstrasse 12 , 10117 Berlin, Germany
[2]Max-Planck Institute for Molecular Genetics

microRNAs (miRNA) are a large class of non-coding RNAs important for the regulation of the proteome mainly through silencing and/or degradation of mRNA. Over 400 miRNA genes have been annotated, they are expressed and function in all cells as regulators of many cellular processes. Several highly tissue specific miRNAs are known to coordinate cell-specific gene expression programs during development.
miR-128 is one of a small group of brain enriched, neuron-specific miRNAs. There is evidence for misregulation of miR-128 in glioma and neuroblastoma as well as other malignancies, but functional characterization of miR-128 in CNS development and function is only beginning. Like the paradigm neuronal miRNA, miR-124, miR-128 is not expressed in radial progenitors but is induced upon neuronal differentiation. Deep sequencing of synaptosomal miRNA revealed high levels of miR-128. Consistent with this expression pattern, we have identified and verified several target mRNAs for miR-128 that are subject to direct translational repression. miR-128 target gene interactions are likely to influence development (FoxP2, Reelin), growth control (Aff4, Casc3, Phf6) and activity (Adora2a, Adora2b, RpsKa5). To better understand miR-128 roles in synaptic plasticity and cortex development we are performing in situ hybridization and gain - and loss -of-function experiments by in utero electroporation in the mouse cortex.

# Neurogenesis from androgenetic and biparental mouse ES cells.

Wanja Wolber[1], Soon Won Choi[2], Sigrid Eckardt[3], John K. McLaughlin[3], Christian Geis[4], Manfred Heckmann[5], Albrecht Müller[2], Anna-Leena Sirén[1]

[1]University of Wuerzburg, Department of Neurosurgery, Wuerzburg, Germany
[2]Center for Experimental Molecular Medicine (ZEMM), University of Wuerzburg, Wuerzburg, Germany
[3]Nationwide Childrens Research Institute, Columbus, Ohio, USA
[4]University of Wuerzburg, Department of Neurology, Wuerzburg, Germany
[5]University of Wuerzburg, Department of Physiology, Wuerzburg, Germany

Uniparental pluripotent embryonic stem (ES) cells enable the study of parent-of-origin-specific influences on tissue development and may serve as a potential source of histocompatible cells for regenerative medicine. Uniparental zygotes with two paternal (androgenetic; AG) or two maternal (gynogenetic: GG; parthenogenetic: PG) genomes are not competent to develop into viable offspring but can form blastocysts from which ES cells can be derived. While many aspects of the in vitro and in vivo differentiation potential of PG and GG ES cells of several species have been studied, the developmental capacity of AG ES cells is much less clear. Here, we investigated the potential of murine AG ES cells to undergo neural differentiation.

We observed that AG ES cells were similar to biparental (normal fertilized: N) ES cells in their capacity to differentiate in vitro into pan-neural progenitor cells (pNPCs) and further gave rise to cells that express a variety of neuronal- and glial-specific markers including ß-III-tubulin, NeuN, TH, PITX3, Synapsin 1, GFAP or O4. Electrophysiological analyses performed in voltage clamp conditions demonstrated the ability to generate sodium driven action potentials. Neural progeny of in vitro differentiated AG ES cells exhibited fidelity of expression of imprinted brain genes. Bisulfite-sequencing for two imprinting control regions suggested that pNPCs predominantly maintained their methylation pattern.

Our results indicate that AG ES cell-derived neural progenitor/stem cells do not differ from N neural progenitor/stem cells in their self-renewal and neural multi-lineage differentiation potential.

# Novel regulatory mechanisms of Schwann cell maturation

André Heinen[1], Nevena Tzekova[1], Hans-Peter Hartung[1], Patrick Küry[1]

[1]Heinrich Heine University, Department of Neurology, Moorenstr. 5, 40225 Düsseldorf, Germany

Schwann cells are the myelinating glial cells of the peripheral nervous system (PNS) and their differentiation is accompanied by a coordinated expression of maturation promoting factors, while proteins which negatively regulate the differentiation program are inhibited. Similar to nerve development, stimulatory and inhibitory factors also regulate Schwann cell redifferentiation and myelination during spontaneous peripheral nerve regeneration. Both, developmental differentiation as well as de- and redifferentiation following peripheral nerve injury require a coordinated switch of the gene expression program and suggest that epigenetic mechanisms might control development and maintenance of the myelin sheath. Epigenetic mechanisms comprise histone modifications such as methylation and acetylation and DNA methylation, controlling the transcriptional availability and thus gene activity, as well as posttranscriptional control exerted by the expression of microRNAs. Several recent studies revealed these mechanisms to be involved in oligodendrocyte maturation, the myelinating glial cells of the central nervous system (CNS), whereas little is currently known about their role in peripheral nerve myelination.

We have recently identified the p57kip2 factor as an inhibitor of both Schwann cell as well as oligodendroglial cell differentiation (Heinen et al., PNAS 2008; Kremer et al., PNAS 2009). Suppression of p57kip2 by means of vector based RNA interference leads to morphological changes and accelerated glial cell differentiation, marked by myelin gene and protein induction and a large scale switch in gene expression towards differentiation. These remarkable changes suggest an involved epigenetic regulatory mechanism, possibly controlling p57kip2 expression.

In the present study, we focused on the function of Polycomb group (PcG) genes, which serve as key components for the recruitment of DNA methyltransferases, histone methyltransferases and deacetylases. We describe the influence of a PcG member on rat Schwann cell maturation. Based on studies with vector based long-term gene suppression and concomitant selection of transfected cells, we observed that a shRNA dependent knockdown of this PcG gene interferes with morphological maturation and that it also blocks myelin gene expression. These effects are accompanied by induction of p57kip2 gene expression, which then might interfere with Schwann cell differentiation and maturation. This assumption is supported by the fact that changes in morphology and gene expression can be reversed by suppression of p57kip2 expression levels. We have therefore identified a novel regulatory mechanism of myelinating glial cell differentiation, which appears to be involved in repressing the activity of naturally occurring inhibitory factors.

# Ontogeny of hippocampal neurogenesis and spatial learning in cyclin D2KO mice

Anne Ansorg[1], Otto W. Witte[1], Anja Urbach[1]

[1]University Hospital Jena, Hans Berger Clinic for Neurology, Erlanger Allee 101, 07747 Jena, Germany

In the adult brain, the ability to generate new neurons is limited to the subventricular zone of the lateral ventricle (SVZ) and the subgranular zone of the hippocampal dentate gyrus (SGZ). Considering the role of the hippocampus in learning and memory, it is likely that these new neurons might play a critical role in the formation and consolidation of spatial memories. Although a multitude of different models for studying these newborn cells gave rise to a better understanding of the underlying mechanisms, the functional relevance of adult neurogenesis still remains ambiguous.

To address this issue, we use a transgenic mouse model, in which the cyclin D2 gene was inactivated by excision of exons I and II (cD2KO)[1]. The three D-type cyclins (cD1, cD2, cD3) are expressed in most proliferating cells where they regulate cell cycle progression by specifically activating cyclin-dependent kinases. They play a major role in fetal and early postnatal brain development, with the expression of cD1 and cD3 ceasing in adult neuronal precursors. Cyclin D2 is thought to be the only D-cyclin expressed in neuronal progenitor cells of the adult brain. Consequently, the lack of functional cD2 in cD2KO mice causes the loss of adult neurogenesis in both the SVZ and the SGZ[2].

In the first study we wanted to determine the precise age at which neurogenesis ceases in these animals by characterizing the kinetics of postnatal hippocampal neurogenesis in cD2KO and wild type (WT) mice. Different groups of WT and cD2KO mice were injected with BrdU (50 mg/kg i.p.; three injections per day for two consecutive days) starting at postnatal day (P) 7, P14, P28, P40, P60, P90 and P260 and sacrificed 28 days later. We found a rapid, age-dependent decline in the number of BrdU-positive cells in the dentate gyrus in both genotypes. In WT mice, the number of BrdU-positive cells in the dentate gyrus decreased successively from about 57.000 cells at P7 to 300 cells at P260. In cD2KO mice, the number of BrdU-positive cells declined from about 22.000 cells at P7 to 26 cells at P260, reaching a virtually stable level as early as P28. Triple-immunofluorescence against BrdU and neuronal (NeuN) and astrocytic (GFAP) markers revealed that the percentage of newborn cells that differentiate into neurons is comparable between cD2KO and WT mice.

In the second study we tested whether newborn granule cells are crucial for spatial learning and memory. Two groups of male CD2KO and WT mice (3-4 months) were trained in the Morris water maze for 12 consecutive days (2 sessions per day; 3 trials per session). CD2KO mice were impaired in spatial learning during the first seven days of training, but reached a similar level as WT mice during the last days of training. Probe trials performed either 2 days or 30 days after the last training trial revealed impairments of cD2KO mice in recent and remote memory retrieval.

The finding that cD2KO mice without adult neurogenesis are impaired but able to learn a spatial learning task suggests that adult born neurons are involved but not indispensable for hippocampus-dependent spatial learning. From the immunohistochemical data we conclude that the proposed transition from developmental (involving all three D-type cyclins) to adult neurogenesis (only depending on cD2)[2] occurs around P28.

This work was supported by the Interdisciplinary Centre for Clinical Research Jena (J15) and BMBF (01GZ0709).

[1] Sicinski et al. Nature 1996, 384: 470-474

[2] Kowalczyk et al. J. Cell. Biol. 2004, 167(2): 209-213

# Overexpression of the Chondroitinsulfotransferases Chst 3, Chst 7 and Ust in Cortical Neural Stem Cells

Denise Harrach[1], Alexander von Holst[1]

[1]University of Heidelberg, Institute for Anatomy and Cell Biology, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

*Introduction:* Chondroitin sulfate proteoglycans (CSPGs) and their sulfation by chondroitin-sulfotransferases (Chsts) appear to play a crucial role for the behaviour of neural stem cells (NSCs) in the embryonic neural stem cell niche in mouse forebrain. It has been shown that the inhibition of the sulfation by sodium chlorate or the degradation of the CSPG glycosaminoglycans by chondroitinase ABC leads to less proliferation and altered cell fate decisions of the NSCs (Sirko, von Holst et al. 2007; Akita, von Holst et al. 2008; Sirko, von Holst et al. 2010). The aim of this study was to examine the influence of an overexpression of distinct Chsts on NSCs behaviour in vitro and in slice cultures.

*Methods:* The proliferation and differentiation of cortical neural stem cells from E13.5 mouse embryos upon forced expression of distinct Chst-EGFP constructs was examined by neurosphere forming assay and differentiation assay in vitro. Further characterisations of the influence of the Chsts overexpression were made in forebrain slice cultures after electroporation.

*Results:* The overexpression of distinct Chsts in the NSCs was functional as revealed by an increased signal for the complex sulfated CS-epitope detected by the monoclonal antibody 473HD. In the differentiation assay an increase in neurogenesis at the expense of gliogenesis was observed.

*Conclusion:* In consistence with previous observations, the sulfation of the CSPGs plays a role in the commitment of the NSCs within the neural stem cell niche and could function as a possible communication platform between the NSCs and their extracellular surrounding in the neural stem cell niche.

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# A role for Reelin and Notch1 cooperation during hippocampal development and in the adult

Mirjam Sibbe[1], Onur Basak[2], Ute Häussler[3], Carola Haas[3], Verdon Taylor[2], Michael Frotscher[1]

[1]University of Freiburg, Institute of Anatomy and Cell Biology / Neuroanatomy, 79104 Freiburg, Germany
[2]Department of Molecular Embryology, Max-Planck Institute of Immunobiology, 79108 Freiburg, Germany
[3]Experimentelle Epilepsieforschung, Neurozentrum Freiburg, 79106 Freiburg, Germany

The large extracellular matrix molecule Reelin is required for normal brain development and function. In the reeler mutant mouse lacking Reelin, an ordered hippocampal radial glial scaffold does not develop and dentate granule cells fail to form a regular, densely packed cell layer. Adult neurogenesis is diminished in the hippocampus, and in several CNS disorders a dysregulation of Reelin is found. A key pathway in radial glial development and stem cell maintenance is the Notch signalling pathway. Our data suggest that Reelin and Notch signalling cooperate during development and in the adult nervous system (Sibbe et al., 2009). Upon Notch1 activation, the intracellular domain (NICD) of the transmembrane receptor is cleaved and translocates into the nucleus acting as a transcriptional activator. We found reduced Notch1 activation in the hippocampus of young postnatal reeler mutant mice most probably due to increased ubiquitination of the NICD. Inhibition of Notch cleavage induced a reeler-like phenotype in hippocampal slice cultures, and rescue of the reeler phenotype by added Reelin is blocked by Notch inhibition. Furthermore, reduction of Reelin after induction of epileptic seizures in a model of temporal lobe epilepsy correlates with a reduction in Notch signalling in the SGZ of the hippocampus. Our data point to a recruitment of Notch1 activity by Reelin signalling to form and stabilize hippocampal radial glia.
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# POSITIVE CORRELATION BETWEEN CLIC1 FUNCTIONAL EXPRESSION AND HUMAN GLIOMA AGGRESSIVENESS

Nicoletta Savalli[1], Matteo Setti[2], Marina Angelini[1], Daniela Osti[2], Giuliana Pelicci[2], Michele Mazzanti[1]

[1]Dept. of Biomolecular Sciences and Biotechnology, University of Milan, Italy
[2] Dept. of Experimental Oncology, IFOM-IEO Campus, Milan, Italy

Oxidative stress overcomes when the equilibrium between oxidant species production and antioxidant capability is disrupted. The presence of redox-sensitive motifs in numerous cell cycle regulatory proteins points out that the loss in the redox control of the cell cycle could lead to aberrant proliferation and then to cancer. Among brain tumors, malignant gliomas are very frequent. They are composed by two cell types: a small population of cells able to self-renew and generate progeny (cancer stem cells, CSCs) and a larger population possessing a limited division capacity and committed to a precise fate (bulk cells). Gliomas are very aggressive tumors because of CSC brain infiltration efficiency and their resistance to chemotherapies due to the enhanced ability to efflux drugs. Therefore, CSCs are the most tumorigenic component of glioma and we have focused our efforts on this small population of cells.

Several forms of glioma and glioblastoma show a higher level of expression of the Chloride Intracellular Channel 1 (CLIC1) compared to normal brains. CLIC1 is a protein mainly localized in the cytoplasm and in the nucleoplasm that is able to translocate into the plasma membrane and into the nuclear membrane where it acts as a Cl- channel. The soluble form of CLIC1 belongs to the glutathione S-transferase superfamily. Upon oxidation, the protein forms a dimer that translocates into the membrane and operates as an ion channel.

We are specifically investigating four human glioblastomas (fourth grade gliomas), which express CLIC1. Human glioblastoma biopsies were cultured in a medium selecting for CSCs that grow as neurospheres. By knocking down CLIC1 protein using siRNA viral infection (siCLIC1), we found that CLIC1-deficient cells migrated about 50% less efficiently than control cells treated with siRNA for luciferase (siLUC). Is this phenotype the result of CLIC1 absence in the plasmamembranes? To solve this task, we performed electrophysiological experiments from perforated patches for both siLUC and siCLIC1 cells. Cl- currents mediated by CLIC1 were estimated by analytical subtraction after addition of a specific inhibitor (IAA94 100 μM). The results showed that siCLIC1 cells did not display IAA94-sensitive currents, while siLUC cells presented the CLIC1-mediated chloride current.

Very interestingly, among the four gliomas that we analyzed there is a direct correlation between the tumor aggressiveness and the percentage of IAA94 sensitive current: the more aggressive is the tumor the more CLIC1 current is present.

Our results suggest that CLIC1 is involved in glioma cells migration. The on-going experiments aim to understand the mechanism that mediates tumor infiltration via CLIC1 functional expression as an ion channel. The investigation on the intracellular pathway involving CLIC1 will be useful to eventually identify other proteins or enzymes that, together with CLIC1, could represent possible pharmacological targets to hamper brain tumor expansion.

# Purinergic receptor-mediated $Ca^{2+}$ signaling in the olfactory bulb and the neurogenic area of the lateral ventricles

Thomas Hassenklöver[1], Philipp Schulz[1], Anna Peters[1], Peter Schwartz[3], Detlev Schild[1,2], Ivan Manzini[1,2]

[1]University of Göttingen, Dep. of Neurophysiology and Cellular Biophysics, Humboldtallee 23, 37073 Göttingen, Germany
[2]DFG Research Center for Molecular Physiology of the Brain (CMPB), University of Göttingen, 37073 Göttingen, Germany
[3]University of Göttingen, Dep. of Anatomy and Embryology, Center of Anatomy, Kreuzbergring 36, 37075 Göttingen, Germany

The capability to detect extracellular nucleotides via purinergic receptors is widespread in a variety of tissues and cell types. Functional purinergic receptors are also expressed in many regions of the central nervous system. Purinergic receptor activation has been associated with many critical physiological processes, amongst others neurotransmission, propagation of calcium waves and regulation of proliferation. Nevertheless, purinergic signaling pathways are still not fully understood and in many tissues information on the physiological role of the purinergic system is missing.
In the present study we have investigated the purinergic system of the anterior telencephalon of larval *Xenopus laevis*. In vertebrates, the anterior part of the telencephalon mainly consists of the olfactory bulb (OB), representing the first relay station for olfactory information processing. But different from higher vertebrates, the lateral telencephalic ventricles of *Xenopus laevis* (Amphibia) expand deep into the anterior telencephalon. The periventricular zone (PVZ) of the lateral ventricles is known to provide a neurogenic niche that generates new neurons for the OB circuitry throughout the whole lifetime of an organism. Using transmission electron microscopy, we investigated the ultrastructural organization of the anterior telencephalon and emphasize the differences between OB and PVZ cells. A BrdU incorporation assay was applied to localize the neurogenic periventricular zone (PVZ) and to quantify the physiological proliferation rate of the PVZ cells. The rate of proliferation was rather high with 42.54 ± 6.65% of all PVZ nuclei being in S-phase and therefore dividing actively. Combining functional $Ca^{2+}$ imaging in acute slice preparations as well as immunohistochemistry we revealed that the anterior telencephalon features a widespread and complex purinergic system. While the cells of the OB almost exclusively express ionotropic P2X purinergic receptor subtypes, PVZ cells express both ionotropic P2X and metabotropic P1 and P2Y receptor subtypes. Application of purinergic agonists triggers stereotypic $Ca^{2+}$ signaling in cells of both regions. Using a nose-brain preparation, we also show that the purinergic receptors expressed in the OB are not evidently involved in the immediate processing of olfactory information.

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# Regulation of astrocyte maturation by the extracellular matrix molecule Tenascin C

Michael Karus[1], Stefan Wiese[3], Andreas Faissner[1]

[1]Ruhr-University Bochum, Cellmorphology and Molecular Neurobiology, Universitätsstraße 150, 44780 Bochum, Germany
[2]Ruhr-Universiy Bochum, International Graduate School of Neuroscience, Universitätsstraße 150, 44780 Bochum, Germany
[3]Ruhr-University Bochum, Molecular Cellbiology, Universitätsstraße 150, 44780 Bochum, Germany

During the development of the central nervous system neural precursor cells give rise to three major cells types, namely neurons, astrocytes and oligodendrocytes. Recent studies in the developing forebrain have shown that the extracellular glycoprotein Tenascin -C (Tnc) regulates the behaviour of neural precursor cells regarding both proliferation and differentiation. To gain insight into possible roles of Tnc for spinal cord development we initially undertook a systematic expression analysis of Tnc both on mRNA and protein level and found that Tnc is expressed by Nestin-, GLAST-, and Vimentin-positive neural precursor cells and GFAP-positive astrocytes during late embryonic development. Its expression coincides with the emergence of an EGF responsive neural precursor cell population. As a consequence this cell population is smaller in the absence of Tnc as revealed by neurosphere formation assays from Tnc mutant cells. BrdU incorporation analysis in combination with in situ hybridisation techniques at E15.5 in the spinal cord revealed an increased proliferation rate of immature astrocytes/astrocyte progenitor cells. We also found an increased proliferation of Tnc -deficient neurosphere cells in the presence of FGF2. Likewise, the addition of affinity purified Tnc suppressed a FGF2 dependent neurosphere formation from wildtype cells. These data suggest that Tnc induces a quiescent state in neural precursor cells both in vitro and in vivo thus promoting astroglial maturation. In line with this, we observed a delayed migration of immature astrocytes from the ventricular zone into the prospective ventral white matter. Furthermore, we found that Tnc deficient astrocyte cultures contained less GFAP positive cells indicating an immature phenotype of the cells. Finally, we also documented alterations in the GFAP expression level in the embryonic and adult spinal cord of Tnc-deficient animals in comparison to their wildtype littermates. Taken together, our data suggest that Tnc is important for the timely maturation of astrocytes in the developing spinal cord.

# Regulation of neurotrophin secretion in hippocampal neurons by CAPS1

Robert Eckenstaler[1], Thomas Munsch[1], Volkmar Lessmann[1], Tanja Brigadski[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipziger Straße 44, 39120 Magdeburg, Germany

The mammalian neurotrophins are important regulators of a variety of functions that are necessary for neuronal development and neuronal plasticity. Like other neuropeptides, neurotrophins are stored in secretory granules and are released upon specific electrical stimulation. In comparison to the wealth of knowledge regarding the molecular machinery meditating synaptic transmitter vesicle exocytosis, the mechanisms of secretory granule exocytosis in neurons are less well understood. To investigate the molecular mechanism of activity-dependent release of neurotrophins, we have designed a new generation of shRNA expression vectors enabling functional knockdown analysis of secretion relevant proteins.

In order to analyse the machinery of secretory granule exocytosis by live cell imaging, dissociated hippocampal neurons derived from mouse were genetically manipulated by simultaneous knockdown of secretion-relevant proteins and knock-in of a GFP labelled neurotrophin (BDNF-EGFP). To perform these simultaneous genetic manipulations, we established a protein knockdown using a new generation of shRNA expressing plasmids. These vectors are based on the transcription of the shRNA of interest, which is further matured to the requested siRNA by the endogenous miRNA processing machinery. To evaluate the knockdown efficiency mediated by the shRNA plasmid, we have designed a shRNA plasmid directed against EGFP. These results enabled us to optimize our construct to design a shRNA plasmid directed against CAPS1 yielding efficient knockdown. CAPS1 is an important vesicle priming factor in secretory granule exocytosis of neuroendocrine cells. Knockdown assays using the respective shRNA plasmid show an efficient knockdown in dissociated hippocampal neurons. In addition, depolarisation induced release of BDNF-GFP from secretory granules was analysed after co-transfection of hippocampal neurons with shRNA plasmid targeted against CAPS1. The role of CAPS1 during secretory granule exocytosis was compared to the role of CAPS1 during exocytosis of synaptic vesicles monitored with FM 4-64 destaining. Taken together, we have established an efficient method for knockdown of proteins to analyse the molecular machinery mediating secretory granule exocytosis.

# Regulators of miRNA biogenesis control neural differentiation

Anna Maria Rohde[1], Duong Nguyen[1], Elisa Cuevas[1], Heiko Fuchs[2], Agnieska Rybak[3], F. Gregory Wulczyn[1]

[1]Charitè Universitätsmedizin Berlin, Germany
[2]Max Planck Institute for Molecular Genetics, Berlin, Germany
[3]Max Delbrück Centrum for Molecular Medicine Berlin, Germany

miRNAs were originally recognized as regulators of developmental timing in C. elegans. In the mammalian CNS, mounting evidence suggests that temporal waves of miRNA expression accompany developmental progressions from neurulation to synaptogenesis. The paradigm miRNA let-7 is an example of an early-on miRNA, and roles for let-7 in the negative regulation of pluripotency and promotion of neurogenesis have been proposed.
Concentrating on highly regulated let-7 target genes in pluripotent cells, we showed that the mammalian homolog of the C. elegans developmental timing gene Lin41 participates with let-7 in a feedback loop that controls the activity of miRNA biogenesis during stem cell differentiation. Lin41 is a Trim family E3 ubiquitin ligase and the founding member of the Trim-NHL subfamily of developmental regulators. We recently demonstrated E3 ligase activity for Lin41, and showed that Lin41 interacts with and ubiquitinates the essential miRNA pathway protein Ago2. Lin41 acts to reduce steady-state levels of Ago2 in early stem cells, thereby reducing both miRNA accumulation and miRNA-mediated silencing activity. These studies reveal that one function of Lin41 in stem cell differentiation is to mediate regulation of the miRNA pathway. Our current efforts combine biochemical and genetic approaches to investigate the role of Lin41 as suppressor of the miRNA pathway during neural tube formation and in adult brain stem cell niches.

# Serotonin Transporter Knock-out and Chronic Mild Stress: Impact on Adult Neurogenesis in the Hippocampus

Margherita Maria Lee[1], Sandy Popp[1], Eva Gerten[1], Antonia Post[1], Marion Winnig[1], Klaus Peter Lesch[1], Angelika G. Schmitt[1]

[1]University of Würzburg, Molecular and Clinical Psychobiology, Clinic and Policlinic for Psychiatry, Psychosomatic and Psychotherapy , Füchsleinstr. 15, 97080 Würzburg, Germany

Psychiatric conditions like depression or fear and anxiety disorders can be triggered by environmental cues such as stress and certain intrinsic aspects such as genetic variations, for instance the length polymorphism of the serotonin (5-HT) transporter (5-HTT) gene promoter. The 5-HTT is located in the pre-synaptic membrane of serotonergic neurons. Its function is the re-uptake of 5HT from the synaptic cleft into the pre-synapse, and it is responsible for termination, recycling and fine-tuning of the serotonin signal. In addition, the 5-HTT is a target of several antidepressants such as the selective reuptake inhibitors (SSRI) as well as for drugs of abuse including methamphetamine (Ecstasy) and cocaine. 5-HTT knockout (KO) mice display an anxious phenotype as well as exaggerated adrenomedullary stress responses and thus are a common animal model for these stress-related disorders. Apart from that, stress exposure has been shown to reduce adult neurogenesis (aN), which can be found in the subventricular zone and in the dentate gyrus (DG) of the hippocampus of many vertebrates including humans. Furthermore, hippocampal aN is discussed to be linked to mental disorders such as schizophrenia and is known to be regulated by a number of factors including 5HT.

In order to evaluate a possible influence of the 5-HTT genotype and stress on adult Neurogenesis (aN), we investigated cell proliferation and neurogenesis in mice of different 5-HTT genotypes that had either remained untreated or had been subject to chronic mild stressed for 21 days. For this purpose we carried out an immunohistochemistry study using the proliferation marker Ki-67 and the neurogenesis marker NeuroD. Prior to sacrifice these mice had undergone a comprehensive behavioral test battery such as elevated plus maze, open field, light dark box, sucrose preference test and forced swim test.

We were able to detect deviations between 5-HTT genotypes regarding neurogenesis in the DG; proliferation however seems to be unaffected by the genotype. The sucrose preference test confirmed the effectiveness of the applied stress paradigm. On the other hand an impact of the 5-HTT genotype on the depression-related behaviour could be demonstrated by use of the forced swim test. Interestingly, activity level of 5-HTT KO mice seems to be elevated in almost all behavioural tests compared to the wildtype control mice.

# THE ROLE OF CORTICAL FEEDBACK SIGNALS IN REGULATING PROGENITOR CELL-FATE SWITCH DURING NEOCORTICOGENESIS

Srinivas Parthasarathy[1], Anjana Nityanandam[1,2], Victor Tarabykin[1,3]

Max Planck Institute for Experimental Medicine, Cortical Development Group, Goettingen, Germany[1] [3]Institute for Cell and Neurobiology, Center for Anatomy, Charite, Berlin, Germany

The layers of the neocortex are comprised of functionally diverse neurons. These neurons are generated sequentially from progenitors located in the germinal zone lining the lateral ventricles(VZ). Their fate and final position in the cortical plate is specified at the progenitor stage before they migrate into the cortical plate. However, not much is known about how the VZ learns how many pyramidal neurons of each type to produce and when the switch from producing one cell type to another should be made. One source of such signals is the cortical plate itself, creating a feedback loop. Recently we identified Sip1 (Smad Interacting protein1) as a transcriptional repressor of several cortical feedback signals which control the timing of sequential fate decisions in the VZ. Sip1 mutants show a precocious shift in corticogenesis wherein upper layer neurons are generated earlier at the cost of deeper layers and gliogenesis starts earlier ending neurogenesis precociously. However, little is known about the cortical signals. We have shown that Fgf9, which is under the negative control of Sip1 can induce precocious gliogenesis in wild type cortices. We have also identified promising candidates that could be influencing the switch from deep layer to upper layer fate.

# The role of orphan nuclear receptor Nurr1 (nr4a2) in layer specification of the cerebral cortex

Dmitriy Lanshakov[1], Victor Tarabykin[1,2]

[1]Charite, Universitaetsmedizin Berlin, Institute of Cell Biology and Neurobiology, NeuroCure Cluster of Excellence, Berlin, Germany.
[2]Max-Planck-Institute of Experimental Medicine, Department of Neurogenetics, Goettingen, Germany.

Nurr1(orphan nuclear receptor) plays a crucial role during the establishment and survival of the dopamineergic system of the brain. It is also expressed in the subplate and glutamatergic layer 6 cells of the cerebral cortex. The subplate is a transient zone containing precocious neurons involved in several key steps of cortical development. In rodent, subplate neurons are among the earliest born neocortical cells.
We characterize the role of Nurr1 in the neocortex and subplate during development using a conditional mutagenesis approach. Nurr1 expression is mutually exclusive with that of Ctip2, a transcription factor necessary and sufficient for the extension of subcortical motor neurons projections.
Ctip2 is ectopically expressed in upper cortical layers of Satb2 knockout mice. Satb2 is a postmitotic determinant for upper-layer neuron specification in the neocortex. In Satb2 -/- animals, upper layers of the cerebral cortex are misspecified and adapt subcortical features. Additionally, Nurr1 is highly upregulated and ectopically expressed in upper layers neurons. We aim to elucidate the interplay of those transcription factors during cortical layer specification.

# The STAR Family Proteins Sam68, Slm-1 and Slm-2 Differentially Regulate Proliferation and Differentiation of Cortical Neural Stem/Progenitor Cells

Alexander von Holst[1], Bettina Bertam[1]

[2] IGSN and Dept. Cell Morphology & Molecular Neurobiology, Ruhr-University Bochum, [NDEF 05/339, D-44780 Bochum Germany

We have identified the signal transducer and regulator of RNA metabolism (STAR) family protein Sam68 as a target of the extracellular matrix glycoprotein Tenascin C (Tnc) by an induction gene trap screen in neural stem cells. The activity of Sam68 is regulated by phosphorylation and overexpression by nucleofection reduced the proliferation of cortical neural stem/progenitor cells. At the same time Sam68 in turn regulates the splicing of Tnc by favoring the inclusion of the alternative spliced fibronectin type III domains. Both, Tnc and Sam68 are expressed in the germinal layers of the developing neuroepithelium and their expression is maintained in the postnatal and adult subventricular zone of the lateral ventricle wall. This implies an auto-regulatory, oscillatory interplay between the neural stem cell niche microenvironment and cortical neural stem/progenitor cells, which may contribute to the precise timing of neurogenesis during development and in the adult neural stem cell niche. Interestingly, the two Sam68 -related genes Slm -1 and Slm -2 are also expressed in the developing forebrain. Forced expression of Slm -1-EGFP and Slm -2-EGFP fusion constructs in cultured neural stem/progenitor cell revealed opposing functions. Slm-1 interfered with proliferation and appeared to favour neurogenesis
while Slm-2 increased proliferation and promoted astrogliogenesis. So, Slm-1 is similar to Sam68 on the cell biological level, whereas Slm-2 appears to have opposing functions. We are currently investigating, if the role of the Sam68 family members is carried by the signal transducing function, the RNA binding and RNA splicing activity or both to define the molecular mechnism(s) that orchestrate maintenance, proliferation and differentiation of cortical neural stem and progenitor cells.

# The transcription factors AP-2ß and AP-2a are required for survival of sympathetic progenitors and differentiated sympathetic neurons

Mirko Schmidt[1], Leslie Huber[1], Afsaneh Majdazari[1], Günther Schütz[2], Trevor Williams[3], Hermann Rohrer[1]

[1]Max-Planck-Institute for Brain Research, RG Developmental Neurobiology, Deutschordenstr. 46, D-60528 Frankfurt/M, Germany[2]German Cancer Research Center, Dept. Molecular Biology of the Cell I, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany[3]University of Colorado Denver, Department of Craniofacial Biology and Cell and Developmental Biology, Aurora, CO 80045, USA

Differentiation of sympathetic neurons is controlled by a group of transcription factors, including Phox2b, Ascl1, Hand2 and Gata3, induced by BMPs in progenitors located in ganglion primordia at the dorsal aorta. Here, we address the function of the transcription factors AP-2ß and AP-2a, expressed in migrating neural crest cells and maintained in sympathetic progenitors and differentiated neurons. The elimination of both *AP-2a* and *AP-2ß* results in the virtually complete absence of sympathetic and sensory ganglia due to apoptotic cell death of migrating neural crest cells. In contrast, only sympathetic ganglia are targeted in the *AP-2ß* knockout, leading to a reduction in ganglion size by about 40% at E10.5, which is maintained up to E16.5, and is also caused by apoptotic death of neural crest progenitors. The conditional double knockout of *AP-2a* and *AP-2ß* in sympathetic progenitors and differentiated neurons of $AP\text{-}2ß^{KO}/AP\text{-}2a^{Ascl1Cre}$ and $AP\text{-}2ß^{KO}/AP\text{-}2a^{DBHiCre}$ mice results in a further decrease in neuron number, leading eventually to small sympathetic ganglion rudiments at P4. These results define two distinct progenitor populations for sympathetic neurons, depending selectively on *AP-2ß* or either on *AP-2ß* or *AP-2a*. The *AP-2a/ß* dependence is maintained in differentiated sympathetic neurons. The elimination of *AP-2ß* also leads to the complete absence of noradrenergic neurons of the Locus coeruleus. In conclusion, our results reveal an essential survival function of *AP-2a/ß* transcription factors for developing peripheral and central noradrenergic neurons and imply AP-2ß as survival factor for neural crest progenitors specified for a sympathetic neuron fate.

# The zinc-finger homeodomain factor Teashirt1 (Tshz1) controls the layering and differentiation of olfactory bulb granule cells

Alistair Neil Garratt[1], Elena Rocca[1], Carmen Birchmeier[1]

[1]Max-Delbrueck-Center for Molecular Medicine, Department of Neurosciences, Robert-Roessle-Strasse 10, 13125 Berlin, Germany

The paired mammalian olfactory bulbs are essential for processing of odor stimuli and relaying this information to the olfactory cortex. Formed initially as evaginations of the rostral telencephalon, they become populated by neurons born either locally within the olfactory bulb ventricular zone, or more distally in the telencephalon. This latter population moves tangentially in a rostral direction before assuming a radial mode of migration within the olfactory bulbs, where it subsequently matures into granule cell and periglomerular cell interneurons. We present here a detailed characterisation of the cell populations within the granule cell layer of the embryonic mouse olfactory bulb, and assign to the zinc-finger homeodomain factor Teashirt1 (Tshz1) an essential role in both the radial migration and the molecular specification of early born, distally generated granule neurons. Such cells arrived within the bulbs of Tshz1 -/- mutant mice, but distributed aberrantly within the radial dimension, forming closely-packed cell aggregates. Within these clusters, Tshz1+ cells failed to express the zinc-finger transcription factors Sp8 and Sall3 and remained in an immature state as defined by the loss of expression of the markers neuN, glutamic acid decarboxylase (GAD)-67, gamma-amino butyric acid (GABA), tyrosine hydroxylase and guanine deaminase (cypin). Tshz1 is the first factor to be characterised, which functionally distinguishes between the differentiation programs of granule cell and periglomerular cell neurons in the developing olfactory bulb. In addition, our analyses indicate that soluble and/or cell surface factors produced by the Tshz1+ outer granule cell lineage regulate the spatial distribution of other neuronal populations within the olfactory bulb.

# Unravelling effects of Tgfß mediated target genes on forebrain neuronal progenitor cells of different developmental stages

Shalaka Dhanraj Wahane[1], Kerstin Krieglstein[1,3], Tanja Vogel[2]

[1]Institute for Anatomy and Cell Biology, Department for Molecular Embryology, Albert-Ludwigs University, Freiburg, Germany
[2]Department of Neuroanatomy, University Medical Centre, Georg August University, Göttingen, Germany
[3]FRIAS, Albert-Ludwigs University, Freiburg, Germany

Transforming growth factor ßs (Tgfßs) are multifunctional cytokines that are expressed in the forebrain, but little is known about the effects they assert on the heterogeneous stem cell population within the telencephalon. Using cortical primary cell cultures of developing mouse brains, this study aims to reveal Tgfß regulated target genes implicated in forebrain development.

Tgfß mediated neurogenesis has been reported to be increased in older (E16.5) embryonic cortical progenitor cells as compared to cells taken from developmental stage E13.5 and adult spheres. Hence, total RNA extracted from Tgfß- and inhibitor -treated cortical progenitor cells E13.5 and E16.5 mice was used, to perform a microarray, to assess genes regulated by Tgfß stimuli to provide cellular and molecular control in these different developmental stages. A 4-way comparison of the data obtained gave 4 sets of Tgfß-regulated genes. The datasets obtained were (1.) Tgfß -mediated regulation at E13.5, (2.) Tgfß -mediated regulation at E16.5, (3.) Tgfß-mediated regulation at E13.5 and not E16.5, and (4.) Tgfß-independent gene regulation between E13.5 and E16.5.
Regulated genes were clustered, based on their functions and parameters such as genes regulating neuronal differentiation, proliferation, apoptosis, transcription factors, and genes expressed in the extracellular matrix. Validation of regulations of these Tgfß target genes using semiquantitative and qRT-PCR corroborated with the fold changes as seen in the microarray. Analysis of the effects of Tgfß on these genes will provide insights in the role of Tgfß-signalling in forebrain development.

With regard to adult spheres cultured from the dentate gyrus of WT mice, analyses for differentiation rates showed no significant increase in neuronal differentiation upon Tgfß treatment. Since we observed that Tgfß mediates neuronal differentiation of early cortical progenitors only after reduction of FoxG1 expression, we are currently investigating whether reduction of FoxG1 expression would also induce a competence of adult spheres to respond with increased neuronal differentiation upon Tgfß signalling.

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# Mechanism of the reversal of neurobehavioral teratogenicity in mice with neural stem cells

Joseph Yanai[1], Adi Pinkas[1]

[1]Hebrew University Medical School, Institute for Medical Research, Israel-Canada, Medical Neurobiology, 12272, 91120 Jerusalem, Israel

Neurobehavioral teratogenicity is a major clinical concern. We established the mechanism by which the teratogen exerts its deleterious effects on cognitive behavior (Morris maze), the abolishment of ACh-induced activation/translocation, which enables us to reverse the deficits using various mean, most recently with neural stem cell (NSC) transplantation, including neonatal cortex -derived and embryonic -derived NSC. A major mechanism by which the NSC exerted their therapeutic action was by enhancing production of endogenous cells in the impaired brain. NSC therapy can be greatly enhanced toward clinical application, by employing adult NSC, which will enable autologous transplantation. Toward this goal, we developed a model which uses subventricular zone (SVZ) cells. Mice were exposed prenatally to chlorpyrifos by injecting their pregnant mothers with the organophosphate on gestational days 9- 18 (3 mg/kg/day, SC). The offspring showed deficits in the Morris maze test, which, consistent with the literature, were limited to females. Both the control and exposed female offspring were transplanted with SVZ cells (or media) on postnatal day (PN) 35. On PN 60, the mice exposed prenatally to chlorpyrifos displayed impaired Morris water maze performance, requiring longer swimming time than controls to reach the platform. However, transplantation of adult SVZ -derived NSC reversed the deficits caused by prenatal chlorpyrifos exposure, in that the transplanted mice no longer differed statistically from controls. The transplanted cells were later identified in the host brain by DiI tracing, and they showed differentiation to cholinergic neurons and astrocytes. Applying autologous transplantation bypasses both the methodological obstacles of immunological rejection and the ethical concerns related to using embryonic stem cells. SVZ transplantation is obviously not clinically applicable, but it serves as a model for more feasible transplantations, such as adult mesenchymal stem cell transplantation, which is the subject of our current investigation.

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# A mechanism for aromatase-dependent homeostasis of hippocampal synapses

Lars Fester[1], Lepu Zhou[1], Nicola Brandt[1], Erik Disteldorf[1], Christiana Ossig[1], Jan Labitzke[1], Wiebke Wilkars[1], Roland Bender[1], Hubertus Jarry[2], Gabriele M. Rune[1]

[1]University Medical Center Hamburg-Eppendorf, Institute of Anatomy I: Cellular Neurobiology, Martinistr. 52, 20246 Hamburg, Germany

[2]University of Goettingen, Department of Experimental Endocrinology, Robert-Koch-Str. 40, Goettingen, Germany

Both form and function of dendritic spines, which serve as postsynaptic partners of the majority of excitatory synapses, are critically dependent on their actin cytoskeleton. Here we found that activity of aromatase, the final enzyme of estadiol synthesis, regulates actin associated proteins. In hippocampal neurons, letrozole, an aromatase inhibitor, downregulates the expression of profilin and synaptopodin and dephosphorylates cofilin, finally resulting in spine loss. The actin-associated protein synaptopodin is additionally tightly associated with the spine apparatus, a spine-specific Ca2+ store and a typical feature of mushroom spines. Consistently, mushroom spines, as defined by spine size and by the presence of a spine apparatus, were primarily affected by inhibition of aromatase activity. Most importantly, on manipulation of Ca2+ transients we found that in hippocampal neurons Ca2+ transients control aromatase activity. Ca2+ release from internal stores, such as the spine apparatus, inhibits aromatase activity, which in turn regulates protein expression. This effect is mediated by estrogen receptor (ER)ß and abolished after Ca2+ channel blockade, in the presence of testosterone, activating aromatase, and ER blockers. The regulation of actin -associated protein expression downstream of the regulation of aromatase activity by Ca2+ transients introduces aromatase activity as a hitherto unknown factor in Ca2+-induced signalling cascades, with regard to the homeostasis of synapses in the hippocampus.

# Activity Dependence of Fine-Scale Synaptic Organization in CA3 Hippocampal Dendrites during Development.

Johan Winnubst[1], Thomas Kleindienst[1], Christian Lohmann[1]

[1]Netherlands Institute for Neuroscience , Synapse and Network Development, Meibergdreef 47, 1105 BA Amsterdam, Netherlands

During brain development, before sensory systems become functional, neuronal networks spontaneously generate repetitive bursts of neuronal activity, which are typically synchronized across many neurons. Such activity patterns have been described in detail on the level of the network, but the effect that this activity has on the fine-organization of synaptic inputs received by an individual neuron remains unclear. By correlating the occurrence of local calcium transients with whole-cell electrophysiology recordings a previous study by Kleindienst et. al. (submitted) was able to identify the activity of individual synaptic sites during spontaneous activity in cultured CA3 pyramidal neurons of neonatal rats. Analysis of the spatio-temporal activity patterns of the detected synapses revealed that synapses that were located close together on the dendritic branch (<20 µm) were significantly more activated in concert then synapses that were farther apart. These findings would indicate that the dendritic tree is organized with subcellular precision and that local clusters of synaptic inputs tend to carry functionally related information. We speculated that this clustering could be an activity-dependant sorting process during development, where neighboring synapses with presynaptic axons that spike simultaneously get specifically stabilized. In this current study we therefore investigated whether spontaneous activity during development is necessary for this kind of fine-scale organization to arise. To achieve this we incubated cultured hippocampal slices in TTX thereby blocking all spiking activity in these slices. Activity patterns of functional synaptic inputs were then determined by combining calcium imaging with whole-cell patch clamp recordings using the previously described method. We found that in control cells neighboring synapses indeed showed higher levels of co-activity then synapses that were farther apart. However, prior incubation with TTX completely abolished this spatio-temporal effect. These findings show that spontaneous network activity acts as an important component in the precise wiring of neural networks and demonstrated for the first time that it is capable of connecting neurons with subcellular precision.

# Activity-dependent maturation of the A17-RBC reciprocal synapse in the mouse retina

Timm Schubert[1], Ed Parker[2], Thomas Euler [1], Rachel O. Wong[2]

[1]Centre for Integrative Neuroscience / Institute for Ophthalmic Research, University of Tübingen, Germany
[2]Dept. of Biological Structure, University of Washington, Seattle, USA

In the mammalian retina, glutamatergic transmission from rod bipolar cells (RBCs) is regulated by feedback inhibition provided by A17 amacrine cells (A17s) at highly localized sites of reciprocal contact between the RBCs and A17s. We investigated how GABAergic and glutamatergic neurotransmission influence the assembly of this reciprocal synapse during development. Two transgenic mouse lines in which neurotransmission is perturbed were used. In a GAD1 retinal knockout (GAD1KO) mouse (Schubert et al., 2010), GABAergic transmission in the retina is impaired. In the Grm6:TeNT mouse, glutamatergic transmission in RBCs is severely reduced (Kerschensteiner et al., 2009).

To determine when during development GABAergic feedback from A17s to RBCs becomes functional, we performed whole-cell recordings from RBCs in the wild type retina and measured AMPA-evoked postsynaptic currents. At P11, AMPA-evoked currents were significantly reduced by DHT (Chavez et al., 2006) indicating that the A17-RBC reciprocal synapse is functional before eye opening (P14).

In the GAD1KO retina, the frequency of GABAergic spontaneous inhibitory postsynaptic currents (sIPSCs) in RBCs was significantly reduced during the early period of synaptogenesis (P11-13) and at maturity (P30), indicating decreased GABAergic transmission throughout neonatal development of the A17-RBC circuit in this mutant. Using the GAD1KO, we investigated how a lack of GABAergic transmission affects the development of GABAergic synapses onto RBC axon terminals. At P11, GABA puffs at RBC axon terminals in the KO showed that feedback is mediated by both GABAA and GABAC receptors, and that the proportion of GABAA and GABAC mediated currents was unchanged. In contrast, in the P30 GAD1KO retina, GABAA receptor-mediated currents were reduced, whereas GABAC receptor-mediated currents were unaltered compared to wildtype. However, AMPA-evoked currents recorded in A17s in the KO retina were normal, suggesting that the density of glutamate receptors on A17 processes was not affected by a lack of GABAergic transmission onto RBCs during development.

We next examined whether glutamatergic transmission regulates the development of the reciprocal RBC-A17 synapse. In the Grm6:TeNT mouse line, the light chain of tetanus toxin, which cleaves the vesicle fusion-protein VAMP2, is expressed under the control of the Grm6 promoter. Whole cell recordings of sIPSCs in RBCs revealed that the formation of synaptic feedback onto RBCs is independent of glutamatergic forward transmission. AMPA puffs onto A17s at P11-13 and P30 evoked GABAA and GABAC receptor-mediated currents in RBCs that resembled those of wildtype RBCs. Also, GABAergic sIPSCs were normal in RBCs lacking glutamatergic transmission.

Together, our observations imply that GABAergic and glutamatergic neurotransmission do not play a crucial role in the initial formation (around P11-13) of the reciprocal RBC-A17 synapse. However, there appears to be a failure in maintaining GABAA but not GABAC receptors on RBC terminals when GABAergic transmission is deficient. Thus, the maintenance of synapses containing GABAA or GABAC receptors appears to be differentially regulated by synaptic activity.

# Alternative transcripts of the songbird BDNF gene

Moritz Hertel[1], Falk Dittrich[1], Carolina Frankl[1], Iwona Ruczynska[1], Anja Lohrentz[1], Antje Bakker[1], Bernd Timmermann[2], Heiner Kuhl[2], Manfred Gahr[1]

[1]Max Planck Institute for Ornithology, Behavioural Neurobiology, Eberhard Gwinner Str/Pforte, 82319 Seewiesen, Germany

[2]Max Planck Institute for Molecular Genetics, Ihnestr. 63-73, 14195 Berlin, Germany

Brain-derived neurotrophic factor (BDNF) plays a pivotal role in the development of song learning in birds. In juvenile male zebra finches the expression of BDNF in the forebrain song control system is estrogen-dependent at the onset of song learning. Further, the estrogen receptor a is expressed in neurons in and near two key song control regions during development. To investigate the potential role of the estrogen receptor a for BDNF expression in songbirds, the genomic sequence of the BDNF gene was determined in two songbird species, the zebra finch and the canary. Genomic sequences close to multiple songbird BDNF 5'-UTRs that were discovered by 5'-RACE-PCR experiments were analyzed for the presence of estrogen responsive elements (EREs). Songbird BDNF 5'-UTRs differed in the degree of sequence similarity to non-songbird (e.g. chicken) BDNF genes. Expression patterns of the different songbird BDNF 5'-UTRs were examined in juvenile and adult zebra finches by the means of semi-quantitative PCRs.

# Analysis of Reelin Effects on early Neuronal Process Differentiation and Polarity

Maurice Meseke[1], Burkhard Baader[1], Eckart Förster[1]

[1]University of Hamburg, Institute of Anatomy I: Cellular Neurobiology, Martinistr. 52, 20246 Hamburg, Germany

During cortical development, the positioning of radially migrating neurons is controlled by the glycoprotein reelin, secreted by Cajal-Retzius cells in the marginal zone. In the reeler mutant mouse lacking reelin expression, cortical neurons fail to migrate to their proper positions. Moreover, numerous malpositioned neurons in the reeler cortex display defects in polarity and process orientation. How reelin signalling influences cytoskeletal dynamics of migrating and differentiating neurons is poorly understood. Recently, it has been shown that reelin, when binding to the reelin receptor apoer2, induces phosphorylation of the actin associated protein cofilin in the leading process of radially migrating neurons, suggesting a stabilizing effect of Reelin on the actin cytoskeleton in the leading processes of migrating neurons. However, this effect only partially describes the role of reelin in organizing cytoskeletal dynamics, since neurons in mutant mice lacking the reelin receptor vldlr show normal cofilin phosphorylation but still display migration defects (see review Förster et al., 2010). To search for novel reelin effects on cytoskeletal components, we studied Reelin effects on process differentiation and orientation of cortical tissue in situ and in dissociated primary cultures of hippocampal neurons. Our results suggest that Reelin effects on cytoskeletal dynamics of differentiating neurons require close interplay of both actin and tubulin components. This work was supported by DFG FO 223/6-1; Ref.: Förster et al. (2010) Europ. J. Neurosci. 31, 1511-1518.

# Analyzing the L4 network in the lamina of Drosophila

Birgit Ahrens[1], Kevin Lüthy[1,2], Shilpa Rawal[1], Ian Meinertzhagen[2], Karl-Friedrich Fischbach[1]

[1]Universität Freiburg, Institut für Biologie III, Schänzlestraße 1, 79106 Freiburg, Germany
[2]Dalhousie University, Halifax, Canada

L4 neurons are the only monopolar cells in the lamina of the fly that invade neighboring cartridges. In the proximal lamina layer, they form reciprocal synapses with both L2 and collateral branches from other neighboring L4 neurons. It is our aim to understand and genetically manipulate the development and function of L4 neurons and their collaterals. The starting point of our research is the finding that these collaterals specifically express the cell recognition protein Kirre/Dumbfounded at their sites of contact with other L4 cells and with L2 neurons in the proximal lamina. Kirre is a member of the irre cell recognition module (IRM) group of proteins, which we have described recently (Fischbach et al., 2009). Using the Gal4/UAS gene expression system we are specifically manipulating the genetic constitution of differentiating L4 and L2 neurons. Cell autonomous and non-autonomous effects are distinguished by analysing single-cell genetic mosaics, using the MARCM method to induce loss of the Gal4 inhibitor Gal80 by means of mitotic recombination. Effects on synapses are being studied at the level of the electron microscope. Our long-term objective is to understand the function of the L4 network in fly vision and its development in the lamina.

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# BMP-receptor signalling enables the formation of large excitatory synapses in the auditory circuit

Le Xiao[1], Nicolas Michalski[1], Ralf Schneggenburger[1]

[1] Laboratory of Synaptic Mechanisms, Brain Mind Institute, École Polytechnique Fédérale de Lausanne (EPFL), 1015 Lausanne, Switzerland

In the brainstem auditory circuit of mammals and birds, excitatory synapses with extraordinarily large size have evolved, which ensure fast membrane potential signalling mediated by large, multiquantal excitatory postsynaptic currents (EPSCs). These synapses form prior to the onset of hearing in a target-cell specific manner; however, the molecular signalling pathways which drive their formation are unknown. Here we identify bone morphogenetic protein (BMP) signalling as an essential signalling pathway in the development of calyces of Held. Through an unbiased genome-wide transcriptome analysis, we identified BMP5 and BMP4 as candidates for diffusible signalling molecules differentially expressed between medial nucleus of the trapezoid body (MNTB; the target area of calyces of Held), and lateral superior olive, a neighboring nucleus which does not receive large synapses. Conditional knock -out (k.o.) of BMP -receptor (BMP -R) 1a in the lower auditory system, together with conventional k.o. of BMP-R1b (BMP-R 1 double k.o.;

# Cofilin and its phosphorylation at Ser3 are essential for the polarization and migration of cortical neurons

Xuejun Chai[1], Li Fan[2], Hong Shao[2], Shanting Zhao[1], Hans Georg Mannherz[3], Michael Frotscher[1]

[1]Institut of Anatomie and Cell Biologie, University of Freiburg, Germany [2]School of Life Sciences, Lanzhou University, China [3]Institute of Anatomy, Ruhr University Bochum, Germany

During brain development, postmitotic neurons migrate away from their birthplace to their final destinations, as they are usually generated at sites distant from their final positions. Neuronal migration is essential for the proper lamination of the cerebral cortex. Migrating neurons show an asymmetrical morphology: a bipolar shape of the cell body with a long, thick leading process and a short, thin trailing process. This polarization is important for migration. The motility of migrating neurons is based on cytoskeletal dynamics that require constant remodelling of the actin and microtubule cytoskeleton. Cofilin is an actin -binding protein that plays an essential role in enhancing actin filament dynamics and reorganization by severing actin filaments. The activitiy of cofilin is reversibly regulated by phosphorylation and dephosphorylation at Ser3, with the phosphorylated form being inactive. Loss of n-cofilin impairs radial neuronal migration, resulting in an altered cortical lamination. To investigate the roles of n- cofilin and its phosphorylation at Serine3 on the migratory process, we transfected postmitotic neurons in the cerebral cortex of mouse embryos by in utero electroporation. For this, different constructs of n-cofilin including point mutations at the serine3 residue were used. Our results showed that overexpression of n-cofilin and point mutation at Ser3 induced loss of polarization of migrating neurons and interfered with the migratory process.

# Local distribution of serotonin-immunoreactive fibres in mushroom body compartments of mature cricket brains

Ashraf Mohamed Ali Mashaly[1], Friedrich-Wilhelm Schürmann[2]

1- Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, KSA
2- Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Universitaet Goettingen, Berliner Str. 28, 37073 Goettingen, Germany

The distribution of serotonin-immunoreactive fibres in the brain of the mature cricket was studied by confocal microscopy. In the cricket Gryllus bimaculatus, 5-HT neurons belong to the class of so-called wide field neurons. They occur in all brain neuropils including portions of mushroom body compartments. Microglomerular parts of the anterior and posterior calyces are supplied by a network of 5-HT fibres. In the anterior calyx, 5-HT fibres with bouton-like specialisations are concentrated in the outer regions of the microglomerular layer. There they occupy marginal parts of a microglomerulus, in the close vicinity of the strong f-actin-phalloidin stained portions which characterize KC II dendritic tips around a central PN-bouton. We interpret these extrinsic 5-HT elements as fourth contributors to a microglomerulus, which is known to contain cholinergic and GABA-ergic extrinsic elements synaptically coupled to intrinsic Kenyon cell dendrites. We tentatively suggest 5-HT fibres to additional synapse with Kenyon cell dendrites of type KC II and III. A 5-HT network is found in the outer parts of the stalk among KC II fibres. Large central areas of KC I and II fibres are devoid of 5-HT immunoreactivity, also lacking in the marginal KC III fibre stalk subcompartment. In the lobes, only parts contain 5- HT fibres. There, the tiny 5-HT fibres are of the varicose fibre type. Especially the compact KC I fibre bundle and adjacent KC II fibres in the lobes lack supply with 5- HT immunostained elements. Interestingly, the distribution of 5-HT fibres is apparently restricted to older, early generated KC II fibres with marginal positions in the mushroom body compartments.

# Developmentally regulated protein synthesis in dendrites

Elmer Rodrigo Antileo Ibarra[1], Peter Landgraf[1], Thilo Kähne[3], Karin Richter[4], Karl-Heinz Smalla[2], Daniela C. Dieterich[1]

[1]Leibniz Institute for Neurobiology Magdeburg, Neurochemistry ,Emmy Noether Research Group Neuralomics,Magdeburg, Germany
[2]Special Lab. Mol. Biol. Techniques, Leibniz Institute for Neurobiology, Magdeburg, Germany
[3]Institute of Experimental Internal Medicine, Otto-von-Guericke University, Magdeburg, Germany
[4]Institute of Biochemistry and Cell Biology, Otto-von-Guericke University, Magdeburg, Germany

Dendrites are extensions of the cell body of neurons specialized for receiving and processing synaptic inputs by providing biochemical compartments for local control of signaling mechanisms. Dendritic responses to synaptic activity rely on the ability of spines to regulate protein synthesis and degradation. The feasibility of local protein synthesis is suggested both by the existence of ribosomes and mRNAs localized in dendrites, and translation of these mRNAs contributes to synaptic plasticity. At present, however, it is unclear if local protein translation in dendrites is a hallmark of mature neurons or if local protein synthesis is an innate feature of dendrites, i.e. present already at early stages during development of neurons, and therefore, possibly involved in synaptogenesis. In this study we investigate the developmental expression and subcellular localization of different components of the translational machinery (ribosomes and MetRS) in dendrites. From very early time points on these components are present along the dendritic processes. At later, more mature stages, cluster-like structures of ribosomes appear associated with synaptic terminals along dendrites. Additionally, biochemical analysis of fractions enriched for synaptic structures indicates that the recruitment of the translation machinery and synaptic components to synapses starts early during development.
In order to analyze these findings in more detail, we identified newly synthesized proteins in isolated, translation-active synaptoneurosomes (SNS) prepared from dissected rat brains at different developmental stages. In electron micrograph images of SNS we are able to identify both presynaptic and postsynaptic compartments as well as astroglial end feeds close to synaptic structures, indicating intact tripartite synapses. Most importantly, SNS are devoid of any nuclei and nuclear proteins. For analysis and identification of newly synthesized proteomes we applied the recently developed BONCAT (bioorthogonal non-canonical amino acid tagging) technology, followed by affinity purification and two- dimensional mass spectrometry. BONCAT capitalizes on the co-translational introduction of the unique chemical azide functionality into newly synthesized proteins via the incorporation of the azide-harboring non- canonical amino acid Azidohomoalanine (AHA) followed by chemo -selective tagging of azide-labelled proteins with an alkyne-affinity tag using copper-catalyzed [3+2]-azide-alkyne-cycloaddition (click chemistry). First results of our analysis identify a diverse spectrum of proteins including postsynaptic, presynaptic and astroglial candidates and suggest that local protein synthesis is not exclusively restricted to mature systems, but also might occur during brain development.

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# *Estrogen-induced gene expression patterns of juvenile bird song control nuclei analyzed with a zebra finch specific microarray*

Benjamin Wasmer[1], Carolina Frankl[1], Antje Bakker[1], Falk Dittrich[1], Manfred Gahr[1]

[1]Max Planck Institute for Ornithology, Department of Behavioral Neurobiology, Eberhard Gwinner Strasse , 82319 Seewiesen, Germany

Development and maintenance of bird song are sex hormone sensitive. However, the gene regulatory mechanisms responsible for these hormonal effects are largely unknown. To address this issue we used a zebra finch specific whole-transcript expression array (Affymetrix, Santa Clara, CA, USA: MPIO-ZF1) that was developed at the Max Planck Institute for Ornithology, Germany. The investigations of estrogen-induced gene expression patterns were conducted with two song control nuclei, the high vocal center (HVC) and the robust nucleus of the arcopallium (RA). Our previous studies with juvenile male zebra finches have shown that the developmental increase of brain-derived neurotrophic factor (BDNF) expression in HVC at the onset of song learning is estrogen-dependent and that its precocious expression can be achieved by an early systemic estrogen treatment. For microarray analyses HVC and RA samples were taken from such early estrogen-treated males before the RA was innervated by HVC projection neurons. In total we found 811 (21) up- and 1566 (14) down-regulated genes in the HVC and 1849 (13) up- and 666 (16) down-regulated genes in the RA of estrogen-treated juvenile males (FDR = 0; expression levels = 0.5 (1), log2). Gene expression data analyses revealed specific biological pathways to be over-represented after estrogen administration that are more prominent in the adult compared to the juvenile song control nuclei. Particularly, we detected an estrogen-responsive cluster of genes that is known to be involved in growth cone guidance. Higher expression of all semaphorins (7 genes) detected was found in the HVC of control birds but in the RA of estrogen-treated birds. Remarkably, up-regulation of semaphorin 3A in RA was found to coincide with an increased expression of its receptor in the HVC.

# Experience-dependent changes in cortical network topology

Markus Butz[1], Huib Mansvelder[1], Arjen van Ooyen[1]

[1]VU Universiteit Amsterdam, Department of Integrative Neurophysiology, De Boelelaan 1085, 1081HV Amsterdam, Netherlands[1]

The brain is not as hard-wired as traditionally thought. Recent studies reveal a large plastic capacity even of the mature brain. Activity is a crucial driving force for neural plasticity as there is a reciprocal interaction between synaptic connectivity and neuronal activity. Connectivity determines the flow of activity and hence the function of the brain's networks on the one hand and, on the other hand, the flow of neuronal activity shapes network connectivity in the developing but also in the adult brain. This includes changes in the connection strengths of existing synapses (synaptic plasticity) but also the formation of new synapses and the breaking and rewiring of existing synaptic connections (structural plasticity). Neurons have a major tendency to keep their activity in a homeostatic regime which they achieve by adapting their synaptic input spectrum. Here we investigate by a theoretical modelling study how the local compensation of lasting changes in neuronal activity due to a focal loss of input (deafferentation) enforces changes in global network topology. We assess networks before and after the deafferentation by means of graph theory. We predict that networks experiencing a permanent change of the input pattern tend to develop towards a more randomized topology and a higher in-betweenness of deafferented neurons. We compare our theoretical findings with recent data from stroke brains. We found that cortical networks compensate in a comparable way from a central brain lesion (stroke) like from lasting changes in experience (deafferentation) which may imply a principal mechanism to compensate for disturbances in neuronal activity homeostasis. We further predict that a small-world topology is most robust against those disturbances as such networks need the shortest time to return to homeostasis. Since brain network topologies can be extracted from functional (fMRI) as well as structural neuroimaging (DTI), our modelling study provides testable predictions for experimental and clinical research.

# Influence of retinoic acid, insulin and 20-hydroxyecdysone on neurite outgrowth and growth cone turning in locust embryonic neurons

Jeyathevy Sivalingam[1], Jörg Mey[1], Katrin Göbbels[1], Peter Bräunig[1]

[1]RWTH Aachen University, Institute of Biology II, Lukasstraße 1, 52074 Aachen, Germany

Identification of molecules modulating neuritic outgrowth is a major goal in the field of neuroscience and is very important for different applications.
Retinoic acid (RA), the active metabolite of vitamin A, plays an important role during development and regeneration in vertebrates, including patterning of the nervous system and neurite outgrowth (Clagett-Dame et al., 2006). In addition, RA appears to induce positive growth cone turning in regenerating neurons of the adult mollusc, *Lymnaea stagnalis*, *in vitro* (Farrar et al., 2009). Also, in the *Locusta migratoria*, retinoic X receptor (RXR) transcripts appear to be present throughout embryogenesis. Locust RXRs bind 9-cis-RA and all-trans-RA with high affinity, similar to human RXRs (Nowickyj et al., 2008). In insects RXR forms the heterodimerization partner for the nuclear ecdysone receptor which binds to 20-Hydroxyecdysone (20-HE). The lipophilic molecule 20-HE is an active moulting hormone, involved in the insect development and metamorphosis. It was already shown, that the presence of 20-HE in the cell culture medium with ganglion explants of locusts enhances the outgrowth of neurites. A combination of 20-HE with insulin increases neurite outgrowth even further (Vanhems et al., 1990). Therefore we also tested insulin in our experiments. However in contrast to vertebrate, little is known of the effects of insulin on invertebrate neurons.
Neurons from *Locusta migratoria* were used to study the modulating effect of RA and the other above-mentioned substances on directed neurite outgrowth and growth cone turning. Using the Dunn chemotaxis chamber the growth behaviour of stimulated neurons was monitored over a time-period of 7 hours. Accordingly, neurons from young adult locust thoracic ganglion and the entire ventral nerve cord of embryos at 50 – 60% development were used. The molecules tested included RA ($10^{-9}$ M), 20-HE (5µg/ml) and insulin (5µg/ml). The outgrowth was monitored with an inverted microscope. Adult locust neurons treated with a linear concentration gradient of RA showed no directional preferences in growth behaviour. Due to the fact that embryonic neurons are in the growth- and pathfinding stage and furthermore RXRs are detected in the embryonic development stages, neurons from embryonic locust nerve cord were used for further studies. Growth cone turning and an enhanced neurite outgrowth is anticipated by the use of RA, 20-HE and insulin.

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# Integration of peripheral and central inputs in the developing visual cortex

Friederike Siegel[1], Christian Lohmann[1]

[1]Netherlands Institute for Neuroscience, Synapse and Network Development, Meibergdreef 47, 1105 BA Amsterdam, Netherlands

The development of neuronal networks is regulated by activity-dependent mechanisms. Already at early stages of development, most brain areas spontaneously generate network activity. For instance, during visual system development, retinal waves synchronously activate neighboring neurons and sweep across large parts of the retina before it is sensitive to light. These patterns of neural activity are thought to be crucial for the formation of neural circuits in the retina and upstream targets of the visual system by means of Hebbian-like mechanisms. Retinal waves are relayed to the primary visual cortex and mediate important aspects of cortical circuit formation. However, little is known about the specific patterns of activation on the cellular level during cortical network events. In addition, how retinal activity contributes to cortical activity patterns in the living animal has not been investigated so far.

To study synchronous activity in the developing cortical network, we labeled populations of neurons in the visual cortex of anesthetized neonatal mice with a calcium-sensitive dye and performed two-photon time-lapse imaging of spontaneous network activity. Subsequently, we modified retinal activity patterns to dissect their contributions to cortical network dynamics. We abolished retinal activity by means of binocular enucleation or increased the frequency of retinal activity by binocular injection of forskolin.

Synchronous network events occurred periodically in the visual cortex of neonatal mice and the patterns of cortical activity were qualitatively distinct. One class of network events was characterized by high cellular participation rate, high synchronicity and high mean amplitude. In contrast, the other class comprised fewer cells, lower synchronicity and lower mean amplitude. After the removal of retinal inputs, specifically the frequency of network events of the second group was reduced. Interestingly, network events of the first class remained unaffected. Accordingly, pharmacologically increasing the frequency of spontaneous retinal waves yielded the opposite result. Only the frequency of cortical network events of the second class increased whereas the frequency of the other group of events was not changed. These results indicate that, already before the onset of vision, retinal inputs have significant effects on the generation of network activity in the developing visual cortex. Furthermore, our data suggest that distinct types of cortical network events arise from different brain regions and cooperate during the maturation of the visual cortex.

# *Investigating the role of Adenosine receptors in growth cone physiology*

Hartmann Harz[1], John-Christian Eilert[1], Michael Hartmann[1], Silvio Bürge[1]

[1]Ludwig-Maximilians-Universität München (LMU), Bio Imaging Zentrum (BIZ), Großhadernerstr. 2-4, 82152 Martinsried, Germany

Growth cones are structures at the tips of growing axons responsible for regulation of motility and guidance of neuronal processes. The intricate signal transduction network of growth cones is influenced by a multiplicity of extracellular molecules. In a recent publication we have shown that extracellular concentration gradients of adenosine can induce growth cone turning of sensory neurons from chicken embryos (E11)[1]. The data indicate that this effect is at least partially mediated by the adenosine receptor $A_{2A}$. However, neither the signal transduction chain(s) nor the intracellular effectors were clarified in that publication. Therefore we investigated the effect of adenosine receptor stimulation on intracellular signalling molecules (cAMP, $Ca^{2+}$) and on the cytoskeleton (F-actin).

The main signal transduction pathway of $A_{2A}$ receptors is the stimulation of the adenylate cyclase via $G_s$ and a subsequent increase of the cytoplasmic cAMP concentration[2]. Therefore it is likely that an adenosine stimulus of growth cones from sensory neurons leads to an increase of the cytoplasmic cAMP concentration. However, other adenosine receptor subtypes like $A_1$ and $A_3$ have an opposite effect on the intracellular cAMP concentration. Here we investigated the cAMP level by using a fluorescence resonance energy transfer (FRET) probe based on an "exchange protein directly activated by cAMP" (Epac1). Here we used an adenovirus based transduction system in order to increase the number of neurons expressing the cAMP sensor protein. In addition we also established the FRET based cAMP measurement method in PC12 cells and other permanent cell lines heterologously expressing different adenosinereceptor subtypes.

It is widely accepted that cytoplasmic $Ca^{2+}$ has a key role in regulating growth cone motility[3]. Although there is little evidence from literature that $A_{2A}$ receptor activation is linked to intracellular $Ca^{2+}$ transients we investigated this possibility. Here we provide evidence that $A_{2A}$ receptor stimulation in turn can induce $Ca^{2+}$ release from intracellular stores.

The peripheral domain of a growth cone is rich in F-actin. One important target of extracellular guidance cues is this cytoskeletal structure[4]. Hence we investigated the effect of adenosine on the F-actin content of growth cones. Here we show that adenosine receptor stimulation increases the amount of phalloidin stainable F-actin in growth cones of chicken sensory neurons.

Altogether the data provided here support the notion that extracellular adenosine in general is influencing growth cone physiology and in particular is involved in regulation of growth cone motility. To which extent these effects have a physiological function in plasticity, development and repair of the neuronal system is matter of further investigation.

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# Long-range axogenesis of neocortical pyramidal neurons requires transcriptional specification by *NEX* and *NDRF*.

Ingo Bormuth[1,2], Tomoko Yonemasu[1], Kuo Yan[1,2], Maike Gummert[1], Ming Zhang[3], Sven Wichert[1], Alexander Pieper[1], Weiqi Zhang[3], Sandra Goebbels[1], Victor Tarabykin[1,2], Klaus-Armin Nave[1], Markus H. Schwab[1]

[1]Max-Planck-Institute of Experimental Medicine, Department of Neurogenetics, Göttingen, Germany.
[2]Charité, Universitätsmedizin Berlin, Institute of Cell Biology and Neurobiology, NeuroCure Cluster of Excellence, Berlin, Germany.
[3]University of Münster, Department of Psychiatry, Laboratory of Molecular Psychiatry, Münster, Germany.

Establishment of long-range fiber tracts by neocortical projection neurons is fundamental for higher brain functions. The molecular control of axon tract formation, however, is still poorly understood. Here, we have identified basic helix-loop-helix (bHLH) transcription factors *NEX* (Neurod6) and *NDRF* (Neurod2) as key regulators of fasciculation and targeted axogenesis in the neocortex. In NEX/NDRF double mutants, fiber tracts of neocortical origin are massively reduced or completely absent. Callosal axons, which are most severely affected, lack expression of the cell adhesion molecule contactin 2, defasciculate in the subventricular zone, and follow random trajectories within the ipsilateral cortex instead of crossing the midline. In contrast to long-range axogenesis, generation and maintenance of pyramidal neurons, initial axon outgrowth, dendritogenesis, and glutamatergic synapse assembly are largely unaffected, and thus under distinct transcriptional control. These findings demonstrate that neocortical projection neurons require transcriptional specification by neuronal bHLH proteins to execute an intrinsic program of remote connectivity.

# Loss of polysialic acid causes thalamocortical pathfinding defects and degeneration of the reticular thalamic nucleus

Iris Röckle[1], Miriam Schiff[1, 2], Birgit Weinhold[1], Herbert Hildebrandt[1, 2]

[1]Hannover Medical School, Institute of Cellular Chemistry, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Center for Systems Neuroscience (ZSN), Hannover, Germany

Polysialic acid (polySia) is a posttranslational modification of the neural cell adhesion molecule NCAM and tightly linked to neural development. Genetic ablation of NCAM ($N^{-/-}$) or the polySia synthesizing enzymes ST8SiaII and ST8SiaIV ($II^{-/-}IV^{-/-}$) leads to a loss of polySia. Surprisingly, however, only polySia-negative, NCAM-positive $II^{-/-}IV^{-/-}$ mice, but not NCAM- and polySia-deficient animals ($N^{-/-}$ or $II^{-/-}IV^{-/-}N^{-/-}$) exhibit a severe neurodevelopmental phenotype characterized by defects of major brain axon tracts, including internal capsule hypoplasia. Here, we demonstrate that misguidance of thalamocortical fibers and deficiencies of corticothalamic connections contribute to the internal capsule defects of $II^{-/-}IV^{-/-}$ mice. In $II^{-/-}IV^{-/-}$ animals, thalamocortical fibers cross the primordium of the reticular thalamic nucleus (Rt) at embryonic day 14.5 normal, but than fail to turn into the ventral telencephalon, thus deviating from their normal trajectory without passing through the internal capsule. At postnatal day 1, a reduction and massive disorganization of fibers traversing the Rt was observed, while TUNEL and cleaved caspase-3 staining indicated abundant apoptotic cell death of Rt neurons at postnatal day 5. Further during postnatal development, the number of parvalbumin-positive Rt neurons was drastically reduced in four week old $II^{-/-}IV^{-/-}$ mice, but not in the NCAM-deficient $N^{-/-}$ or $II^{-/-}IV^{-/-}N^{-/-}$ triple knockout animals displaying no internal capsule defects. Thus, degeneration of the Rt in $II^{-/-}IV^{-/-}$ mice may be a consequence of malformation of thalamocortical and corticothalamic fibers providing major excitatory input into the Rt. Indeed, apoptotic death of Rt neurons could be induced by lesioning corticothalamic fibers on whole brain slice cultures. We therefore propose that degeneration of the Rt in polysialylation-deficient, NCAM-positive $II^{-/-}IV^{-/-}$ mice is caused by defective afferent innervation due to thalamocortical pathfinding defects.

# Most cerebellar oligodendroglia have an extracerebellar origin

Nora Mecklenburg[1], Constantino Sotelo[1], Salvador Martínez[1]

[1]Intitute of Neuroscience, Alicante, Experimental Neurobiology, Campus de San Juan, 03550 San Juan de Alicante, Spain

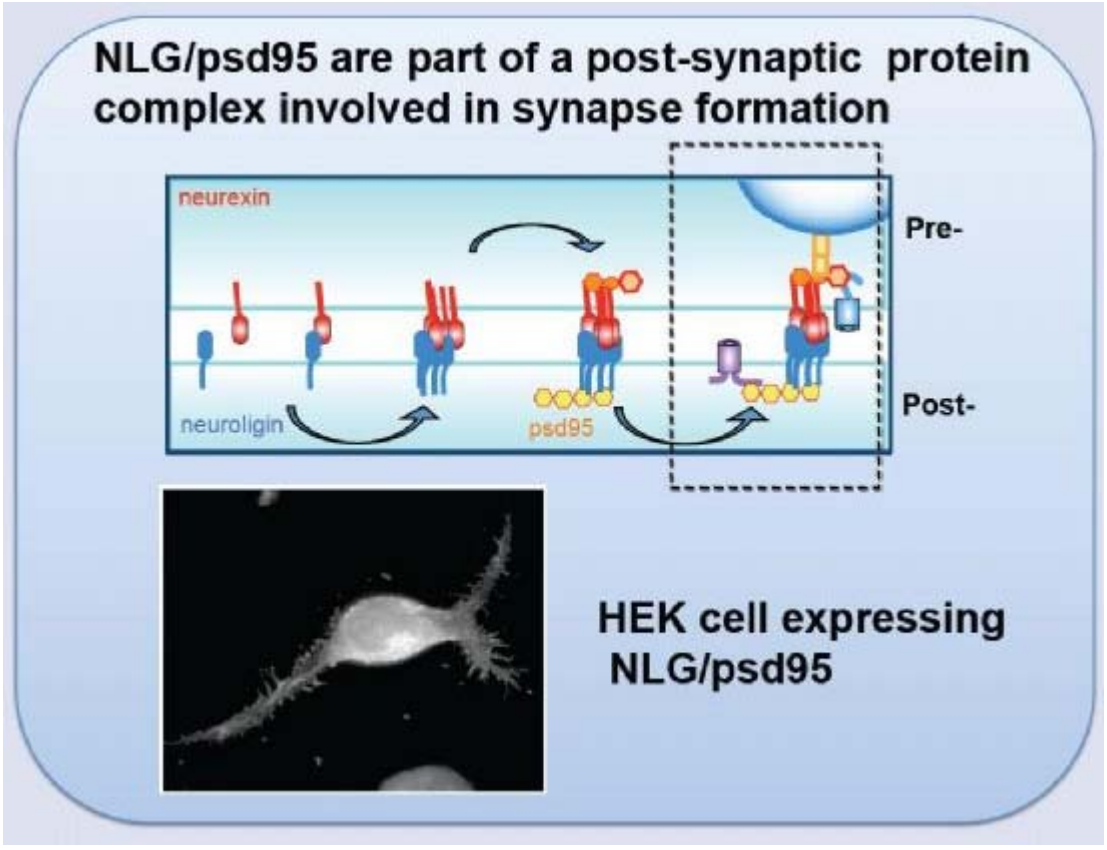
While the origin of oligodendroglia in the prosencephalon and spinal cord has been extensively studied, the origin of cerebellar oligodendrocytes has been only partially analyzed. To investigate where cerebellar oligodendrocytes generate and which migratory pathways they follow to reach their adult ultimate locations, in-ovo transplants were performed using the quail/chick chimeric system. The chimeric embryos were developed up to HH45 (19 days) to map the localization of donor cells and analyze their phenotype by immunohistochemistry staining. We found that mesencephalic homotopic and homochronic transplants generated cellular migratory streams from the grafted epithelium into the host cerebellum, crossing the isthmic borders and entering to the cerebellum via the velum medullare into the ventral region of the central white matter. Mapping the final location of these mesencephalic cells showed that they are located in all layers of the cerebellar cortex except the external granular layer. From their entry into the cerebellum, they were mainly accumulated at the central and folial white matter, as well as in the superficial regions of the internal granular layer and around Purkinje cells. At this later location, the donor cells were positive for Vimentin and acquired Bergmann glial features. At the other locations, particularly in the white matter, they were positive for PLP and Olig2 with oligodendrocytic identity. The combinatory analysis of the different grafts allowed us to propose a fate map of chick cerebellar oligodendroglia at neural tube stage. Most oligodendrocytes originate from the central alar plate of the mesencephalic vesicle.

# Neuroligin-1/psd-95 interactions induce cell morphology changes via lipid domain nucleation.

Maja Kaiser[1], Nicole Mende[1], Sophie Pautot[1]

[1]Center for Regenerative Therapies Dresden , AG Pautot, Tatzberg 47/49, 01307 Dresden, Germany

Thus far, neuroligin-1 (Nlg-1) has been known as a post-synaptic adhesion signaling membrane protein involved in initiating synaptic contact. Nlg-1 has previously been shown to trigger presynaptic differentiation in a neurexin expressing axon. Here, we are reporting that nlg-1 might also play a role in cell morphology changes. Indeed, when we co-expressed in HEK-293 nlg-1 with psd-95, a scaffolding protein which binds nlg-1 PDZ domain, we have observed extensive cell morphology changes. Co -transfected cells exhibited long expansions resembling dendritic branches, as well as a significant increase in cell surface area. We could destabilize these expansions by adding a PI3K inhibitor. After addition of wortmannin, we observed a retrograde transport of both nlg-1 and psd-95 from the branches toward the cell body. Depletion of the membrane cholesterol lead to the same observation suggesting that the formation of these branches are due to lipid membrane domain formation around nlg-1 clusters. These cholesterol rich domains enable the recruitment of PI3K, which in turns promotes the growth and the maintenance of these expansions. Moreover, the extent of the growth appeared to be related to the relative level of expression of nlg -1 and psd -95. Measure of nlg -1 and psd -95 expression level in hippocampal neurons culture showed similar level when neurons started branching out seeking their synaptic partners. These results taken together suggest that nlg -1, when clustered by psd -95 at the cell membrane, contributes to the formation of membrane lipid domain where proteins involved in cell expansion are recruited leading to formation of "dendrite like" branches.



# Neuromorphogenesis depending on the actin nucleator Cobl requires complex formation with the F-BAR protein syndapin I

Lukas Schwintzer[1], Rashmi Ahuja[1], Julia Grimm[1], Nicole Koch[1], Michael M. Kessels[1], Britta Qualmann[1]

[1]Universitätsklinikum Jena, Friedrich-Schiller-Universität Jena, Institut für Biochemie I, Nonnenplan 2, 07743 Jena, Germany

Actin cytoskeletal functions in vivo require careful control in time and space. Cortical actin dynamics plays a critical role in neuromorphogenesis. In developing primary hippocampal neurons, the formation of the dendritic arbor is dependent on the actin nucleation factor cordon-bleu (Cobl) (Ahuja et al., 2007). The underlying coordination of Cobl's actin nucleating activity, however, has remained elusive. Here we describe that syndapin I, an N-WASP/Arp2/3 complex-interacting F-BAR domain protein that is crucial for proper development of neuronal morphology (Dharmalingam et al., 2009), interacts with Cobl. Coprecipitations with various Cobl mutants demonstrate that syndapin I binds to two proline-rich regions within the N-terminal half of Cobl. These interactions are direct and depend on the SH3 domain of syndapin I – a domain also critically involved in eliciting syndapin I-mediated increases in dendrite number and dendritic branching. Coimmunoprecipitations demonstrate that the syndapin I/Cobl complexes are formed in vivo. Reconstitutions in intact cells suggest that syndapin I is able to recruit Cobl to membranes. Furthermore we present data addressing role of Cobl and syndapin I in neuromorphogenesis. Coexpression analyses show that both proteins promote dentritogenesis. Importantly, Cobl-mediated functions in neuromorphogenesis critically rely on syndapin I. Our work provides urgently awaited insights into the molecular basis for proper neuronal network formation.

# Neuronal growth cones and neurite extension require M6-glycoproteins

Patricia de Monasterio-Schrader[1], Ursula Fünfschilling[1], Miso Mitkovski[2], Agnieszka Z. Burzynska[1], Matthias Klugmann[1], Leda Dimou[3], Sergi Papiol[1], Klaus-Armin Nave[1], Hauke B. Werner[1]

[1]MPI of Experimental Medicine, Dept. of Neurogenetics, Germany
[2]MPI of Experimental Medicine, Two-Photon and Confocal Microscopy, Germany
[3]Physiological Genomics, Institute of Physiology, Ludwig-Maximilians University Munich, Munich, Germany

Neuronal function depends on the developmental outgrowth of their cellular processes led by structurally and molecularly specialized growth cones. The tetraspan transmembrane glycoprotein M6A has been implicated in neurite extension because of its increased abundance at the onset of neurite outgrowth *in vivo*, its particular enrichment at the leading edge of growth cones, and according to overexpression studies *in vitro*. On neuronal growth cones, M6A and its homolog M6B have an overlapping but non-identical distribution. For the analysis of neuronal development in the absence of M6-proteins we have generated single-mutant M6A$^{null}$ and M6B$^{null}$ mice, as well as M6A$^{null}$*M6B$^{null}$ double-mutants. At the cellular level, we found that the chronic lack of both neuronal M6-proteins significantly impaired neurite extension of cultured cerebellar granule cells as well as cortical neurons. Moreover, mutant cortical neurons did not react to EphrinA5 induced growth cone collapse. Our results imply that M6A defines a novel, F-actin free structural compartment at the growth cone tip that exerts adhesive forces to the substrate. Taken together, M6-proteolipids organize the structure of neuronal growth cones that is prerequisite for their normal reaction to guidance cues and neurite extension.

# Neuronal morphology is controlled by the interplay of Cobl and Abp1

Natja Haag[1], Lukas Schwintzer[1], Rashmi Ahuja[1], Julia Grimm[1], Britta Qualmann[1], Michael M. Kessels[1]

[1]Friedrich-Schiller University Jena, Institute for Biochemistry I, Nonnenplan 2, 07743 Jena, Germany

The shaping of neuronal morphology during differentiation and circuit formation critically relies on dynamic remodelling of the actin cytoskeleton. On the subcellular level, this requires a careful regulation of actin filament formation at the cell cortex, which is driven by actin nucleation factors. In developing primary hippocampal neurons, the actin nucleator cordon-bleu (Cobl) is essential for proper formation of the dendritic compartment [1].The underlying fine-control of Cobl's actin nucleating capacity, however, largely remains elusive.

Here we describe that Abp1, an N-WASP/Arp2/3 complex-interacting and F-actin-binding protein that is crucial for precise development of neuronal morphology [2-4], directly interacts with Cobl in vitro via SH3/PxxP motif association. Furthermore, coimmunoprecipitations demonstrate that Abp1/Cobl complexes are formed in cells. Fractionations of brain tissue suggest that a sub-pool of both Abp1 and Cobl is associated with the plasma membrane as it would be required for Abp1/Cobl complexes shaping neurons. To explicitly prove the role of Abp1/Cobl protein complexes in dendritogenesis, we exploited the increased dendrite formation and dendritic branching induced by an excess of Cobl and asked whether this phenotype would be critically dependent on the Cobl interaction partner Abp1. Abp1-RNAi indeed was fully sufficient to suppress the effects of Cobl on dendritic arborisation. Likewise, it was possible to suppress the Cobl-mediated effects by quenching the protein with overexpressed Abp1 SH3 domain. Interestingly, histological analyses of Cobl expression in the brain revealed high amounts of Cobl in Purkinje cells. These cells are marked by an elaborate dendritic arbor and also show Abp1 expression. In order to address putative Cobl functions in dendritogenesis in a relatively intact brain environment, we used a gene gun to biolistically transfected Purkinje cells within cerebellar slice cultures with RNAi plasmids against both Cobl and Abp1. Cobl-deficient Purkinje cells displayed a strongly impaired arborization. The number of dendritic branching points was significantly reduced. Lack of Abp1 caused a similar morphological phenotype. As knock-down of both proteins phenocopies each other, Cobl and Abp1 both seem to be critically involved in the dendritic arborization of Purkinje cells.

Summarized, our data thus strongly suggest that Abp1 is crucial for cell morphological changes brought about by the Cobl-actin nucleation machinery and that Cobl/Abp1 complexes play a critical role in proper development of the elaborate dendritic arbor of Purkinje cells – a prerequisite for the high connectivity in the cerebellar cortex and for the implementation of complex tasks, such as control and coordination of movements.

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# Nitric oxide affects injury-induced neuritogenesis and synaptogenesis of both nitrigic and non-nitrigic neurons

Ria Mishaal Cooke[1], Volko A Straub[1]

[1]University of Leicester, Cell Physiology and Pharmacology, University Road, LE1 9HN Leicester, United Kingdom

Nitric oxide (NO) is recognised as having an important function at stages in an animal's life when neurons grow and connect, illustrated by the dynamic regulation of nitric oxide synthase (NOS) expression during the embryonic development of vertebrates (mammals, fish and amphibians) and invertebrates (insects and molluscs). The significant influence of NO on elements of neuritogenesis and synaptogenesis appears to be evolutionarily conserved at least among invertebrates (e.g. *Drosophila*, *Manduca* and *Schistocerca*). While the importance of NO is now widely accepted, the precise nature of its role remains unclear. To complement our previous studies that have shown evidence for an effect of NO on the growth of isolated cultured buccal neurons from the pond snail *Lymnaea stagnalis* (Cooke and Straub, 2008), the current study is focussed on elucidating the effect of NO on injury-induced growth and synapse formation of *Lymnaea* neurons in organotypic cultures of the intact buccal ganglia. Here, we show that *Lymnaea* buccal motor neurons exhibit extensive growth and the formation of novel connections 1-4 days after proximal dorsal buccal nerve crush, comparable to previous reports of regeneration and synapse formation in homologous neurons in a closely related snail, *Helisoma* (e.g. Hadley et al, 1982). We were interested to investigate if NO had similar or differential effects on cells which endogenously produce NO versus those that do not. Our work focussed on the nitrigic B2 neuron and the non-nitrergic B1 neuron. We found that blocking endogenous NO production with the NOS inhibitor 7-nitroindazole affects neuritogenesis following injury of both B2 and B1 neurons. We also observed changes in the time course and strength of existing and injury-induced novel synapses between these neurons. In addition to the effects of blocking NOS activity, we also present results for the effects of NO donors and the soluble cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ). These data show that the effect of NO is ubiquitous and not limited to those cells which endogenously produce NO.

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# NO slowdown by CO: dual regulation of neuronal migration by gaseous messengers

Sabine Knipp[1], Gerd Bicker[1]

[1]University of Veterinary Medicine Hannover, Cell Biology, Bischofsholer Damm 15/102, 30173 Hannover, Germany

During development of nervous systems, neuronal cell migration is controlled by multiple guidance cues. We have previously shown that the gaseous messenger molecules nitric oxide (NO) and carbon monoxide (CO) regulate cell motility in an insect enteric nervous system (ENS) (Development 2003, 130: 3977-3987; Development 2009, 136: 85-93).

The development of the locust ENS provides a useful model for studying the cell biology of migration. It is formed by neurons that arise in three neurogenic zones on the stomodeum and migrate considerable distances towards their mature positions. The neurons of the midgut plexus travel in a mode of chain migration on four defined pathways across the whole midgut. Although the precise guidance factors have not been completely identified, the longitudinal midgut musculature seems to be involved (Dev Dyn 2009, 238: 2837-2849)

Migration of enteric neurons on the midgut is facilitated by transcellular NO signaling via activation of soluble guanylyl cyclase (sGC) and elevation of cGMP levels. We could also identify the Rho kinase ROCK as a putative downstream cytoskeletal regulator for enteric neuron migration.

A second gaseous messenger, carbon monoxide, is inhibiting enteric neuron migration. Furthermore, CO provides a slow down signal to regulate the intercellular distance in the chain of migratory neurons. Pharmacological blocking of CO release leads to a significant divergence of the cell bodies along the migratory pathway. Time lapse video analysis reveals that CO affects the migratory speed of enteric neurons by interfering with their stop-and-search behavior. Again, one of the downstream targets of CO may be sGC. Like NO, CO is able to activate sGC in enteric neurons, although less effective. Thus, we propose a dual-process hypothesis in which the activation of the sGC enzyme by differential CO and NO binding coordinates midgut neuron migration.

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# Spinal cord – motor cortex coculture model: a new technique to study neuronal regeneration in vitro

Martin Pohland[1], Jürgen Kiwit[2], Jana Glumm[1,2]

Institute of Cell Biology and Neurobiology, Center for Anatomy, Charité-Universitätsmedizin Berlin[1] Department of Neurosurgery, HELIOS Klinikum Berlin, Klinikum Buch [2]

## Introduction

After mechanical traumas cortical axons have a substantial potential for axonal growth and regeneration. Today several hippocampal, as well as spinal, in vitro lesion models are used to investigate neuronal differentiation, axonal growth and path finding (Bonnici et al., 2008; del Rio et al., 2010). Here we describe a new cytoarchitecture-preserving slice coculture technique to analyse neuronal regeneration and axonal outgrowth between the motor cortex and the spinal cord.

## Methodes and Materials

Spinal cord (sc) from postnatal (P0-P3) C57Bl/6 mice and motor cortex (mc) dissected from Bl6.GFP pups (P0-P3) expressing green fluorescent protein (GFP) under beta-actin promoter control were chopped either in a sagittal longitudinal plane for the sc or in a coronal plane for the mc. Afterwards the medial cortex zone was orientated to the rostral end of the spinal cord and incubated up to two weeks.

## Results

Using nonfluorescent pups as medulla donors and constantly GFP-expressing heterocygote mice as cortex givers, we can easily distinguish ingrowing cortical neurons in non-fluorescent wild type tissue. Our data shows ingrowing fibers and growth cones which are already detectable after 1 day in vitro (DIV). Moreover, the rate of growth was measured using confocal microscopy. In addition, immunhistochemical staining after 1, 3 and 6 DIV suggest a strong neuronal outgrowth and not only a reestablishment of cortical fibers but also their connections by means of microscopical analysis. Furthermore we were able to design a capable evaluation-matrix.

## Conclusion

Thus, this in vitro method offers possibilities to test axon-regenerative properties of determined compounds or treatments and could provide an important tool to answer a variety of questions in the field of neuronal regeneration.

# The role of 5-HT7/G12 signaling pathway in developmental regulation of morpho- and synaptogenesis in hippocampal neurons

Fritz Kobe[1], Daria Guseva[2], Michael Mueller[1], Diethelm W Richter[1], Evgeni Ponimaskin[2]

[1]University of Goettingen, CMPB / Neuro- and Sensory Physiology, Humboldtallee 23, 37073 Goettingen, Germany
[2] Medical School Hannover, Cellular Neurophysiology, Germany

Serotonin (5 -hydroxytryptamine or 5- HT) is an important neurotransmitter regulating a wide range of physiological and pathological functions via activation specific 5-HT receptors. In addition to its well-established role in neuronal communication, 5- HT has been shown to be involved in many aspects of neural development, such as neurite outgrowth, regulation of neuronal morphology, growth cone motility and dendritic spine shape and density. However, the molecular downstream mechanisms are still poorly understood. We have recently shown that the activation of the 5- HT7/G12 signaling results in increased neuronal outgrowth. In present study we demonstrate the importance of this pathway for the spine formation, synaptogenesis and synaptic plasticity. Interestingly, these effects were presented only in young mice and abolished in adult animals. In order to elucidate these findings we focused on the expression profile for both 5- HT7 receptor and Ga12 protein in the mice hippocampus during the different stages of postnatal development using real -time PCR and immunohistochemistry. We found that the 5-HT7 receptor transcripts were strongly expressed at the early postnatal stages (P2 and P6) and down regulated (up to 9 fold) during the later development. A similar expression profile was also obtained for the Ga12 protein. Immunohistochemical analysis also revealed higher expression of the 5-HT7 receptor during early postnatal stages in comparison to P90. Notably, we obtained a high degree of co-localization between the 5-HT7 receptor and 5-HT containing varicosities at P2 and P6 stages, but not at P90.
Taken together, our results provide evidence that the 5-HT7 receptor and Ga12 proteins display dynamic expression patterns characterized by the strong and transient expression during the early postnatal stages of hippocampal development. Such changes in expression level might be a possible mechanism of morpho- and synaptogenesis modulated by 5-HT7/G12 signaling during development. Consistent with electrophysiological results we observed increase in both the number of total AMPA receptor-positive puncta as well as in AMPA receptors at synapses. These data suggested that stimulation of the 5-HT7/G12 signaling pathway results in an increased number of excitatory synapses of hippocampal neurons and can play an important role for the formation and function of initial neuronal networks.

# L1CAM ubiquitination facilitates its lysosomal degradation

Michael K. Schaefer[1]

[1]University of Freiburg, Institute of Anatomy and Cell Biology, Albertstr. 17, 79104 Freiburg, Germany
[2]University of Bonn, Institute of Animal Sciences, Katzenburgweg 9a, 51335 Bonn, Germany

The cell adhesion molecule L1CAM is implicated in several processes in the developing and adult nervous system. Mutations in the human L1CAM gene cause various neurological conditions, jointly referred to as L1 syndrome. Intracellular trafficking of L1CAM is important for cell migration, neurite growth and adhesion. We demonstrate here that L1CAM is ubiquitinated at the plasma membrane and in early endosomes. Mono-ubiquitination regulates L1 intracellular trafficking by enhancing its lysosomal degradation. We propose that L1's ubiquitination might be an additional mechanism to control its re-appearance at the cell surface thereby influencing processes like neurite growth and cell adhesion.

# Developmental expression of cell surface molecules in the locust

René Eickhoff[1], Michael Stern[1], Gerd Bicker[1]

[1]University of Veterinary Medicine Hannover, Div. of Cell Biology, Bischofsholer Damm 15/102, 30173 Hannover, Germany

Mushroom bodies are multimodal integration centers that have been identified in a wide range of arthropods and some annelids. Developmental studies on mushroom bodies have in most detail been carried out in the holometabolous insect species *Drosophila melanogaster* and *Apis mellifera*. Far less is known about the formation of mushroom bodies in hemimetabolous insects. Here we examine the embryonic and larval morphogenesis of the mushroom bodies in *Locusta migratoria*, a well established invertebrate model organism for the analysis of cellular mechanisms of nervous system development. To analyze neurogenesis and neurite outgrowth, we use cytochemical markers of newborn neurons, cell proliferation markers, and phalloidin staining of polymeric actin. However, our main focus is the expression patterning of cell surface glycoproteins such as Semaphorin Ia and Fasciclin I. These molecules have originally been identified in the locust and are known to play pivotal roles in nervous system development in both locusts and *Drosophila*.

At the onset of development neuropils of the developing olfactory pathway express the cell recognition molecule Semaphorin Ia. During late embryonic stages semaphorin 1a expression in the mushroom bodies becomes restricted to a subset of Kenyon cells in the core region of the pedunculus. This is also true for adults. Fasciclin I expression in the olfactory pathway appears later than Semaphorin Ia expression. Larval and adult mushroom bodies reveal strong Fasciclin I immunoreactivity. Neurogenesis in the mushroom bodies of the locust is not fully completed after embryonic development. Kenyon cell progenitors proliferate throughout all larval stages. Newborn Kenyon cells that grow out into the core of the pedunculus express Semaphorin 1a but not Fasciclin I in L1 larvae.

We also address the question whether nitric oxide synthase (NOS) localization and activity implicates a role for the messenger molecule nitric oxide in mushroom body development. Cytochemical markers for NOS do not provide conclusive evidence for a role of this enzyme during embryonic development of Kenyon cells.

# Poster Topic

# T3: Developmental Cell Death, Regeneration and Transplantation

T3-1A 6-OHDA-injection into the nigrostriatal pathway of mice leads to a phenotypic shift of striatal neurons into tyrosine hydroxylase immunoreactive neurons
*Stefan Jean-Pierre Haas, Martin Duckert, Andreas Hilla, Oliver Schmitt, Andreas Wree*

T3-2A BHLH Transcription Factors of the NeuroD Family are essential for differentiation and survival of cortical pyramidal neurons.
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T3-1B Human unrestricted somatic stem cells (USSC) in spinal cord injury: Characterization of directed migration, paracrine neurotrophic support, axon regeneration, and functional improvement
*Jessica Schira, Marcia Gasis, Veronica Estrada, Marion Hendricks, Christine Schmitz, Nadine Hamacher, Torsten Trapp, Fabian Kruse, Gesine Kögler, Peter Wernet, Hans Werner Müller*

T3-2B Implantable mechanical microsystem enhances axon regeneration after complete spinal cord injury in the rat
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T3-4B Improved intrathecal infusion method designed for rodent models of spinal cord injury
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T3-5B An *in vitro* model for scar formation to study the mechanisms of scar-reducing treatments used in spinal cord injury
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*Tamara I. Rabe, Frèdèrique Varoqueaux, Gundula Griesel, Andreas Kispert, Peter H. Burbach, Ahmed Mansouri*

T3-2C Taxol facilitates axon regeneration in the mature CNS
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T3-3C Translational Research in Axon Regeneration: Locomotor Recovery after Systemic Administration of Deoxyribozyme to XT-1 mRNA after a Moderate Contusion of the Adult Rat Spinal Cord
*Martin Oudega, Owen Chao, Roderick Bronson, Donna Avison, Alexander Marcillo, Andres Hurtado, William Buchser, Barbara Grimpe*

T3-4C GDF-15 deficiency leads to Schwann cell and motoneuron loss in adult mice.
*Sabrina Walter, Klaus Unsicker, Jens Strelau*

T3-5C Colloids as mobile substrates for the implantation and integration of differentiated neurons into the mammalian brain
*Sophie Pautot, Dennis Jgamadze, Daniel Stone, Jamie Berger, David Schaffer, Ehud Isacoff*

# 6-OHDA-injection into the nigrostriatal pathway of mice leads to a phenotypic shift of striatal neurons into tyrosine hydroxylase immunoreactive neurons

Stefan Jean-Pierre Haas[1], Martin Duckert[1], Andreas Hilla[1], Oliver Schmitt[1], Andreas Wree[1]

[1]Medical Faculty/University of Rostock, Institute of Anatomy, Gertrudenstrasse 9, 18057 Rostock, Germany

We established a mouse model of Parkinson's disease by a unilateral injection of 6-OHDA into the medial forebrain bundle. In these mice, contrary to Hemiparkonsian rats, we documented the appearance of tyrosine hydroxylase (TH) containing neurons in the dopaminergic deafferentiated striatum. This is of special interest, because TH is the pacemaker enzyme of dopamine synthesis. In this study we investigated whether these TH-ir neurons were newly generated or if reprogramming mechanisms of existing striatal neurons were leading to a phenotypic shift.

C57BL/6-mice (n=16) were lesioned by a stereotactic 6-OHDA-injection into the right medial forebrain bundle (2 µl containing 5 µg 6-OHDA). Three days prior to the lesion all animals received daily injections of the S-phase marker BrdU for up to one week. One group of animals was perfused four days after lesion. Three months after lesion apomorphine-induced rotations were evaluated in the second group to document successful lesions and finally these mice were also perfused with 3.7% PFA and immunohistochemistry to visualize TH-ir neurons or glial cells in combination with BrdU-ir cell nuclei in brain sections was performed with both groups.

Four days after lesion the unilateral dopaminergic cell loss in the substantia nigra and the loss of TH-ir terminal fibers in the striatum was clearly detectable. At this time point numerous TH-ir neurons with various processes could be observed in the lesioned striatum. Three months post lesion the unilateral dopaminergic cell loss in the substantia nigra and the decreased amount of TH-ir fibers in the striatum was more pronounced. This unilateral striatal dopaminergic deafferentiation was also demonstrated by the numbers of apomorphine-induced contralateral rotations (16.4 ± 2.5 rotations per min). No TH-BrdU co-localized neurons 4 days postlesion or after three months postlesion were detected in the lesioned striatum. Here, only glial cells co-expressed BrdU. The amount of BrdU-ir cells in the lesioned hemisphere was significantly higher in the lesioned striatum, compared to the intact striatum where only single glial cells contained BrdU.

TH-ir neurons in the lesioned striatum were detectable very early post-lesion and did not contain BrdU. Therefore, these now appearing cells have not been generated by adult neurogenesis. However, the fact that postmitotic neurons in the adult brain perform a phenotypic shift is a very interesting phenomenon. Moreover, because TH is the pacemaker enzyme of dopamine synthesis the further question is if these cells express more dopaminergic markers and are able to compensate the lesion effect. This issue and the question from which pool of existing striatal neurons the TH-ir neurons were derived remains unclear and will be further investigated.

# BHLH Transcription Factors of the NeuroD Family are essential for differentiation and survival of cortical pyramidal neurons.

Kuo Yan[1], Ingo Bormuth[1,2], Tomoko Yonemasu[1], Sandra Goebbels[1], Victor Tarabykin[1,2], Klaus-Armin Nave[1], Markus H. Schwab[1]

[1]Max-Planck-Institute of Experimental Medicine, Department of Neurogenetics, Goettingen, Germany.

[2]Charite, Universitaetsmedizin Berlin, Institute of Cell Biology and Neurobiology, NeuroCure Cluster of Excellence, Berlin, Germany.

Basic helix-loop-helix (bHLH) transcription factors regulate biological differentiation processes ranging from cell determination to complex organ development. NeuroD (Neurod1), NDRF (Neurod2), and NEX (Neurod6) are closely related neuronal bHLH transcription factors expressed in pyramidal neurons following similar spatial and temporal pattern. Disruption of either one gene in mice does not impair pyramidal neuron development but may well lead to differentiation defects and loss of other large cell populations (e.g. of hippocampal granule neurons in case of NeuroD inactivation).

We analyzed NeuroD*NDRF*NEX triple mutant mice and found strong effects on pyramidal neuron differentiation and survival. The hippocampal CA1-3 fields are aplastic. The neocortex is reduced in size and the strict organization of pyramidal neurons in cortical columns and layers is impaired.

NeuroD, NDRF and NEX function highly redundant in pyramidal neuron differentiation. Either one of the three has the capability to serve as key regulator of pyramidal neuron survival.

# Cell loss and autophagy in the extra-adrenal chromaffin organ of Zuckerkandl are regulated by glucocorticoid signaling

Andreas Schober[1], Rosanna Parlato[2], Katrin Huber[1], Ralf Kinscherf[3], Günther Schütz[2], Klaus Unsicker[1]

[1]University of Freiburg, Institute of Anatomy & Cell Biology II, Department of Molecular Embryology, Albertstr. 17, D-79104 Freiburg, Germany

[2]German Cancer Research Center, Division of Molecular Biology of the Cell II, INF-280,69120 Heidelberg, Germany

[3]University of Marburg, Institute of Anatomy & Cell Biology, Robert-Koch-Str. 8, D-35037 Marburg, Germany

Zuckerkandl's organ (ZO) contains the largest accumulation of extra -adrenal chromaffin tissue in mammals, which disappears postnatally by modes which are still enigmatic. It has long been known that extra-adrenal chromaffin tissue in other locations and SIF cells in sympathetic ganglia persist postnatally, but undergo hypertrophy when treated with glucocorticoids (GC). Whether this reflects a pharmacological or a physiological role of glucocorticoids was not clear. Using mice with a conditional deletion of the glucocorticoid receptor (GR) gene under the control of the dopamine beta hydroxylase (DBH) promoter we now present the first evidence for a physiological role of GC signaling in the postnatal maintenance of the ZO. Postnatal cell loss in the ZO is accompanied by autophagy. Electron microscopy reveals autophagic vacuoles and autophago-lysosomes. Additionally, lysosomal (cathepsin-D) and macrophage (BM-8) markers were significantly increased in the ZO of GR-deficient as compared to wild type mice. Moreover, postnatal death of chromaffin cells in the ZO was significantly accelerated in mice lacking the GR. In summary, our results indicate that extra-adrenal chromaffin cells in the ZO show signs of autophagy, which accompany their postnatal numerical decline controlled by GR signaling. Currently, we are studying putative causal relationships between cell loss and autophagy in this system.

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# Growth differentiation factor-15 (GDF-15) in peripheral nerve injury

Petar Charalambous[1], Xiaolong Wang[1], Andreas Schober[1], Jens Strelau[2], Frank Bosse[3], Hans Werner Müller[3], Klaus Unsicker[1]

[1]Institute of Anatomy and Cell Biology, Department of Molecular Embryology, Albertstr. 17, 79104 Freiburg, Germany
[2]Department of Neuroanatomy and Interdisciplinary Center for Neurosciences IZN, University of Heidelberg, INF 307, Heidelberg, Germany
[3]Molecular Neurobiology Laboratory, Heinrich-Heine-University Düsseldorf, 40223 Düsseldorf, Germany

GDF-15 is a distant member of the TGF-ß superfamily, which we discovered by screening EST data bases for conserved TGF-ß consensus sequences (1). GDF-15 is widely expressed in the body, most prominently in liver, lung, kidney, and exocrine glands (2). Functions assigned to GDF-15 to date are mostly related to cancer biology and progression, hematopoiesis, inflammation, and cardioprotection. In the brain we found GDF-15 to be expressed ubiquitously, yet at relatively low levels. One prominent neural function relates to its capacity to promote survival of embryonic and adult lesioned midbrain dopaminergic neurons (3). In the cryo-lesioned cortex GDF-15 is highly upregulated in neurons and sporadically in microglial cells (4). We have generated a GDF-15 knockout mouse, which has revealed progressive postnatal motor and sensory neuron loss (5).

GDF-15 is also expressed in the peripheral nervous system, as e.g. in dorsal root ganglia (DRG) and peripheral nerves, where it is synthesized and released by Schwann cells. This is consistent with one feature of the GDF-15 knockout phenotype, peripheral hypermyelination.

To further address putative functions of GDF-15 in peripheral nerves we have performed sciatic nerve transections in adult rats at mid-thigh level and determined GDF-15 mRNA and protein levels in the DRG as well as in 4 portions of the sciatic nerve, two proximal and two distal of the lesion site.

Our preliminary results suggest that at day 1 post-lesion GDF-15 mRNA is increased on average 7-fold in the DRG, 10-fold proximally, but only 2-fold distally to the lesion. GDF-15 mRNA levels in the DRG and proximal section return to almost control levels on day 7, whereas levels in the distal nerve increase to approximately 5-fold. We conclude that GDF-15 may operate as a "lesion factor" both in the central and peripheral nervous systems, yet its precise roles remain to be determined.

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# Human unrestricted somatic stem cells (USSC) in spinal cord injury: Characterization of directed migration, paracrine neurotrophic support, axon regeneration, and functional improvement

Jessica Schira[1], Marcia Gasis[1], Veronica Estrada[1], Marion Hendricks[1], Christine Schmitz[1], Nadine Hamacher[1], Torsten Trapp[2], Fabian Kruse[1], Gesine Kögler[2], Peter Wernet[2], Hans Werner Müller[1]

[1]Neurochemical Laboratory, Neurology, Heinrich Heine University Duesseldorf, Moorenstr. 5, 40225 Duesseldorf, Germany, [2]Institute for Transplantation Diagnostics and Cell Therapeutics, Heinrich Heine University Medical Center, 40225 Duesseldorf, Germany

Spinal cord injury (SCI) results in permanent loss of axons, scar formation and consequently functional disability. One approach to promote axonal regeneration is to transplant cells with the capacity to protect the endogenous tissue and/or to stimulate axon growth. Actually, different stem cell types have been grafted into animal models and humans with SCI. Due to inconsistent results, it is still an open question which stem cell type will prove to be therapeutically effective. Thus far, stem cells of human sources grafted into spinal cord mostly included barely defined heterogeneous mesenchymal stem cell populations derived from bone marrow or umbilical cord blood. Here, we have used a well-defined unrestricted somatic stem cell (USSC) population isolated from human umbilical cord blood and transplanted into an acute traumatic SCI of adult immunosuppressed rat. Stereotactical grafting of native USSC into the vicinity of a dorsal hemisection injury at thoracic level Th8 yielded massive migration of these stem cells to the lesion center. In vitro under-agarose chemotaxis assays revealed that the attraction of USSC to the injured spinal cord tissue is hepatocyte growth factor-mediated. After transplantation into the injured spinal cord, USSC accumulated within the lesion area where they survived for at least three weeks without neural differentiation. Histological analysis showed significantly enhanced axon ingrowth into the lesion site five weeks after grafting as assessed by anterograde tracing. In addition, USSC conditioned medium efficiently increased neurite outgrowth of rat embryonic dorsal root ganglion explants and primary cortical neurons comparable with the capacity of astrocyte conditioned medium. Importantly, long-term behavioral studies (16 weeks) including three different locomotor tests (open field BBB locomotor score, horizontal ladder walking test, CatWalk gait analysis) demonstrated significant functional benefits following USSC transplantation. The observed functional improvement correlated well with reduced tissue loss or augmented tissue sparing and stimulation of regenerative axon growth. To accomplish the beneficial effects, neither neural differentiation nor long-lasting persistence of the grafted human stem cell appears to be required. The secretion of neurite outgrowth promoting factors in vitro further suggests a paracrine mechanism underlying the beneficial effects of USSC in SCI. Given the highly supportive functional characteristics in SCI, production in virtually unlimited quantities at GMP-grade and lack of ethical concerns, USSC appear to be a suitable human stem cell source for clinical application in CNS injuries.

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Correspondence: J.schira@uni-duesseldorf.de

# Implantable mechanical microsystem enhances axon regeneration after complete spinal cord injury in the rat

Veronica Estrada[1], Nicole Brazda[1], Christian Voss[2], Christine Schmitz[1], Klaus Seide[3], Nils Weinrich[3], Jörg Müller[2], Hans Werner Müller[1]

[1]Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf, Moorenstr. 5, 40225 Düsseldorf, Germany
[2]Institute of Micro Systems Technology, Hamburg University of Technology
[3]BG Trauma Hospital Hamburg

After a spinal cord injury, the distal part of an axon is often separated from its proximal part, resulting in the degeneration of the distal part and subsequent loss of the respective function. Furthermore, the fibrous scar which forms after a spinal cord lesion – with a collagen IV meshwork being the substrate binding several inhibitory molecules - is a major impediment for axonal regeneration. We have previously developed a pharmacological treatment to transiently suppress the formation of the collagenous fibrous scar which includes the local application of an iron chelator to inhibit a key enzyme of collagen IV-biosynthesis in acute spinal cord injury of the adult rat (Hermanns et al., J Neurosci Methods, 2001). Treated rats showed axonal regeneration, neuroprotection of projecting pyramidal neurons and functional recovery in different motor tasks after acute SCI (Klapka et al., Eur J Neurosci. 2005).
In the present study, we used a mechanical microconnector (Figure A), composed of poly(methyl methacrylate) (PMMA), which was developed for the purpose to reconnect the separated stumps in an injured spinal cord. It is further intended to be used for prolonged application of pharmacological substances via an internal channel system.
Animals received a complete spinal cord transection at level Th8/9. Subsequently to the lesion, the mechanical microconnector was inserted into the gap which formed between the two stumps. After the microconnector was positioned correctly into the spinal cord, the *dura mater* was sutured, and the spinal cord tissue was brought into the hollow space of the connector via transient application of negative pressure with an external vacuum pump. Control animals received the total spinal cord transection plus suture of the *dura mater*, but no connector implantation.
Functional outcome was measured weekly using the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale. Animals which received total spinal cord transection and subsequent microconnector implantation reached BBB scores of 8, relating to sweeping movements of both hind limbs or, respectively, plantar placement of the hind paws with no weight support.
Histological examination was performed at the time points of 2, 5 and 12 weeks post lesion and microconnector implantation (wpi). In the lesion area, we investigated tissue histocompatibility of the connector device, scar formation, and vascularization at 2 wpi. After 5 or 12 wpi regenerative axon growth of varying neuronal populations involved in motor and sensory functions was analyzed via immunohistochemical staining (neurofilament, Calcitonin gene-related peptide, serotonin, tyrosine hydroxylase) or BDA-labeling (corticospinal tract), respectively. Besides vascularization we observed increased amounts of axon fibers in the lumen of the microconnector (Figure B) compared to the scar area of control animals.
In future experiments the mechanical microconnector will be tested for its suitability to deliver pharmacological substances, e.g. iron chelator and cAMP, to suppress scarring, protect from secondary tissue degeneration and promote axon regeneration.

These results show, for the first time, the use of a mechanical microsystem to successfully reconnect separated stumps of a completely transected spinal cord leading to promotion of regenerative axon growth.

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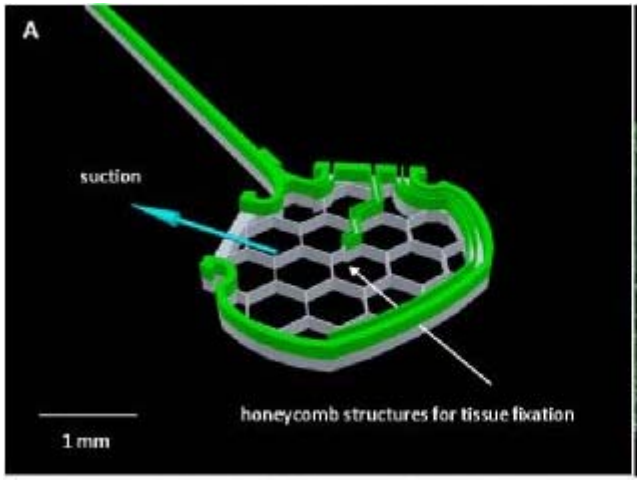
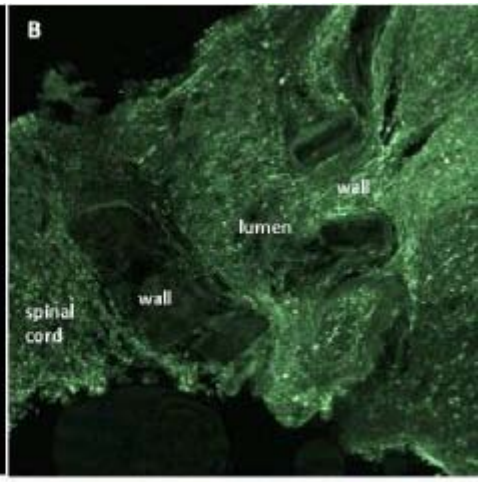



A: Schematic overview of the mechanical micro-connector




B: Neurofilament-positive axons in the connector lumen at 5 wpI

# Identification and Characterization of regeneration-associated genes (RAGs) by paradigm-specific gene expression profiling of injured PNS

Katharina Malik[1], Marcia Gasis[1], Mark Boras[1], Hans Werner Mueller[1], Frank Bosse[1]

[1]Mol. Neurobiology Laboratory, Dept. of Neurol., Heinrich-Heine-University, Duesseldorf, Germany

In order to identify regeneration-associated genes we very recently performed a comprehensive series of mRNA expression studies at 8hrs, 1d, 2d, 7d and 21d after rat sciatic nerve injury. Expression profiles of three distinct lesion paradigms of the sciatic nerve were obtained by Affymetrix GeneChip hybridization: (i) during Wallerian degeneration and spontaneous regeneration after peripheral nerve crush, (ii) of chronically axotomized neurons after nerve transection combined with ligation of nerve stumps, and (iii) during delayed regeneration of neurons following reanastomosis (coaptation) of previously cut nerve stumps.
Paradigm- and time point-specific clustering of lesion-induced mRNA regulation enabled us to identify numerous new putative regeneration-associated genes (RAGs) that exactly reflect expression patterns of well established genes with crucial functions in successful axonal regeneration.

In order to characterize selected new putative RAGs we started cellular localization analysis in vivo as well as functional tests in vitro. Interestingly, cultures of neuronal F11 cells showed differential mRNA expression of a large proportion of the selected putative RAGs under neurite outgrowth promoting culture conditions. We successfully characterized the cellular distribution of such selected putative new RAGs and revealed expression in large and small diameter sensory DRG neurons in vivo. Even more, our immunostainings revealed distinct sub-cellular protein translocations of single RAG candidates which may be cell differentiation/neurite outgrowth dependent.
To assess whether our RAG candidates may affect neurite outgrowth, specific constructs were generated to recombinantly modify candidate gene expression levels in cultured F11 cells under different growth conditions. Quantitative RT-PCR as well as western blots were used to study functionality of generated constructs. In fact, comprehensive transient overexpression and/or suppression studies with several RAG candidates revealed their impact on both neurite formation and neurite outgrowth. In particular, transient overexpression of a specific transcription factor was sufficient to stimulate the generation of neurite-like processes under proliferating culture condition and to promote neurite outgrowth. In contrast, transient knock-down of this RAG candidate resulted in a robust decrease of both total neurite number and neurite length suggesting important role(s) of this transcription factor in the context of axonal regeneration.

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# Improved intrathecal infusion method designed for rodent models of spinal cord injury

Brigitte König[1], Nicole Brazda[1], Hans Werner Müller[1]

[1]Molecular Neurobiology Laboratory, Department of Neurology, University of Düsseldorf, Moorenstr.5, 40225 Düsseldorf, Germany

Intrathecal infusion is a widely used method for local delivery of pharmacological drugs directly to spinal cord injuries in patients and preclinical animal models. However, there is evidence that the infusion device could cause additional damage to the spinal cord and could be occluded by fibrotic scarring. Here we have compared three intrathecal drug delivery methods (brain infusion kit, intrathecal catheter infusion and a fixation-improved intrathecal catheter infusion) to suppress axon growth inhibitory lesion scarring in a rat model of traumatic spinal cord injury at thoracic level T8. The improved intrathecal catheter infusion (see Figure) consisting of (1) a firmly fixed catheter at the level of a tube reducing adaptor to an autologous fat cushion, which is placed after laminectomy of T11 over the spinal cord and fixed on the surrounding tissue of T11, and (2) a catheter that is routed on top of the dura mater prior to insertion into the subarachnoid space close to the lesion site turned out to be the most appropriate application method. It caused least spinal cord damage and resulted in highly significant scar reduction when the iron chelator BPY -DCA (2,2` -bipyridine-5,5`-dicarboxylic acid) was infused by an osmotic minipump.

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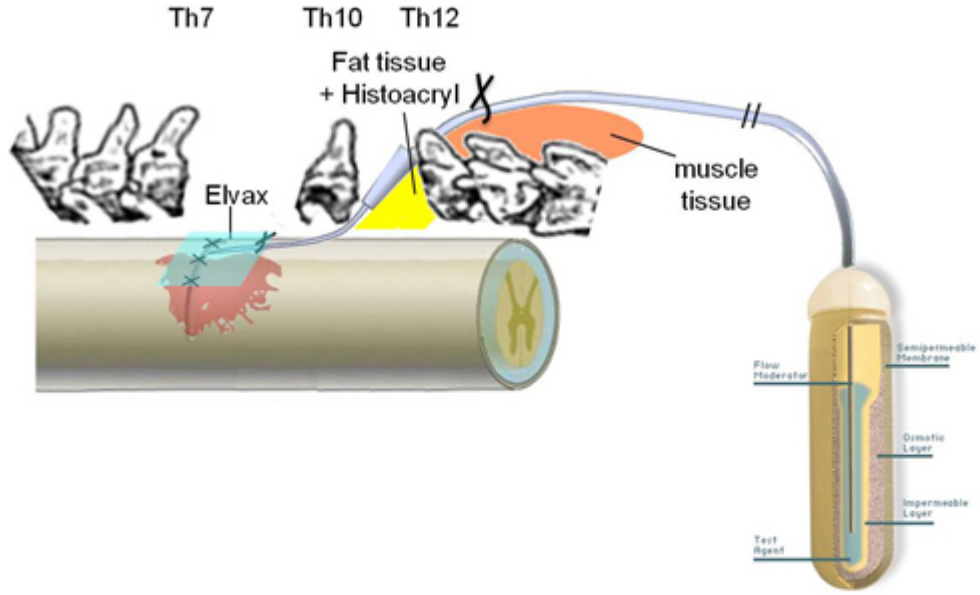


Figure: Schematic illustration of the improved intrathecal catheter infusion after dorsal hemisection of rat spinal cord at thoracic level 8. The catheter infusion device was connected to an Alzet osmotic minipump.

# An *in vitro* model for scar formation to study the mechanisms of scar-reducing treatments used in spinal cord injury

Christina Francisca Vogelaar[1], Stefanie Krafft[1], Brigitte Ziegler[1], Hans Werner Müller[1]

[1]Molecular Neurobiology Laboratory, Department of Neurology, Moorenstrasse 5, 40225 Duesseldorf, Germany

Spinal cord injury leads to permanent damage of axon tracts and impairment of sensory and motor functions. Lesion-induced fibrous scarring is considered a major impediment for regeneration of injured axons in the CNS. The collagen-rich basement membrane acts as a scaffold to axon growth inhibitory factors, including chondroitin sulphate proteoglycans (CSPGs), semaphorins (Sema), and ephrins. In our laboratory, a pharmacological treatment was developed, transiently suppressing fibrous scarring (Klapka et al, 2005). This "anti-scarring treatment" (AST) consists of local application of an iron chelator and cyclic AMP, inhibiting collagen synthesis by invading fibroblasts. AST treatment stimulated regeneration of various axon tracts (corticospinal and rubrospinal tract, serotonergic, catecholaminergic and sensory axons), leading to improvements in locomotor recovery.

In order to study the molecular mechanisms of AST treatment, an *in vitro* model for scar formation is necessary. To date, models involved mixed cultures of a growth inhibitory and a growth permissive cell type, studying axon growth behaviour across the border between the cells. However, in these models, no active process of scar formation takes place, so these cannot be used to study scar-reducing treatments. Recently, a new model for scar formation was developed by Kimura-Kuroda et al (2010), in which fibroblasts and astrocytes in co-culture formed scar-like clusters after addition of TGF-ß1. We reproduced the model in our lab. We investigated in more detail the mechanisms of scar formation. Incubation with BrdU simultaneously with TGF-ß1 revealed that cluster precursors are already visible at 6 hours after TGF-ß1 application, which developed into mature clusters by 1-7 days. Clusters were partly formed by fibroblast proliferation, but also by migration of fibroblasts into the clusters. Live cell imaging will be performed to elucidate the balance between cell migration and cell division. Immunohistochemical analysis showed that mature clusters contain extracellular matrix (ECM) molecules and growth inhibitory proteins, like various CSPGs (NG2, neurocan, phosphacan, CS-56), sema-3A, and tenascin-C.

We are currently using this scar formation model to test the scar-reducing properties and mechanisms of AST and related scar-reducing treatments. We are studying various scar-reduction mechanisms, like the effects of treatment on proliferation, apoptosis, migration, and inhibitor expression. Some of the tested treatments resulted in a reduction in the number and size of clusters. We will investigate whether the treatments stimulate growth of cortical and dorsal root ganglion axons on the normally inhibitory clusters. We will use the model to study and improve existing treatments, but also to find new treatment strategies.

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# Role of the transcription factor Uncx4.1 in midbrain neurogenesis

Tamara I. Rabe[1], Frèdèrique Varoqueaux[2,3], Gundula Griesel[1], Andreas Kispert[4], Peter H. Burbach[5], Ahmed Mansouri[1,2]

[1]Max Planck Institute for Biophysical Chemistry, Molecular Cell Differentiation Group , Am Fassberg 11, 37077 Göttingen, Germany

[2]DFG-CMPB

[3]Max Planck Institute for Experimental Medicine, Molecular Neurobiology, 37075 Göttingen – Germany

[4]Medizinische Hochschule Hannover, Institut für Molekularbiologie, 30625 Hannover - Germany

[5]Rudolf Magnus Institute of Neuroscience, Department of Neurology, University Medical Center Utrecht, 3584 CG Utrecht – The Netherlands

The homeobox gene Uncx4.1 is expressed in the developing embryo and is confined to the mesodermal and ectodermal lineages. In the central nervous system Uncx4.1 is detected in the spinal cord, the mesencephalon, and the telencephalon.
In the ventral midbrain Uncx4.1 is found to localize with dopaminergic (DA) neurons at E11.5 of gestation. Using global and conditional loss -of-function mouse mutants we found that the number of tyrosine hydroxylase (TH)-positive cells are reduced in the absence of Uncx4.1. This is corroborated by the decreased expression of Pitx3 and DAT. A thorough molecular marker analysis will be presented providing strong evidence for a crucial role of Uncx4.1 in DA neuron differentiation.

# Taxol facilitates axon regeneration in the mature CNS

Marco Leibinger[1], Vetrivel Sengottuvel[1], Anastasia Andreadaki[1], Dietmar Fischer[1]

[1]Ulm University, Experimental Neurology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Mature retinal ganglion cells (RGCs) cannot normally regenerate axons into the injured optic nerve, but can do so after activating the intrinsic regenerative program by lens injury. However, the outcome of this regeneration is still limited by inhibitors associated with the CNS myelin and the glial scar. The current study demonstrates that Taxol markedly enhanced neurite extension of mature RGCs and PC12 cells by stabilization of microtubules and desensitized axons towards myelin and CSPG inhibition in vitro without reducing RhoA activation. In vivo the local application of Taxol at the injury site of the optic nerve enabled axons to regenerate beyond the lesion site, but did not affect the intrinsic regenerative state of RGCs. Furthermore, Taxol treatment markedly increased lens injury mediated axon regeneration in vivo, delayed glial scar formation, suppressed CSPG expression, and transiently reduced the infiltration of macrophages at the injury site. Microtubules stabilizing compounds such as Taxol might be promising candidates as adjuvant drugs in the treatment of CNS injuries particularly when combined with interventions stimulating the intrinsic regenerative state of neurons.

# Translational Research in Axon Regeneration: Locomotor Recovery after Systemic Administration of Deoxyribozyme to XT-1 mRNA after a Moderate Contusion of the Adult Rat Spinal Cord

Martin Oudega[1], Owen Chao[2], Roderick Bronson[3], Donna Avison[4], Alexander Marcillo[5], Andres Hurtado[6], William Buchser[7], Barbara Grimpe[8]

[1]University of Pittsburgh, Medical School, Departments of Physical Medicine and Rehabilitation, Neurobiology, and Biomedical Engineering Center for the Neural Basis of Cognition Center for Neuroscience, 200 Lothrop Street, Pittsburgh, PA 15213-2536 Pittsburgh, USA

[2]Heinrich Heine University, University Medical Center, Department of Experimental Psychology, Düsseldorf, Germany

[3]Dana Faber/Harvard Cancer Center, Rodent Histopathology Core, Boston, Massachusetts, USA

[4]University of Miami, Leonard M. Miller School of Medicine, Division of Veterinary Resources, Miami, FL, USA

[5]University of Miami, Leonard M. Miller School of Medicine, The Miami Project to Cure Paralysis, Animal and Behavioral Core Facility, Miami, FL, USA

[6]International Center for Spinal Cord Injury, Hugo W. Moser Research Institute at Kennedy Krieger and Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA

[7]University of Pittsburgh, Cancer Institute, Imaging Cytometry Facility, Pittsburgh, PA, USA

[8]Heinrich Heine University, University Medical Center, Department of Neurology, Düsseldorf, Germany

Endogenous and therapy-induced axonal regeneration is limited after a contusion injury to the adult spinal cord. One major reason that leads to failure of functional restoration is the inhibitory nature of the lesion scar. Reducing the axon-growth inhibitory nature of the lesion environment holds promise to enhance functional recovery after spinal cord injury.

Here, we investigate whether systemic administration of the deoxyribozyme to xylosyltransferase-1 (XT-1) mRNA influences motor and sensory function after a moderate contusion of the adult rat thoracic spinal cord (MASCIS; 10g x 12.5cm). XT-1 is the enzyme that initiates glycosamino glycan (GAG-) side chain formation on proteoglycans (PGs) such as chondroitin sulfate (CS-), dermatan sulfate (DS-) or heparan sulfate (HS-) PGs. Thus, treatment with deoxyribozyme to XT-1 would prevent the formation of these axon growth-inhibitory PGs and increase endogenous repair after spinal cord contusion. Various concentrations of deoxyribozyme were tested to generate a dose-response-curve. We found that systemic administration of the deoxyribozyme to XT-1 compared to saline treatment improved horizontal ladder walking. Rats were also tested for changes in overground locomotor function using the BBB rating scale. The deoxyribozyme treatment did not change sensory function as measured with mechanical allodynia and the Tail-Flick-Test. Importantly, we tested blood and organs from all the rats used in this study and found no signs of toxicological side effect with any of the used deoxyribozyme concentrations. Our results suggest that endogenous repair in adult rats with a contused spinal cord was increased after systemic treatment with deoxyribozyme to XT-1 mRNA. The treatment with deoxyribozyme was safe even at high doses. The data underlines the potential to repair spinal cord injury using deoxyribozyme to XT-1.

# GDF-15 deficiency leads to Schwann cell and motoneuron loss in adult mice.

Sabrina Walter[1], Klaus Unsicker[2], Jens Strelau[1]

[1]University of Heidelberg, Neuroanatomy, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany
[2]University of Freiburg, Department of Embryology, Albertstr. 17,79104 Freiburg

Growth/differentiation factor-15 (GDF-15) is a distant member of the TGF-beta superfamily which is widely expressed in the central and peripheral nervous system (BÃ¶ttner et al. 1999 a,b). Our previous studies showed that GDF-15 exhibits prominent neurotrophic effects on embryonic midbrain dopaminergic neurons in culture and 6-hydroxydopamine lesioned adult dopaminergic neurons in the substantia nigra.
In order to investigate the physiological relevance of GDF-15 in promoting the survival of neurons we generated and analyzed GDF -15-deficient mice. These mice exhibit progressive postnatal losses of spinal, facial, and trigeminal motoneurons, which reach a 20% maximal deficit at 6 months of age and are accompanied by losses of motoneuron axons and significant impairment of motor skills. Sensory neurons in dorsal root ganglia are also reduced by 20%, whereas sympathetic neurons are not affected (Strelau et al. 2009).
GDF-15 is expressed in peripheral nerves, expressed and secreted by Schwann cells (SC), and retrogradely transported in the adult sciatic nerve.
SC numbers in motor nerves are significantly decreased in adult GDF-15 deficient mice whereas no difference was observed during the early postnatal myelination period. Strikingly, despite the reduced numbers of SCs, the remaining axons in the femoral nerve of adult GDF-15 deficient mice are wrapped in a thickened myelin sheath. This hypermyelination phenotype is accompanied by increased expression of the myelin genes MAG, MBP, PMP22 and P0. Changes in nodes of Ranvier, internodal length or nerve conductance could not be detected. In further studies, we compared *in vitro* myelination in SC-DRG neuron co-cultures from GDF-15 deficient and wild-type mice. However, we could neither detect differences in the thickness of the myelin sheath nor in the expression of myelin protein encoding transcripts between the different SC/DRG neuron combinations used (SC wt/DRG wt; SC GDF-15$^{-/-}$/DRG wt; SC wt/DRG$^{-/-}$; SC$^{-/-}$/DRG$^{-/-}$).
Taken together, our data suggest that GDF -15 is a trophic factor for sensory and motoneurons *in vivo*. Whether deregulation of myelination is a causative or an accompanying effect of neuron losses in GDF-15 deficient mice has to be addressed in future experiments.

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# Colloids as mobile substrates for the implantation and integration of differentiated neurons into the mammalian brain

Sophie Pautot[1], Dennis Jgamadze[1], Daniel Stone[2], Jamie Berger[2], David Schaffer[2], Ehud Isacoff[3]

[1]TUD, CRT-Dresden, Tatzberg 47-49, 01307 Dresden, Germany
[2]BioEngineering, UC Berkeley,CA, USA
[3]MCB, UC Berkeley, CA, USA

Neuronal degeneration and the deterioration of neuronal communication lie at the origin of many neuronal disorders. A major effort to treat such diseases has been focused on developing cell replacement therapy. Current cell culture techniques are not compatible with the transplantation of differentiated primary neurons, because these adhere tightly to substrates and are very sensitive to shear and tear. Here, we demonstrate that primary hippocampal neurons from embryonic or postnatal rats that are grown on colloids can be successfully transplanted into the hippocamal brain region of adult rats. The neurons survive and elaborate long processes that extend from the surface of the colloid into the host brain tissue. By transfecting the colloid -borne neurons with cDNAs encoding the light -gated glutamate receptor and a fluorescent protein before transplantation, the marked transplanted neurons are endowed with the ability to be driven to fire by remote optical control. 1-2 weeks after transplantation calcium imaging of brain slices from the recipient animal demonstrates that optical excitation of the transplanted neurons elicits activity in the host neurons, indicating the formation of functional transplant-host synaptic connections. The work paves the way for neuronal circuit repair by transplantation of genetically engineered, differentiated primary neurons.

# Poster Topic

## T4: Neurotransmitters, Retrograde messengers and Cytokines

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*Salim Sharopov, Chase Kantor, Junya Kuribayashi, Klaus Ballanyi, Heiko.J Luhmann, Werner Kilb*

T4-3C Quantitative Analysis of Neuropeptides in the Brain of *Aedes aegypti*
*Anna Reifenrath, Kunhi P. Siju, Christian Wegener, Susanne Neupert, Jörg Kahnt, Bill S. Hansson, Frank Hauser, Reinhard Predel, Rickard Ignell, Joachim Schachtner*

T4-4C Role of central serotonin in sleep regulation and circadian rhythmicity
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T4-5C VESTIBULAR CEREBRO -CORTICAL PROCESSING AND THALAMO-CORTICAL NEUROTRANSMISSION IN RATS BASED ON MICRO-PET DATA
*Nadine Schabbach, Elena Lange, Ulla Stier, Stefan Reuss*

T4-6C Different positive allosteric modulators specific for hmGlu$_2$ share an identical binding site
*Hannah K. Delille, Rajathees Rajaratnam, Wilfried Braje, Hervé Geneste, Liliane Unger, Mario Mezler*

T4-7C Characterization of the role of CXCL12/CXCR4 signalling in the development and survival of midbrain dopaminergic neurons
*Amparo Tolosa, Kerstin Krieglstein*

# Arginase and arginine decarboxylase – where do the gate keepers of polyamine synthesis reside in rat brain?

Daniela Peters[1], Jana Berger[2], Christian Derst[3], Rüdiger W. Veh[4], Gregor Laube[5]

[1]Charité - Universitätsmedizin Berlin, Institut für Integrative Neuroanatomie, Philippstr. 12, 10115 Berlin, Germany

Polyamines are important regulators of basal cellular functions but also serve highly specific tasks in the mammalian brain. With this respect, polyamines and the synthesizing and degrading enzymes are clearly differentially distributed in neurons vs. glial cells and also in different brain areas. The synthesis of the diamine putrescine may be driven via two different pathways. While in the "classical" pathway urea and carbon dioxide are removed from the amino acid arginine by arginase and ornithine decarboxylase, the alternative pathway, first removing carbon dioxide by arginine decarboxlyase and then urea by agmatinase, may serve the same purpose. However, the intermediate product of the alternative pathway, agmatine, is an endogenous ligand for imidazoline receptors and may serve as a neurotransmitter.

In order to evaluate and compare the expression patterns of the two gate keeper enzymes arginase for the classical pathway and arginine decarboxylase for the alternative pathway, we generated polyclonal, monospecific antibodies against arginase (I/II) and arginine decarboxylase. Using these tools, we immunocytochemically screened the rat brain and compared the expression patterns of both enzymes in cerebral cortex, hippocampus, striatum, thalamus, hypothalamus, cerebellum and brain stem on the regional, cellular and subcellular level.

In contrast to other polyamine pathway enzymes, like ornithine decarboxylase and spermidine synthase, arginine decarboxylase and arginase are both widely expressed in rat brain neurons. In cerebral cortex and hippocampus, principal neurons and putative interneurons were clearly labeled for both, arginine decarboxylase and arginase. Labeling, however, was strikingly different in these neurons with respect to the subcellular localization of the enzymes. While with antibodies against arginine decarboxylase the immunosignal was distributed throughout the cytoplasm, arginase -like immunoreactivity was preferentially localized to mitochondria. Using an arginase II-specific sequence for further purification of the pan-arginase antibody, both arginase isoforms could be largely separated.

Given the apparent congruence of both enzyme distributions with respect to certain cell populations like CA1 pyramidal neurons it seems likely that the synthesis of agmatine rather than putrescine may be the major purpose of the alternative pathway of polyamine synthesis, while the classical pathway supplies putrescine and spermidine/spermine in these neurons.

# Cellular properties of Neuropeptide S-expressing neurons

Kay Jüngling[1], Jörg Lesting[1], Reiner Reinscheid[2], Hans-Christian Pape[1]

[1]Institute for Physiology I, Westfälische Wilhelms-Universität, Robert-Koch Str. 27a, 48149 Münster, Germany

[2]Department of Pharmaceutical Sciences, University of California Irvine, Irvine, CA, USA

The recently discovered Neuropeptide S (NPS) and its receptor (NPSR) constitute a novel transmittersystem in the central nervous system. The NPS consists of 20 amino-acids and binds to the NPSR, a member of the family of G-protein coupled receptors (GPCRs), and thereby activating intracellular signal-cascades (e.g. Increase of intracellular Ca2+- and cAMP-levels). Various publications describe the effects of NPS on the behaviour of mice and rats. Espacially the reduction of general anxiety and the facilitation of fear extinction after local or systemic NPS-injection has drawn much attention on this transmittersystem, although its effect on neuronal circuits is less clear. In addition only little is known about the NPS-source within the brainstem. It has been shown, that NPS-expressing neurons in mice are located near the locus coeruleus (LC) and near the lateral parabrachial nucleus, but their morphological and electrophysiological properties remaind elusive. We made use of a novel transgenic mouse-line, expressing EGFP fused to the signal-sequence of NPS in neurons, which show endogeneous NPS-expression. These neurons can be identified in the brainstem nuclei by their green fluorescence and thus can be targeted for detailed analysis. Moreover, we have used Cholera toxin Alexa 594 to retrogradly stain NPS -expressing neurons, which project into the amygdala, the paraventricular thalamic nucleus and the subiculum. In this first approach we analyzed properties of NPS-EGFP positive neurons near the LC. In addition, we investigated the effects of the corticotropin-releasing factor (CRF) on the intrinsic properties of NPS-EGFP expressing neurons.

# Characterization of glutamatergic vesicle acidification and refilling dynamics in hippocampal neurons

Magalie Martineau[1], Jürgen Klingauf[1]

[1]Zellbiophysik, Institut für Medizinische Physik und Biophysik, Universität Münster, Robert-Koch Straße 31, 48149 Münster, Germany

The refilling of synaptic vesicles with neurotransmitters is potentially a rate-limiting step in neurotransmission. Biochemical investigations on isolated vesicles indicate that all vesicular transport depends on a proton electrochemical gradient ($\Delta\mu H+$) generated by the vacuolar-type H+-ATPase. $\Delta\mu H+$ consists of two components: the membrane potential ( $\Delta\Psi$) and the pH gradient ($\Delta pH$), the ratio of which influences unevenly the vesicular uptake of different neurotransmitters.
In the process of vesicular refilling not only proton ions are of uttermost importance. Indeed, chloride ions allow isolated synaptic vesicles to increase $\Delta pH$ thus to acidify by collapsing $\Delta\Psi$, and influence vesicular glutamate transport velocity and final content. Recently, reconstitution of vesicular glutamate transporters in liposomes has led to two opposite models where chloride concentration influences the transport and storage of glutamate inside vesicles either through a chloride conductance in the transporter itself or through an allosteric regulation of the transport (Schenck et al., 2009; Juge et al., 2010). These studies strengthen the role, still ambiguous, of chloride in vesicular acidification and filling.
Yet, the dynamics of vesicle recycling in intact neurons are still largely unknown. Here we studied the kinetics of vesicular reacidification in live cultured hippocampal neurons using synaptopHluorin, the pH-sensitive variant of GFP (pHluorin) coupled to the luminal domain of synaptobrevin 2 as well an antibody against the vesicular GABA transporter labelled with cypher5E, a pH-dependent variant of the cyanine dye Cy5. Utilizing Rose Bengal, an inhibitor of the vesicular glutamate transporters, we have investigated the relationship between vesicular glutamate refilling, the establishment of $\Delta\mu H+$, and the initial intravesicular chloride concentration just after retrieval by endocytosis. Our results surprisingly indicate that vesicular acidification is not an independent step preceding transmitter refilling but that in a preserved environment generation of $\Delta\mu H+$ and neurotransmitter loading in newly endocytosed synaptic vesicles are strongly coupled, i.e. no proton gradient can be built up in the absence of transmitter transport.

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# GABA depolarizes immature neocortical neurons in the presence of the ketone body ß-hydroxybutyrate

Knut Kirmse[1], Otto W. Witte[1], Knut Holthoff[1]

[1]Universitätsklinikum Jena, Experimentelle Neurologie, Hans-Berger-Klinik für Neurologie, Erlanger Allee 101, 07747 Jena, Germany

A large body of evidence suggests that the neurotransmitter γ-aminobutyric acid (GABA) undergoes a developmental switch from being predominantly depolarizing-excitatory to predominantly hyperpolarizing-inhibitory. Recently published data, however, point to the possibility that the presumed depolarizing mode of GABA action during early development might represent an artifact due to an insufficient energy supply of the in vitro preparations used. Specifically, addition of the ketone body DL-ß-hydroxybutyrate (ßHB) to the extracellular medium was shown to prevent GABA from exerting excitatory effects. Applying a complementary set of minimally invasive optical and electrophysiological techniques in brain slices from neonatal mice, we here investigated the effects of ßHB on GABA actions in immature cells of the upper cortical plate. Fluorescence imaging revealed that GABA -mediated somatic $[Ca^{2+}]$ transients, that required activation of $GABA_A$ receptors and voltage-gated $Ca^{2+}$ channels, remained unaffected by ßHB. Cell-attached current-clamp recordings showed that, in the presence of ßHB, GABA still induced a membrane potential depolarization. To estimate membrane potential changes quantitatively, we employed cell-attached recordings of voltage-gated potassium currents and demonstrate that the GABA -mediated depolarization was independent of supplementation of the extracellular solution with ßHB. We conclude that, in vitro, GABA depolarizes immature cells of the upper cortical plate in the presence of the ketone body ßHB. Our data thereby support the general concept of an excitatory-to-inhibitory switch of GABA action during early development.

# How dead are "dead-end" vesicles: can the exocytosis of unreleasable vesicle be induced?

Sandra Magin[1], Mathias Pasche[1], Ulf Matti[1], Detlef Hof[1], Jens Rettig[1], Ute Becherer[1]

[1]Universität des Saarlandes, Physiologisches Institut, Gebäude 59, 66421 Homburg / Saar, Germany

$Ca^{2+}$-dependent exocytosis of secretory vesicles comprises several steps: docking of the vesicles to the plasma membrane, priming to render the vesicles release-competent and fusion with the plasma membrane. Total internal reflection fluorescence microscopy (TIRFM) enables the real-time visualization of vesicles near the plasma membrane as they undergo changes from one functional state to the other. Nofal et al. (2007, J Neurosci, 27:1386-95) showed in chromaffin cells, that the mobility of large dense core vesicles (LDCVs) correlate with these functional states. Indeed docked vesicles display a caged mobility, whereas primed vesicles are completely immobile. We found that 80% of the LDCVs at the plasma membrane are stationary immediately before secretion, while the remaining 20% show slightly higher mobility. Furthermore these 20% exhibit a delayed secretion, thus correspond to vesicles which were only docked prior to the stimulus and primed on the run. However, some immobile, thus putatively primed, LDCVs could not be released by classical methods of stimulation. These vesicles can be referred as “dead-end vesicles”. Currently, we investigate this dead-end state.

# Immunostaining reveals at least six subpopulations of olfactory local interneurons contributing to the *Apis mellifera* antennal lobe network

Jens Bierfeld[1], Natalia Charlina[1], M. Gabriele Pszolla[1], Giovanni Galizia[1], Sabine Kreissl[1]

[1]University of Konstanz, FB Biologie, AG Galizia, Fach 624, 78457 Konstanz, Germany

As foragers on flowers, worker honeybees (*Apis mellifera*) are able to learn odors from a virtually endless variety. The honeybeeÕs olfactory system, therefore, has to accomplish a complex processing task. Olfactory stimuli induce activity in the olfactory receptor neurons, which convey stimulus information from the antennae to olfactory glomeruli within the antennal lobe, the first olfactory center in the honeybee brain. Here, local interneurons (LNs) interconnect all glomeruli, and form a dense neural network, which shapes the combinatorial representation of olfactory stimuli. Projection neurons (PNs) relay the result to higher order brain centers. Several functions have been proposed for LNs, including: mediating lateral inhibition, generating PN synchrony, refining temporal response properties of PNs, and modulating gain control. LNs differ in their complement of active compounds such as neurotransmitters and neuropeptides, as well as in morphology. Knowing the numbers of local interneurons that contribute to the network, and their functional subtypes is crucial for understanding the computational capacity and olfactory coding in this system and will provide valid information for computational modeling attempts.

We counted approx. 3,200 cell bodies of PNs and LNs around each antennal lobe, a number about 1,500 smaller than reported previously. With a reported number of 800 projection neurons we calculate that the network consists of 2,400 LNs. GABA is believed to be the main inhibitory transmitter in the antennal lobe. Thus, we counted the number of GABA immunoreactive (GABA-ir) cells and found about 1,250 such neurons. 420 of these were also immunoreactive for an antibody raised against the neuropeptide tachykinin (TK). This covered the whole TK-ir population of LNs in the antennal lobe.

Next, we counted histaminergic cells (His). We found about 254 prominently stained His-ir LNs in each antennal lobe. In our preliminary data we observed that 10% of TK-ir neurons also showed His-immunoreactivity.

20 GABA-ir cells stained also against the neuropeptide allatostatin A, while showing no immunoreactivity against tachykinin.

Together, these results indicate that the total population of 2,400 local interneurons in the antennal lobe of the honeybee is made up by at least six subpopulations: (1) GABA and TK containing neurons, (2) GABA and allatostatin containing neurons, (3) His-ir neurons which may or may not contain GABA, (4) His-ir neurons that are also TK-ir and GABAergic, (5) GABA containing neurons without a known co-transmitter, (6) neurons with a transmitter yet to be identified. Interestingly, subpopulations (1) Ð (5) are all likely to exert inhibitory action on their postsynaptic partners. All found subpopulations likely contribute to the shaping of olfactory response patterns by adding functional diversity and plasticity to the neuronal network within the antennal lobe of the honeybee.

# In vitro effects of Substance P on subpopulations of central amygdala neurons from GAD67-GFP mice

Héctor Romo-Parra[1], Christine Strippel[1], Ludmila Sosulina[1], Hans-Christian Pape[1]

[1]University Hospital Münster, Institute for Physiology I, Robert Kochstr. 27a, 48149 Münster, Germany

Aim: Substance P (SP) is one of the most abundant peptides in the central nervous system and has been implicated in stress regulation and affective and anxiety -related behaviour. Furthermore, SP and its neurokinin 1 (NK1) receptor has been found within brain areas involved in the regulation of stress and anxiety responses including the main output of the amygdala complex, for instance, the central amygdala (CE). Despite the amount of data concerning SP little is known about the cellular mechanisms of this neuropeptide action in CE. At least two types of neurons have been well described electrophysiologically and neurochemically in the CE. Taken advantage of this classification and based on GAD67-GFP mice that yield a most reliable labelling of GABAergic neurons, here we differentiated between GABA- and non GABAergic neurons by: i) the expression of NK1 receptor *in situ* ii) the morphology of the cell recorded; iii) the electrophysiological electrotonic and electrogenic parameters and iv) the response upon SP application *in vitro*.

Methods: Curent - and voltage -clamp whole -cell recordings in CE neurons from GAD67-GFP mice were carried out. Electrotonic and electrogenic properties were characterized upon hyperpolarizing and depolarizing pulses in current clamp mode. SP effects on firing frequency and on holding current shift were studied. Recorded neurons were filled with biocytin for a further morphology-based classification.

Results: A total of 125 CE neurons recorded were subdivided in two groups: GFP-positive GABAergic and GFP-negative non-GABAergic neurons. Both groups differed in resting membrane potential, AHP and in sag ratio values. Morphologically, GFP-positive biocytin-stained neurons showed ovoid, pyramidal or fusiform somata and differed in dendritic orientation. Dendrites of these cells were smooth, aspinous or covered with variably sized varicosities. On the contrary, dendrites from GFP -negative cells were covered with numerous spines (n=3). Bath application of SP (300nM) induced three main effects on GFP-positive neurons (n=19): 1) depolarization (control = -78.52 +/- 2.2 mV; SP = -75.80 +/- 2.2 mV); 2) increase in action potential (AP) firing frequency (control = 2.2 +/- 0.2 Hz; SP = 4 +/- 0.5 Hz) and 3) increase in input resistance (control = 397.5 +/- 50.9 MO vs SP = 495.4 +/- 61.4 MO) associated with drop in membrane conductance. These effects were prevented by the inclusion of the NK1 receptor antagonist L -822429 in the bath. In contrast GFP -negative neurons (n=11) showed: 1) hyperpolarization (control = - 74.05 +/ - 2.8 mV; SP = - 76.98 mV +/ - 2.6 mV) reduction in AP firing frequency (control = 2.33 +/ - 0.4 Hz; SP = 0.83 +/ - 0.24 Hz) with no change in input resistance, upon SP (300 nM) application. SP applied during voltage ramps (- 70 to - 140 mV) showed in GFP-positive neurons moderate inward rectification current with a reversal potential ($E_{rev}$) at - 101.5 +/- 2.8 mV (n = 10) that was not modified by TTX (1 µM). While SP effect on GFP -negative neurons displayed an $E_{rev}$ at - 73.75 +/ - 4.1 mV (n=10) wich was not significant different from the $E_{rev}$ induced by the $GABA_A$ agonist muscimol (10 µM; n=9).

Conclusion: The present data demonstrate cell type-specific direct and indirect effects of substance P in the mouse central amygdala.

# Interrelations between monoaminergic afferents and NPY-immunoreactive interneurons in the rat laterobasal amygdala: light- and electron microscopic findings

Maria Roswitha Bonn[1], Henning Schwert[1], Elisabeth Van Bockstaele[2], Esther Asan[1]

[1]University of Wuerzburg, Institute of Anatomy and Cell Biology, Koellikerstr. 6, 97070 Wuerzburg, Germany
[2]Thomas Jefferson University, Department of Neuroscience, 900 Walnut Street, Philadelphia, PA 19107, USA

GABAergic interneurons in the rat lateral (La) and basolateral (BL) nuclei contribute to inhibitory circuits which significantly influence amygdaloid emotion processing. Interneuron subpopulations can be distinguished in both nuclei according to their expression of peptides and calcium binding proteins. Neuropeptide Y (NPY) and parvalbumin (PV) are produced by separate subpopulations, with the comparatively small subset of NPY interneurons supposedly subserving particularly important anxiolytic functions. The dense, heterogeneous monoaminergic innervation of La and BL presumably modulates the activity both of excitatory projections and of inhibitory intrinsic circuits of the nuclei. Previous light microscopic dual immunolabeling studies documented numerous appositions of serotonin (5-HT)/serotonin transporter(5HTT)-immunoreactive(ir) serotonergic and scarce appositions of tyrosine hydroxylase(TH)-ir dopaminergic axons on NPY-ir somata and proximal dendrites in La and BL. Similar patterns were found in the present study for serotonergic/dopaminergic perisomatic appositions on different types of PV-ir interneurons of the La and BL, indicating non-selective monoaminergic innervation of interneuron subpopulations. Double in situ hybridization showed coexpression of different 5-HT-receptor subtype (5-HT1A, 5-HT2C) mRNAs in subpopulations of NPY mRNA-producing interneurons, ongoing coexpression analyses of dopamine receptor subtypes have not as yet yielded conclusive results, possibly due to low receptor mRNA expression levels. Immunoelectron microscopy showed that NPY-ir somata and dendrites were contacted by numerous terminals forming symmetric and asymmetric synapses. Symmetric synapses of NPY-ir terminals were observed upon unlabeled somata and dendrites of different calibers. First double labeling analyses documented that TH- and NPY-ir terminals contacted unlabeled somata as common targets. Ultrastructural evidence for direct synaptic contacts between TH-ir and NPY-ir elements was rare. Only occasionally were direct membrane appositions observed between a TH-ir axon and an NPY-ir neuron or axon, but these lacked a detectable synaptic specialization in the plane of section analyzed. Our findings indicate significant serotonergic and minor dopaminergic influence on NPY-producing interneurons of the rat laterobasal amygdala. The monoaminergic innervation patterns appear similar for NPY-producing and other interneurons. Additional analyses of receptor expression will help to clarify whether monoaminergic transmission might elicit differential responses in the various interneuron subpopulations via different receptor subtypes. The ultrastructural analyses support an inhibitory nature of NPY-containing elements in the La and BL since NPY-ir terminals were found to form symmetric synapses. Further comparative quantitative analyses of interrelations of dopaminergic and serotonergic afferents with NPY-ir and other interneurons will yield more detailed insights into the emotion-related inhibitory circuits in La and BL.

# Monitoring spatial and temporal dynamics of second messenger molecules using modified genetically encoded sensor proteins

Annika Christina Meisenberg[1], Arnd Baumann[1]

[1]Forschungszentrum Juelich GmbH, Institute of Structural Biology and Biophysics, Wilhelm-Johnen-Strasse, 52425 Juelich, Germany

Second messenger molecules, e.g. cAMP, cGMP or $Ca^{2+}$ essentially contribute to the transmission and amplification of signals originating from intercellular communication. Furthermore, they are important mediators for intracellular signal processing. Here, second messengers participate in the regulation or modulation of e.g. differentiation, gene transcription as well as ion channel or enzymatic activity.
Hence investigation of second messenger -dependent signaling is of great interest. Analyzing the spatial and temporal dynamics of these molecules is a challenging task and such studies hold promise to give further insight into intracellular signal transduction and processing. Until recently synthetic $Ca^{2+}$-sensitive fluorescent dyes like Fluo-4 (Gee et al., 2000) or Fura-2 (Grynkiewicz et al., 1985) were applied to image cellular $Ca^{2+}$ dynamics. Synthetic dyes, however, suffer from some limitations: cell-type specific targeting or long term measurements are almost impossible to achieve (Hires et al., 2008). The dynamics of second messenger molecules like cAMP can not be adressed at all due to the lack of small organic fluorescent dyes specifically binding to cAMP. Genetically encoded sensor proteins (GESP) are promising alternatives to overcome these limitations. GESPs are tailored from protein domains that specifically bind to $Ca^{2+}$ ions or cAMP. These domains are fused to reporting elements, which are often derivatives of fluorescent proteins.
Here we report on sensor proteins for a subset of second messengers. The sensors were modified such that they are targeted to different subcellular compartments. Subcellular distribution of the respective sensor proteins was investigated by fluorescence microscopy and immunohistochemical staining. The functionality of the modified sensors was validated by imaging experiments after transient expression in appropriate cell lines. The chosen cell lines allowed to specifically maniplulate either of the second messenger concentrations under investigation. Having this set of sensor proteins at hand we are going to precislsly determine second messenger concentrations at different subcellular locations. This approach will substantiate our understanding about the molecular dynamics occuring during signaling processes at the cellular level.

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# Monoaminergic including cholinergic neurons express the TASK-3 potassium channel throughout the rostrocaudal axis of the rat brain

Christiane Marinc[1], Christian Derst[1], Rüdiger W. Veh[1]

[1]Charité, Centrum 2, Institut für Integrative Neuroanatomie, Philippstraße 15, 10115 Berlin, Germany

Monoaminergic and cholinergic systems are important regulators of a variety of vegetative and emotional functions. Neurotransmitter release in these systems is crucially regulated by cellular excitability. It has become obvious that members of the two-pore potassium ($K_{2P}$) channel family significantly contribute to neuronal potassium resting currents. TASK-3 (TWIK-related-acid-sensitive-K+ channel 3, KCNK9), a member of the $K_{2P}$ channel family shows a wide distribution throughout the brain. Especially high levels of TASK-3 mRNA have been found in cholinergic neurons and neurons of the Locus coeruleus and the raphe nuclei.
We generated and purified a polyclonal monospecific antibody against rat TASK-3 protein and used it to study TASK-3 expression in monoaminergic including cholinergic neurons. TASK-3 immunoreactivity was very strong in somatic and visceral motoneurons. Furthermore, cholinergic neurons of the basal nucleus of Meynert also displayed a prominent TASK-3 expression. Immunocytochemical analysis of additional areas in the rat brain revealed a strong expression of TASK-3 channels in serotoninergic neurons of the dorsal raphe and noradrenergic neurons of the locus coeruleus and a minor expression in histaminergic neurons of the tuberomammillary nucleus. In the dopaminergic system strong TASK-3 expression was found in the VTA, whereas TASK-3 immunoreactivity in the substantia nigra compacta was only weak. Immunofluorescence double-labeling experiments with appropriate marker enzymes confirmed the expression of TASK-3 in cholinergic, serotoninergic and noradrenergic neurons.
TASK-3 is regulated by a variety of stimuli, including inhibition by acidification and activation of a variety of $G_q$-coupled receptors, conferring regulatory mechanisms to neurons expressing the channel. The strong expression in cholinergic and monoaminergic neurons suggests TASK-3 as a potentially important regulator of neuronal excitability and therefore neurotransmitter release in these systems.

# Nitric Oxide modulates plasma membrane properties: a novel mechanism for morphological differentiation?

Sven Hippe[1], Yekbun Adiguzel[1], Christian Grote-Westrick[1], Rolf Heumann[1,2]

[1]Ruhr-University Bochum, Department of Molecular Neurobiochemistry, Universitätsstraße 150, 44780 Bochum, Germany
[2]Research Department of Neuroscience, Ruhr-University Bochum, Universitätsstraße 150, 44780 Bochum, Germany

Nitric Oxide (NO), a small signaling molecule, is known to regulate diverse cellular processes in mammals. It is produced as a radical within the cell by nitric oxide synthase (NOS) and it modifies protein properties via nitrosylation of cysteine residues or prosthetic heme groups. Because it is a hydrophobic, gaseous molecule, NO diffuses freely and is primarily located within the plasma membrane, the hydrophobic compartment of the cell (Shaw & Vosper 1977).
The extensive biophysical consequences of this compartmentalization of NO have been largely neglected in research until now. However, based on our recent observation that NO increases plasma membrane fluidity in living cells we show strong evidence for a direct and protein -independent NO membrane interaction (Grote-Westrick et al. to be submitted).
Here we investigate the consequences of the novel NO effect for morphological and cellular differentiation. By the use of NOC18 as NO donor and L-NAME as NOS inhibitor we observe changes of nerve growth factor induced morphological differentiation in PC12 cells. In order to be able to discriminate between protein and membrane mediated effects we use benzyl alcohol (BA) as a non-NO compound affecting the fluidity of plasma membranes more selectively.
Further studies on neurosphere derived Nestin/GFAP positive cells demonstrate that NO affected membrane fluidity results in enhanced cellular migration and formation of outgrowths. By inhibition of NOS activity we found enhanced neurosphere formation which is in line with current literature (Packer et al. 2003; Covacu et al. 2006; Torroglosa 2007). Ability of NO and BA to block this enhancement suggests a role of membrane fluidity in this context.
Overall, our results are compatible with the assumption that NO promotes morphological differentiation as a membrane active substance. Furthermore, reduction in membrane fluidity seems to have a critical role in terms of 'stemcellness'.

# Patterns of expression of both NO-Guanylyl Cylclase isoforms in the mouse hippocampus

Angela Neitz[1], Evanthia Mergia[2], Elisabeth Petrasch-Parwez[3], Doris Koesling[2], Thomas Mittmann[1]

[1]Johannes Gutenberg University , Institute of Physiology and Pathophysiology, Duesbergweg 6, 55128 Mainz, Germany
[2]Ruhr University University , Institute of Pharmacology, Universitätstr. 150, 44801 Bochum, Germany
[3]Ruhr University University , Institute of Neuroanatomy, Universitätstr. 150, 44801 Bochum, Germany

Since more than 20 years nitric oxide (NO) is known to be implicated in various biological processes in the brain. Most of its effects are mediated by two guanylyl cyclase NO-activated receptors (NO-GC1, NO-GC2), leading to a subsequent increase of intracellular cGMP levels. In the hippocampus this signaling pathway is known to plays a key role in specific processes of memory consolidation and/or retrieval, like long-term potentiation (LTP) or long-term depression (LTD). In a previous study we reported that both NO -GC receptors are necessary for LTP induction in the hippocampal CA1 region, since knock-out mice deficient in either one of the two NO-GCs failed to express any LTP. This clearly demonstrates the involvement of both NO-GCs in synaptic transmission. However, their physiological functions and their expression level in various cell types is still a matter of debate and rarely known. Therefore, we performed immunohistochemical analysis in two hippocampal regions, the CA1 area and the dentate gyrus. We used two NO-GC knock-out mice models to differentiate the isoforms more precisely than conventional antibodies can do.
An expression of NO-GCs was visible in both, CaMKII a+-pyramidal cells and Gad67+- inhibitory neurons, but we observed region specific differences in the distribution of the expression levels. Since GABAergic neurons are highly heterogenous in terms of their morphological and neurochemical properties, we used neuropeptides, calcium binding proteins and the enzyme nNOS for a further characterization. Both NO-GC isoforms are expressed in Parvalbumin+- and CCK+-interneurons preferentially innervating the somata and proximal dendrites of pyramidal cells. They are also expressed in Calretinin+ - and VIP+ -interneurons targeting mostly other inhibitory cells. On the synaptic level we found NO-GC2 exclusively expressed in postsynaptic terminals of both cell types in the stratum radiatum of the CA1 region. In contrast, NO-GC1 was present in excitatory and inhibitory pre- and postsynaptic sites. Together, these results show a widespread distribution of both NO-GCs, being present in excitatory and inhibitory neurons. However, they differ in their local and synaptical expression pattern, suggesting the involvement in different signaling cascades.

# Presynaptic modulation of adenosine release in the cerebellum

Boris Klyuch[1], Nicholas Dale[1], Mark Wall[1]

[1]University of Warwick, School of Life Science, Coventry, United Kingdom

The neuromodulator adenosine plays a critical role in many physiological processes within the central nervous system. However, the mechanisms of extracellular adenosine production are still often unclear. In our previous work, using microelectrode biosensors, we have shown that focal stimulation (20 Hz train) in the molecular layer of the cerebellum causes action potential and $Ca^{2+}$-dependent adenosine release (Wall & Dale, 2007). Following the partial block of presynaptic $K^+$ channels (by 4-aminopyridine, 4-AP), adenosine release could be evoked by a single stimulus (Klyuch et al., 2010). We have investigated how these two forms of adenosine release (train and single stimulus) are modulated by presynaptic receptors.

Activation of $GABA_B$, $A_1$ or mGluR4 receptors reduced train-induced adenosine release (by ~ 65, 50 and 40 % respectively). High expression of $A_1$ and $GABA_B$ receptors by parallel fibre terminals and the unique location of mGluR4 receptors (on parallel fibre terminals) strongly suggests that parallel fibre are either the source of adenosine or at least their activity is required for adenosine release. The inhibition of presynaptic A1 receptors (with 8-CPT) increased adenosine release by ~ 90% demonstrating that tonic activation of $A_1$ receptors depresses adenosine release (Wall & Dale, 2007). In contrast, blocking mGluR4 receptors (with MSOP) or $GABA_B$ receptors (with CPG52432) had little effect on adenosine release (10-20 % increase). This data suggests that tonic activation of adenosinergic but not glutamatergic or GABAergic receptors plays a major role in the regulation of train-induced adenosine release under normal conditions.

Single spike-induced adenosine release (in 60 μM 4-AP) was abolished by the activation of $A_1$ or $GABA_B$ receptors and was reduced by ~75% following mGluR4 activation. Block of mGluR4 receptors had a small effect on adenosine release (~ 25% increase), whereas block of $A_1$ or $GABA_B$ receptors markedly increased adenosine release (by 200-250%). We suggest that mGluR4 receptors play a minor role in the regulation of single spike induced adenosine release. The large effects of $A_1$ and $GABA_B$ receptor antagonists suggest that there is an increased tone of GABA and adenosine in the presence of 4-AP. In conclusion, adenosine release is complex and, like classical neurotransmitters, is controlled by the interplay of several presynaptic modulators.

# Proepileptic effect of methylxanthines in a whole-hippocampus preparation of immature rats

Salim Sharopov[1], Chase Kantor[2], Junya Kuribayashi[2], Klaus Ballanyi[2], Heiko.J Luhmann[1], Werner Kilb[1]

[1]University Medical Center Mainz, Institut of Physiology and Pathophysiology, Duesbergweg 6 , D-55128 Mainz, Germany

[2]Department of Physiology, Heritage Medical Research Center, University of Alberta, Edmonton, Canada T6G 2S2

In human preterm infants, the methylxanthines caffeine and theophylline are routinely administered to stabilize respiration. However, recent publications demonstrated changes in the EEG of preterm infants following administration of methylxanthines. In order to investigate, whether these substances promote epileptic seizures, we performed field-potential recordings from the CA1 region of in-toto hippocampal preparations of P1-4 and P8-10 rats. In this preparation we analyzed the effect of theophylline and caffeine on postsynaptic field potential responses (FP), evoked by electrical stimulation in the CA3 region, and tested also whether they can induce spontaneous epileptiform discharges.

In P8-10 hippocampi theophylline at concentrations of 2.5, 5 and 10 mM potentiated the FP responses by about 30% and additional polysynaptic components appeared at theophylline concentration of = 0.5 mM. In P1-4 hippocampi no significant effect of bath-applied theophylline on the FP responses could be observed. Electrical stimulation induced epileptiform discharges at theophylline concentration of = 2.5 mM in the P1-4 hippocampi and at concentrations of = 1 mM in the P8-10 hippocampi. Caffeine had no significant effect on FP responses in the P8-10 hippocampi, but in the P1-4 hippocampi reduced the amplitude of these events by more than 30% at caffeine concentration of = 5 mM. While in the P1-4 hippocampi no electrically evoked epileptiform activity could be observed even at 10 mM caffeine, such electrically evoked epileptiform discharges occurred at caffeine concentrations of = 1 mM in the P8-10 hippocampi. Both theophylline and caffeine also induced spontaneous epileptiform discharges with an ictal-like appearance. Such ictal-like spontaneous discharges were observed in 81% of the P1-4 hippocampi at theophylline concentrations of = 5 mM and in 19%, 63%, 81% and 94% of the P8-10 hippocampi at theophylline concentrations of 1, 2.5, 5 and 10 mM, respectively. Caffeine induced ictal-like discharges only in 6% of the hippocampi at a concentration of 10 mM, while in the P8-10 hippocampi ictal-like discharges were observed in 6%, 22% and 56% of the preparations at 2.5, 5 and 10 mM, respectively.Neither the amplitude nor the duration or the frequency of discharges within ictal-like events showed a significant dependency on the theophylline or caffeine concentration.Similar results were obtained regarding caffeine and theophylline effects on spontaneous early network oscillations in the neocortex and hippocampus of horizontal slices from newborn rats. Additional pharmacological experiments revealed that both caffeine and theophylline enhanced the frequency of epileptiform discharges also in the presence of the noncompetitive $GABA_A$ antagonist picrotoxin (30 μM).

In summary, these results indicate that both theophylline and caffeine at higher concentrations modify basal synaptic transmission in the CA1 region. The effects on postsynaptic responses and spontaneous epileptiform discharges were less pronounced for caffeine, suggesting that this substance may be potentially advantageous for therapeutic applications in preterm infants.

# Quantitative Analysis of Neuropeptides in the Brain of *Aedes aegypti*

Anna Reifenrath[1], Kunhi P. Siju[2], Christian Wegener[1], Susanne Neupert[3], Jörg Kahnt[4], Bill S. Hansson[5], Frank Hauser[6], Reinhard Predel[3], Rickard Ignell[2], Joachim Schachtner[1]

[1]Tierphysiologie, Philipps Universität Marburg, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany
[2]Chemical Ecology, Swedish University of Agricultural Sciences, Alnarp, Sweden
[3]Institut für Allgemeine Zoologie, Friedrich-Schiller-Universität Jena, Germany
[4]Max Plank Institut für Terrestrische Mikrobiologie, Marburg, Germany
[5]Evolutionäre Neuroethologie, Max Planck Institute für Chemische Ökologie, Jena, Germany
[6]Institute of Biology, University of Copenhagen, Denmark

The yellow fever mosquito, *Aedes aegypti*, is the major vector of several arboviral diseases, e.g. dengue fever and yellow fever. The gonotrophic cycle of *Ae. aegypi* females consists of distinct behavioral and physiological phases, including host seeking, blood feeding and oviposition. These changes in behavior depend on changes in the processing of sensory information, especially the processing of olfactory information. A multitude of chemical signaling molecules modulates processing of odor information in the central nervous system, including the primary olfactory centers of insects, the antennal lobes (AL). The most occurring and diverse group of neuromodulators, in vertebrates and invertebrates, are the neuropeptides. In the present study we analyzed neuropeptides in the antennal lobes and the central brain of female *Ae. aegypti* at different times during the gonotrophic cycle using direct MALDI-TOF mass spectrometric peptide profiling. For a quantitative analysis of changes in the concentration of specific neuropeptides we used isotope labeled peptides as internal standards.

# Role of central serotonin in sleep regulation and circadian rhythmicity

Valentina Mosienko[1]

Max-Delbrueck Center for Molecular Medicine, Berlin-Buch

Serotonin (5-hydroxytryptamine, 5-HT) is a monoaminergic neurotransmitter implicated in a variety of physiological processes. Biosynthesis of serotonin is limited at the first step by two distinct tryptophan hydroxylase enzymes, TPH2 and TPH1. In the serotonin-synthesizing neurons in midbrain, pons and medulla oblongata 5-HT synthesis is initiated by TPH2, while this step is done by TPH1 in the pineal gland and enterochromaffin cells of the gastrointestinal tract. Recently, by genetically ablating Tph2 we generated mice lacking serotonin in the central nervous system (Tph2-/-, Tph2-deficient mice).

Serotonin is extensively entangled in fundamental aspects of sleep and circadian rhythm regulation. Using Tph2-deficient mice as a model with altered brain serotonin we assessed the role of the serotonergic system in the regulation of sleep and in the adaptation of circadian rhythms of blood pressure (BP), heart rate (HR), and locomotor activity (LA) to light cycle shift. The parameters were measured for 3 days under basal conditions (6 am-6 pm light phase; 6 pm-6 am dark phase), followed by 7 days of inverted light (6 pm-6 am light phase; 6 am-6 pm dark phase) and 7 days of free run (24 hr darkness).

Using electroencephalogram and electromyogram recordings we detected increased slow wave sleep (SWS) and decreased active wake (AW) during late afternoon in Tph2-deficient mice under basal condition confirming a crucial role of central serotonin in promoting arousal. Moreover using telemetry we detected in Tph2-deficient mice a 2 hr shift in acrophase for LA and BP in comparison to wild type animals.

Under reversed light conditions Tph2-deficient mice could easily adapt to changed light, showing the same diurnal rhythm as wild type animals with increased SWS duration during the light phase, and elevated AW during the dark phase, as well as with adjusted acrophases for BP, LA, and HR.

Under free-running conditions Tph2-/- mice exhibited the same sleep-wake profile as under normal light, staying less awake during late afternoon then control mice.

Our findings suggest an important role of central serotonin in sleep control. However, the central serotonergic system seems to be not critical for the establishment of circadian rhythms and adaptation processes upon light changes in mice.

# VESTIBULAR CEREBRO-CORTICAL PROCESSING AND THALAMO-CORTICAL NEUROTRANSMISSION IN RATS BASED ON MICRO-PET DATA

Nadine Schabbach[1], Elena Lange[1], Ulla Stier[1], Stefan Reuss[1]

[1]University Medical Center, Institute of Microanatomy and Neurobiology, Langenbeckstr. 1, Building 708, 55131 Mainz, Germany

Purpose: Our previous studies using functional brain imaging (Positron-Emission-Tomography, PET) showed activation of distinct cortical and thalamic areas upon vestibular stimulation. Anterograde and retrograde tracings were conducted to visualise the connections of these cortical and subcortical regions. Brain sections containing successfully labelled structures were used to investigate putative transmitters involved in vestibular thalamo-cortical processing, i.e. the catecholaminergic and opioidergic systems.
Methods: Corresponding to the PET data, the anterograde tracer Phaseolus vulgaris-Leucoagglutinin was injected into specific nuclei of the thalamus. The retrograde tracer Fluorogold was applied to respective cerebro-cortical regions. All selected regions are part of the vestibular network. For immunohistochemistry, sections were incubated with antibodies against transmitters, synthesis enzymes or receptors. The immunoreactions were visualised by fluorescence-conjugated secondary antibodies.
Results: The anterograde tracing showed labelled punctuate structures in the amygdala, the somatosensory and the cingulate cortex, often in close vicinity of neurons that expressed the μ-opioid receptor. Neuronal somata labelled by the retrograde tracer were found in the latero-dorsal, ventroposterior-lateral and -medial nuclei of the thalamus. Most of their neuronal somata exhibited immunoreactivity to beta-endorphin. Immunofluorescence for tyrosine-hydroxylase, the marker enzyme for catecholaminergic synthesis, was found neither in the labelled cortical nor in the thalamic structures.
Conclusions: Our neuronal tracing studies showed that brain structures identified by PET to be part of the vestibular system are connected, and suggest that the opiodergic system is involved in transmission.

# Different positive allosteric modulators specific for $hmGlu_2$ share an identical binding site

Hannah K. Delille[1], Rajathees Rajaratnam[1], Wilfried Braje[1], Hervé Geneste[1], Liliane Unger[1], Mario Mezler[1]

[1]Neuroscience Research, GPRD, Abbott, 67061 Ludwigshafen, Germany

The pathology underlying schizophrenia is only partly understood. Besides dopaminergic and serotonergic pathways, also glutamate is thought to play an essential role. Efforts have been made to develop drugs targeting specific metabotropic glutamate receptor (mGlu) subtypes, a goal easier to achieve through subtype selective positive allosteric modulators (PAMs). Previously, three amino acids of $hmGlu_2$ (Ser688, Gly689, and Asn735) have been identified to be crucial for $hmGlu_2$ PAM cellular signaling. Furthermore, transposition of these amino acids into $hmGlu_3$ was shown to allow allosteric modulation of $hmGlu_3$ activity by $hmGlu_2$ PAMs (Schaffhauser *et al*., 2004; Rowe *et al*., 2008).

Using the $hmGlu_2$ PAM Abbott-1 as the radioligand we demonstrate that $hmGlu_2$-S688/G689/N735 are directly involved in PAM binding, and that the PAM binding site is created in the $hmGlu_3$-L697S/V698G/D742N receptor. Several $mGlu_2$ PAMs, like BINA and LY487379, are shown to compete for binding to the PAM binding site with Abbott-1. Although, the affinity of LY487379 to the created binding site in $hmGlu_3$-L697S/V698G/D742N appeared to be lower. An increase in $B_{max}$ of orthosteric ligands at $hmGlu_2$ by PAMs has been described. Additionally, binding of Abbott-1 to $hmGlu_2$ was potentiated by orthosteric agonists, while an antagonist had no effect. This describes reciprocal allosteric modulation of both binding sites.

In summary, our data demonstrate that allosteric modulators bind to $mGlu_2$ at a site formed by the amino acids Ser688, Gly689, and Asn735, as has been suggested from functional studies, and show that othosteric agonists can influence the binding of PAMs to the allosteric site.

# Characterization of the role of CXCL12/CXCR4 signalling in the development and survival of midbrain dopaminergic neurons

Amparo Tolosa[1], Kerstin Krieglstein[1, 2]

[1]Albert-Ludwigs-University Freiburg, Institute of Anatomy and Cell Biology. Department of Molecular Embryology, Albertstraße 17, 79104 Freiburg, Germany

[2]FRIAS, Albert-Ludwigs-University Freiburg, Freiburg, Germany

The identification of mediators in the development of dopaminergic neurons is a crucial step for the developing of treatments based in transplants of dopaminergic cells obtained *in vivo*.
Chemokine CXCL12 regulates an diversity of processes in both CNS and peripheral immune system, including migration, survival, proliferation, and intercellular communications. CXCL12 and its receptor CXCR4 are constitutively expressed in dopaminergic (DA) neurons of the substantia nigra (SN) in adult and interestingly, the SN of Parkinson disease subjects exhibits higher expression of CXCR4 and CXCL12 expression than control subjects, despite the loss of dopaminergic neurons. Nevertheless, the potential role of CXL12 and its receptor in the development of midbrain DA neurons has not been established yet.

The main objective of this work is to determine if CXCL12 signalling is involved in the development and survival of midbrain DA neurons.

*In vitro* experiments with E14 mouse mesencephalic primary culture and *in vivo* experiments on chicken embryos were performed in order to check the effects of chemokine CXCL12 and inhibitors of its signalling in the development of midbrain.

Preliminary results show an increase in the survival of embryonic midbrain DA neurons after treatment with chemokine CXCL12.

Our results indicate a potential neurotrophic role of chemokine CXCL12 in the development of midbrain DA neurons

# Poster Topic

# T5: G Protein-linked and other Receptors

T5-1A Differential expression of $GABA_B$ receptors and their effector Kir3 channels in cholecystokinin- and parvalbumin-containing interneurons
*Daniel Althof, Anna Gross, S. A. Booker, Michael Frotscher, Imre Vida, Akos Kulik*

T5-2A Examinations on the pathophysiological role of the cholinergic system in the $dt^{sz}$ mutant hamster
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# Differential expression of $GABA_B$ receptors and their effector Kir3 channels in cholecystokinin- and parvalbumin-containing interneurons

Daniel Althof[1], Anna Gross[1], S. A. Booker[4], Michael Frotscher[1], Imre Vida[4], Akos Kulik[1]

[1]Institute of Anatomy and Cell Biology, Department of Neuroanatomy, University of Freiburg, Germany
[2]Spemann Graduate School of Biology and Medicine, University of Freiburg, Germany
[3]Faculty of Biology, University of Freiburg, Germany
[4] Neuroscience and Molecular Pharmacology, FLBS, University of Glasgow, UK

Neuronal activity in mammalian cortical networks is controlled by GABAergic inhibitory interneurons. Of particular interest are the perisomatic targeting basket cells, expressing either cholecystokinin (CCK+) or parvalbumin (PV+), which are specialized to control the output of principal cells: the CCK+ neurons function as a modulatory device for the precisely timed firing during oscillatory activity, whereas PV+ basket cells are seen as rhythm generators. Inhibitory interactions among basket cells are crucial for their network functions that are primarily mediated by ionotropic GABAA receptors. However, very little is known regarding the contribution of metabotropic GABAB receptors to these interactions. We investigated the distribution of GABAB receptors and their effector Kir3 channels in dendritic shafts of CCK+ and PV+ cells of the rat hippocampus using immunofluorescence staining and high-resolution immunoelectron microscopy.
At the light microscopic level, strong immunostaining for the GABAB1 receptor subunit was detected in CCK+ cells, whereas immunoreactivity for the protein was weak in PV+ neurons. Electron microscopic analysis revealed that immunogold particles for the receptor subunits were consistently present on the extrasynaptic plasma membrane of dendritic shafts of both cell types. Quantitative analysis further demonstrated a higher density of the protein on the dendrites of CCK+ than on dendrites of PV+ cells.
The effect of postsynaptic GABAB receptors is primarily mediated by G-protein-coupled inwardly rectifying K+ (Kir3) channels, resulting in slow inhibitory postsynaptic potentials. Double -immunofluorescence labelling revealed a consistent expression of Kir3 channel subunits in both cell types: the Kir3.3 protein was found to be the predominant subunit in CCK+ cells, whereas PV+ neurons showed the highest immunoreactivity for Kir3.1.
Differences in the density of GABAB receptors and in the expression of Kir3 channel subunits suggest a differential regulatory role of these proteins in the integration of synaptic inputs, which may underlie the distinct functions of CCK+ and PV+ interneurons in network operations in the hippocampus.

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# Examinations on the pathophysiological role of the cholinergic system in the *dt*$^{sz}$ mutant hamster

Jagoda Kuschka[1], Sinisa Smiljanic[1], Melanie Hamann[1], Angelika Richter[1]

[1]Institute of Pharmacology and Toxicology, Department of Veterinary Medicine, Freie Universität Berlin, Koserstraße 20, 14195 Berlin, Germany

Dystonia, a common movement disorder, is characterized by sustained involuntary contractions of opposing muscles causing twisting movements and abnormal postures. Although anticholinergic drugs which are selective for the muscarinic acetylcholine receptor subtype 1 (M1) are effective in the treatment of dystonias, the underlying pathophysiological mechanisms of this clinical observation are not clarified. Thus, the therapeutical outcome after the empirical application of M1 antagonists is heterogeneous and the administration is often afflicted with a high incidence of severe adverse effects. There is growing evidence that antagonists of the muscarinic acetylcholine receptor subtype 4 (M4) could have a great therapeutic potential in hyperkinetic movement disorders with a lower incidence of side effects. In the here presented study, we therefore studied the effects of the M1 antagonist trihexyphenidyl and of tropicamide, an antagonist with preferential activity for M4, in the *dt*$^{sz}$ hamster, a rodent model of paroxysmal dystonia, to further clarify the pathophysiological role of the cholinergic system in dystonia. Furthermore, the administration of the M1 antagonist pirenzepine, which does not penetrate the blood-brain-barrier, should elucidate if the antidystonic efficacy of M1 anticholinergics in patients may be mediated by peripheral effects at the neuromuscular end-plate.

Previous studies in the *dt*$^{sz}$ hamster indicated that a single intraperitoneal application of trihexyphenidyl did not reduce the severity of dystonia, but delayed the onset of a dystonic episode. The acute systemic injection of tropicamide (15, 30 and 60 mg/kg) yielded comparable results in the present study. A combined systemic treatment with 15 mg/kg tropicamide and 10 mg/kg trihexyphenidyl tended to reduce the severity of dystonia, but did not retard the latency to onset, whereas the administration of 30 mg/kg tropicamide and 15 mg/kg trihexyphenidyl was sufficient to exert a delay to the onset of dystonia and to reduce its severity. However, a further increase of doses (60 mg/kg tropicamide and 30 mg/kg trihexyphenidyl) did not result in more pronounced antidystonic effects. The intraperitoneal administration of pirenzepine (25 and 50 mg/kg) did not lead to antidystonic effects. It is known from patients that treatment with anticholinergics requires a long-term administration for best therapeutic results. Therefore, trihexyphenidyl or tropicamide alone or combined were daily injected over a time period of 21 days. The severity of dystonia was not affected by the long-term treatment with trihexyphenidyl or tropicamide alone but the latency to onset of a dystonic episode was retarded by the application of tropicamide. Only the combined application of both compounds led partially to a reduced severity of dystonia. The long-term treatment with pirenzepine failed to exert antidystonic effects.

In order to examine if *striatal* M1 and M4 receptors are pathophysiologically involved in the effects which were observed after systemic administration of trihexyphenidyl and tropicamide, mutant hamsters received bilateral microinjections of trihexyphenidyl or tropicamide alone (each 200, 400 and 800 ng/0.5 µl/hemisphere) and the combination of both compounds (200+200, 400+400 and 800+800 ng/0.5 µl/hemisphere) directly into the striatum. However, a significant reduction of the severity of dystonia and also a delay to onset of dystonic episodes were not observed.

In summary, the present results did not show a critical pathophysiological role of the cholinergic system in the dystonic syndrome of the *dt*$^{sz}$ hamster since antidopaminergic or GABAmimetic compounds led to much more pronounced effects in previous studies. Nevertheless, it can not be excluded that antagonists with a higher selectivity for the M1 and the M4 receptor, which are not available so far, might exert more pronounced effects.

This study was supported by the *Dystonia Medical Research Foundation*.

# Modulatory Role of GABA and $GABA_B$ Receptors in Cockroach Salivation

Stefanie Blankenburg[1], Wolfgang Blenau[1]

[1]University of Potsdam, Institute of Biochemistry and Biology, Zoophysiology, Karl-Liebknecht-Str. 24-25, 14476 Potsdam-Golm, Germany

Saliva, produced in the salivary gland, initiates in general the digestion of food. Despite of various studies on the control of salivation in different insect species, the comprehension of the neuronal and hormonal control of salivation is more than fragmentary.
In the cockroach *Periplaneta americana*, salivary secretion of the acinar-type salivary glands is controlled by the nervous system. The glands are innervated by two salivary neurons (SNs) originating from the subesophageal ganglion (SEG). SN1 contains dopamine as a neurotransmitter. SN2 has recently been shown to contain γ-aminobutyric acid (GABA; Rotte *et al.*, 2009). The application of GABA during salivary duct nerve stimulation enhances the electrical response of acinar cells and increases fluid and protein secretion. The effect on electrical response can be mimicked by the application of $GABA_B$ receptor agonists and blocked by $GABA_B$ receptor antagonists. The pharmacological evidence for the involvement of $GABA_B$ receptors in mediating the effects of GABA has to be substantiated by the molecular identification of these receptors.
The aim of the current study is to molecularly identify, characterize and localize the $GABA_B$ receptor subtypes in *P. americana*. We used PCR with degenerate primers followed by RACE-PCR in order to isolate cDNA fragments encoding for $GABA_B$ receptor subtypes 1 and 2 (PeaGB1 and PeaGB2). The deduced amino acid sequences reveal not only characteristics common to all G protein-coupled receptors but also show a high degree of conservation with vertebrate and invertebrate $GABA_B$ receptor subtypes. The distribution of the $GABA_B$ receptor transcripts in the brain and the SEG of *P. americana* was investigated by using *in situ*-hybridization to cryosections. PeaGB2 mRNA is highly expressed in various brain areas and in two cell clusters within the SEG and in the soma rind of the SEG.

References:
Rotte C *et al.* (2009) J. Exp. Biol. 212, 126-136.

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# Release of Neuropeptide S and mechanisms of receptor activation

Frank Erdmann[1], Kay Jüngling[1], Hans-Christian Pape[1]

[1]Westfälische Wilhelms-Universität Münster, Institut für Phyiologie 1, Robert-Koch-Str. 27a, 48149 Münster, Germany

Neuropeptide S (NPS) and its cognate receptor constitute a newly discovered transmitter system in the CNS involved in fear acquisition and memory. Behavioral studies in mice have shown an anxiolytic effect as well as an enhanced fear extinction after NPS -administration into the amygdala. The 20 aminoacid peptide is conserved among vertebrates and synthesized in two distinct nuclei of the brainstem. The NPS receptor (NPS-R) is highly expressed in the amygdala and has been characterized as a G-protein coupled receptor which triggers both, G-protein s as well as G-protein q activity. Accordingly, studies have shown that heterologous expression of the NPS-R in HEK 293 cells results in an increase in cytosolic calcium concentration as well as an activation of the cAMP-dependent pathway after NPS application.

Despite these findings, little is known about the molecular mechanisms underlying NPS release or perception of the NPS-signal by the NPS receptor. Moreover, the exact intracellular signalling pathways following receptor activation are only poorly understood.

To investigate these issues, we use dissociated neuronal cell cultures for transfection and expression of full length preproNPS fused to eGFP as well as the human NPS receptor. This allows us to study mechanisms underlying transport and release of NPS as well as activation of the NPS-R using imaging techniques.

# Serotonin Receptor 1A-modulated Glycine Receptor alpha 3 Phosphorylation controls Breathing in Mice

Till Manzke[1], Marcus Niebert[1,2], Uwe R. Koch[1,2], Steffen Vogelgesang[1,2], Swen Hülsmann[1,2], Evgeni Ponimaskin[1,2], Ulrike Müller[6], Trevor G. Smart[5], Robert J. Harvey[4], Diethelm W. Richter[1,2]

[1]Dept. Neuro- and Sensory Physiology, University of Göttingen, Göttingen, Germany

[2]DFG Research Center Molecular Physiology of the Brain (CMPB), Göttingen, Germany

[3]Dept. Child and Adolescent Psychiatry, University of Göttingen, Göttingen, Germany

[4]Dept. Pharmacology, The School of Pharmacy, London, UK

[5]Dept. Neuroscience, Physiology & Pharmacology, University College London, London, UK

[6]Institute for Pharmacy and Molecular Biotechnology IPMB, University of Heidelberg, Germany

Rhythmic breathing movements originate from a dispersed respiratory network in the medulla and pons. Here, we demonstrate that rhythmic activity of this network is significantly affected by the phosphorylation status of the glycine receptor a3 subtype (GlyRa3), which critically controls glutamatergic and glycinergic neuronal discharges. The receptor is specifically sensitive to serotonergic modulation, which is vital when respiratory network operation has to adapt to varying environmental conditions. We demonstrate that a serotonin receptor type 1A (5-HTR1A)-specific modulation occurs directly through GlyRa3 receptors inducing their dephosphorylation, which augments inhibitory glycine-activated chloride currents. The 5-HTR1A - GlyRa3 signaling pathway is distinct from opioid receptor signaling and efficiently counteracts opioid-induced depression of the breathing and consequential apnea. This rescue of breathing, paradoxically, originates from enhanced glycinergic synaptic inhibition not only of glutamatergic neurons, but also of glycinergic neurons causing disinhibition of the neurons they innervate. Together these effects change respiratory phase alternations and ensures rhythmic breathing in vivo. Such protection is absent in GlyRa3-deficient mice, which show an irregular respiratory rhythm under baseline conditions and in which systemic 5-HTR1A activation fails to remedy opioid-induced respiratory depression. Delineation of this novel 5-HTR1A - GlyRa3 signaling pathway offers mechanistic basis for pharmacological treatment of opioid-induced apnea and other breathing disturbances caused by disorders of inhibitory synaptic transmission, such as hyperekplexia, hypoxia/ischemia and brainstem infarction.

# The physiological role of dopamine receptors in the fruit fly Drosophila melanogaster

Thomas Roeder[1], Flora Stephano[1], Samar El-Kholy[1]

[1]University of Kiel, Zoology, Olshausenstraße 40, 24098 Kiel, Germany

Dopaminergic signalling is of central importance for a great variety of different aspects of behaviour and homeostasis. In addition, impairments in dopaminergic signalling may lead to severe diseases, with Parkinson's disease as the most prominent one. The effects of this amine are mediated by a set of G-protein coupled neurotransmitter receptors, belonging either to the D1- or D2-group. In the fruit fly Drosophila melanogaster, a total of three different receptors are known to transmit the physiological effects of this compound. These receptors are the DopR1, DopR2 and D2R receptors, also belonging to either of the large subgroups of dopamine receptors.
To evaluate their expression patterns within the central nervous system, we generated transgenic flies to allow promotor driven expression of marker molecules. Using this approach, we were able to compare the expression profiles within the brains of larvae and adults. Whereas DopR2 (also known as DAMB) is primarily present in the mushroom bodies, the other receptors show a more complex expression pattern. Especially the DopR1 receptor appears to be present in numerous different neurons. Some of them were identified by colocalization experiments. Using an antibody against the pigment dispering factor (PDF), we were able to show that this receptor is highly present in clock neurons of the fly, presumably to modulate the circadian rhythms of the fly. In addition, the receptor could also be colocalized with the dopamine decarboxylase, the enzyme that produces the transmitter itself. This, the DopR1 receptors within these neurons are potential autoreceptors.
Regarding the expression of the third dopamine receptor (D2R), we also observed a complex expression pattern with high levels of expression in the optic lobes, namely the medulla, but also in the central complex.
These studies are currently complemented by physiological studies using corresponding knockout and RNAi lines.

# Investigating molecular and physiological functions of GPRC5 receptors

Thomas Pelz[1], Stefan Kurtenbach[3], Bastian Toetter[1,2], Sonja Oberland[1,2], Eva M. Neuhaus[1,2]

[1] FU-Berlin, Fachbereich Biologie, Chemie, Pharmazie, Takustr. 3, 14195 Berlin, Germany.

[2] NeuroScience Research Center, Charité, Universitätsmedizin Berlin, 10117 Berlin, Germany.

[3] Department of Cell Physiology, Ruhr-Universitaet Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany

The four members of the receptor group 5 within the family C of G protein-coupled receptors (GPCR), termed GPRC5A-D, have been discovered about a decade ago, but to date neither interacting cytosolic or membrane proteins nor GPRC5-activating ligands have been identified for any of them. GPRC5 receptors lack a large extracellular, N-terminal domain that contains both a ligand-binding site and a site for protein oligomerisation in the other family C GPCRs, which makes it even more difficult to predict a putatively receptor-binding set of interactors. It has been shown that the GPRC5 receptors are transcriptionally regulated by retinoic acid (RA) *in vitro*, why they have formerly been named retinoic acid-inducible genes (RAIG). RA is crucially involved in the development and maintenance of organisms, especially the nervous system. We could show that GPRC5B is regulated by RA *in vivo*. Large-scale sequence analysis revealed exclusive expression in vertebrates. Considering this and the localisation of the receptor in distinct regions of the brain involved in neurogenesis, it is tempting to anticipate important implications on vertebrate development and/or physiologically relevant neuronal processes.
In our approach we use the split-ubiquitin-system, which provides a large-scale assay where protein interaction with integral and membrane-associated proteins can be analysed. Thereby we are able to determine putative interactions of murine and human GPRC5B. Furthermore we perform BRET (bioluminescence resonance energy transfer) assays with all of the GPRC5 receptors and some putatively interacting proteins. To screen for receptor-binding ligands, we use the Calcium Imaging technique along with a set of molecules with different chemical properties.

# Poster Topic

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# A switchable ratiometric sensor for reactive oxygen species based on voltage-gated sodium channels

Ehsan Nematian[1], Enrico Leipold[1], Benjamin Borowski[1], Sophie Neugebauer[1], Toshinori Hoshi[2], Stefan H. Heinemann[1]

[1]University of Jena, Biophysics, Hans-Knöll-Str. 2, 07745 Jena, Germany
[2]University of Pennsylvania, Department of Physiology, Philadelphia, PA, USA

Reactive oxygen species (ROS) are mediators of cell signaling, play roles in host defense, and, particularly when present at high concentration, give rise to pathophysiological phenomena such as degenerative diseases. Therefore, knowledge on the generation of ROS, their distribution in and between cells and, ultimately, their molecular targets is essential for understanding ROS-related biological processes. Several low-molecular weight fluorescent probes are available to detect ROS in cells, but many of them are either slow and/or difficult to calibrate. The recently introduced genetically encoded fluorescence proteins roGFP2 and HYPER represent a considerable improvement as they yield ratiometric signals reporting on disulfide redox state or $H_2O_2$ concentration, respectively. However, these probes rely on excitation light, which produces ROS itself, they are prone of bleaching, and their sensitivity to ROS is difficult to adjust. Here we introduce a genetically engineered voltage-gated sodium channel (roNaV) with a cysteine residue in the inactivation motif (M1305C in the intracellular linker connecting domains III and IV, based on rat skeletal muscle channel NaV1.4) as a ratiometric ROS sensor when combined with whole-cell patch-clamp recordings. The degree of fast inactivation provides a ratiometric signal (steady-state current divided by peak current) directly related to the modification of the introduced cysteine and, hence, is independent of the degree of protein expression in the host cell. The time constant of modification is a linear function of ROS concentration as exemplified by extracellular application of the oxidant chloramine T to roNaV-expressing HEK293 cells. The signal is reversible as application of DTT restores inactivation completely. The sensitivity is similar to that of roGFP2, but the dynamic range of the ratiometric signal is about twice as large (about 0.02 to 0.80 vs 0.10 to 0.30). Using roNaV, we could demonstrate and quantify ROS production in HEK293 cells exerted by epifluorescence excitation with blue (450-490 nm) and even with green (510-560 nm) light (20x dry objective). Another advantage of roNaV over other available ROS sensors is that it can be switched between *ON* and *OFF* states by altering the membrane potential and, hence, by populating the channels in either activated (ROS-sensitive) or inactivated (ROS-protected) states. Using this property, we estimated the lifespan of a major component of ROS, liberated from flashlight-irradiated Lucifer Yellow loaded into HEK293 cells, to below 200 ms.

# Adaptation and Information Transmission in a Convergent Sensory Network

Ulrike Ziehm[1], Jan Benda[2]

[1]Freie Universität Berlin, Neuroinformatics & Theoretical Neuroscience and BCCN Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

[2]Ludwig-Maximilians-Universität München, Department of Biology and BCCN München, Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany

The adjustment of neuronal sensitivity to the mean intensity of a stimulus is a common feature in many sensory systems and practically all levels of sensory processing. Little, however, is known about the cellular mechanisms underlying such adaptive reshaping of the response properties of neurons within a network.

To investigate the effects of several adaptation mechanisms on the transmission of sensory input we simulated a small network of spiking neurons consisting of 30 sensory units that converge onto a single higher order neuron. This network architecture resembles for instance early stages of auditory processing in grasshoppers and crickets. Individual sensory units have sigmoid rate-level-functions with narrow dynamic ranges, similar sound-frequency tuning, and similar adaptation properties, but differ in response threshold so that the large range of physiologically relevant intensities is partitioned across the population.

As possible adaptation mechanisms we simulated different combinations of spike frequency adaptation in the input population as well as in the output neuron and short-term synaptic plasticity. The relation between sensory input and output-neuron response is analyzed for constant and time varying stimuli. Remarkably, the resulting rate-level functions differ from the ones observed in the cricket auditory system, unless a sigmoidal function is applied to the total synaptic input to the output neuron. By means of Fisher-information estimated from onset and steady state responses to constant step stimuli and mutual information between stimulus and neuronal response for time varying stimuli we discuss the impact of the different adaptation mechanisms on the signal transmission properties of the convergent network.

# Analysis of native phosphorylation sites of the K+-Cl--cotransporter KCC2

Maren Weber[1], Anne Ripperger[1], Kerstin Harms[1], Juanying Ye[2], Ole Nørregaard Jensen[2], Hans Gerd Nothwang[1], Jens Schindler[1]

[1]University of Oldenburg, Neurogenetics Group, Carl-von-Ossietzky-Str. 9-11, 26129 Oldenburg, Germany
[2]University of Southern Denmark, Protein Research Group, Campusvej 55, 5230 Odense M, Denmark

In mature neurons of the central nervous system, chloride outward transport is mediated by the K+-Cl--cotransporter KCC2. Its transport activity is essential for Cl- homeostasis and is required for the inhibitory effect GABA and glycine. In immature neurons, KCC2 is expressed in a transport-inactive state. Various kinases have been reported to associate with KCC2 or to modulate its transport activity. However, the knowledge of native phosphorylation sites of KCC2 is rare.

To identify native phosphorylation sites of KCC2, we performed immunoprecipitations of KCC2 and analyzed the protein by mass spectrometry. In total, we identified nine different native phosphorylation sites of KCC2. Three of them have not been reported yet. Seven phosphorylation sites were in the C-terminal region and two phosphorylation sites were in the N-terminal region of KCC2.

We individually mutated all identified phospho-Ser/Thr residues on rKCC2b to Ala and Asp attempting to mimic phospho and dephospho states of the protein. Transport activities of wildtype and mutant KCC2 were determined after transfection in HEK293 cells by thallium uptake. Preliminary data indicate site-specific effects of KCC2 phosphorylation on the transport activity. So far, we analyzed six phosphorylation sites. Two of the dephospho mimicking mutants, rKCC2b S1025A and rKCC2b S1026A, showed reduced uptake of thallium compared to wildtype rKCC2b. One phospho mimicking mutant, rKCC2b S25D, had a decreased transport activity compared to wildtype rKCC2b.

After heterologous expression in COS7 cells, we didn't observe obvious differences in the subcellular distribution of the mutants compared to wildtype KCC2.

Further analyses will reveal the impact of all phosphorylation sites on KCC2 transport.

# Analysis of the P2X3 agonist binding site by alanine substitutions

Thomas Riedel[1], Mandy Bodnar[1], Nanette Messemer[1], Sara Wiese[1], Peter Illes[1]

[1]Universität Leipzig, Rudolf-Boehm-Institut für Pharmakologie und Toxikologie, Härtelstraße 16-18, 04107 Leipzig, Germany

Purinergic P2X receptors belong to the family of ligand-gated ion channels. They are non-selective cation channels, activated by extracellular ATP. One of the seven members of the P2X receptor family, the P2X3 receptor, is localized at the plasma membrane of sensory neurons and is involved in pain perception. Therefore, this receptor is a possible target for new drugs in pain treatment. The development of such drugs can be supported by an exact knowledge of the receptor structure and function. There are many hints to the ATP binding site, but the interaction of the P2X receptor with its agonists and antagonists remains still unknown. In this study we investigated the influence of single alanine substitutions in the supposed ATP binding site of homomeric human P2X3 receptors, expressed in HEK293 cells, by means of whole cell patch clamp recordings. The amino acid residues in the this binding pocket were sequentially replaced by alanine. Modifications in the receptor binding site change the concentration -response dependency as well as the current kinetics during fast pulsed agonist applications. Based on this fact, we were able to distinguish binding from gating, conductance and desensitisation effects using a markov model that describes the complete channel behaviour by a matrix of rate constants. The results where also checked for consistency with a structural hP2X3 model that we developed from the known zebra fish P2X4 crystal structure in closed state. The side chains of the most influential amino acids were found to point to the binding pocket or are involved in inter-subunit hydrogen bonds in this model.

# Are there functional P2X receptors in adult adherent neural progenitor cells (NPCs)?

Nanette Messemer[1], Christin Kunert[1], Heike Franke[1], Peter Illes[1], Patrizia Rubini[1]

[1]Universität Leipzig, Rudolf-Boehm-Institut für Pharmakologie und Toxikologie, Härtelstraße 16-18, 04107 Leipzig, Germany

Adult neurogenesis takes place in two major regions of the mammalian brain, in the dentate gyrus of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles. The aim of this project was to elucidate whether cultured adherent neural progenitor cells (NPCs) of the subventricular zone (SVZ) of adult mice express functional P2X receptors (P2XR).

NPCs were prepared from the SVZ of adult mice. After 7 days, neurospheres were dissociated and brought into adherent culture. Whole-cell patch clamp recordings and calcium imaging were used to characterize the functional expression of P2XR, which was further supported by immunocytochemical stainings.

In these adherent cultures we identified the P2X7R as the predominantly expressed member of the P2X family. Inward currents of ATP and the preferential P2X7R agonist Bz-ATP were largely increased in a reduced divalent cation environment. The responses evoked by Bz-ATP and ATP decreased with the age of the adherent cultures. This current reduction could be prevented when growth factors (EGF and FGF-2) were reapplied every day.
The use of unspecific (PPADS) and specific antagonists (A438079, BBG) reduced agonist evoked responses of the P2X7R in patch clamp and $Ca^{2+}$ imaging experiments. A P2XR agonist with selectivity for other subtypes (alpha,beta-meATP, P2X1,3) did not alter the calcium signal respectively the current. Subtype-specific antagonists (TNP-ATP, P2X1-3; NF449, P2X1) did not change the calcium signal caused by Bz-ATP, but TNP-ATP reduced the Bz-ATP response in patch clamp experiments. Moreover $Zn^{2+}$ and basic / acidic pH modulated the Bz-ATP evoked signals in patch clamp as well as in $Ca^{2+}$ imaging experiments.
Using NPCs derived from P2X7R knockout mice, indicated that the Bz-ATP or ATP induced responses are mainly due to the activation of P2X7R.

These results demonstrate that the P2X7R is functionally expressed in adherent cultures of NPCs from the SVZ. Furthermore growth factors may play a pivotal role in the expression of this receptor subtype in the NPCs.

# Astroglial cells of rodent brain slices express in situ functional purinergic P2X7 receptors

Anna Leichsenring[1], Thomas Riedel[1], Heike Franke[1], Claudia Heine[1], Peter Illes[1], João Filipe Oliveira[2]

[1]Universität Leipzig, Rudolf-Boehm-Institute of Pharmacology and Toxicology, Härtelstraße 16 - 18, 04107 Leipzig, Germany
[2]University of Minho, Life and Health Sciences Research Institute (ICVS), Braga, Portugal

The purpose of the present study was to investigate whether astrocytes in the rodent central nervous system (CNS) express functional P2X7 receptors (P2X7R).
For our investigations we used freshly prepared 200 micrometer thick slices of rodent brain and spinal cord. By means of whole cell patch-clamp recordings, electrophysiologically identified astrocytes in the prefrontal cortex (PFC), the oriens layer of the hippocampal CA1 region and the spinal cord substantia gelatinosa (SG) were analyzed. Benzoylbenzoyl ATP (BzATP) - a preferential P2X7R agonist - and ATP caused inward currents in astroglial cells in all investigated tissues. Amplitudes of BzATP and ATP responses were strongly increased in a low divalent cationic external solution and the use of the specific P2X7R antagonist A438079 caused a concentration dependent reduction of the inward current. These results were underlined by excised patch recordings, immunohistochemical detection of the P2X7R protein in astrocytes and recordings in CNS tissues of the respective knockout mice.
In conclusion, we were able to show the functional expression of the P2X7R in rodent astrocytes in situ. Our results contrast sharply with the previous view that native astroglial cells do not express purinergic P2X7R. We suggest that P2X7Rs in astrocytes are not involved in the physiological cross talk with neurons but are sensors of pathologically high ATP concentrations released by brain injury or metabolic limitation. By means of in vitro and in vivo models of ischemia, epilepsy and neuropathic pain, we will further elucidate the functional involvement of glial P2X7R in CNS pathophysiological conditions.

# Brominated pyrroleimidazoles and sesquiterpenes from marine sponges as tools or cell physiology: Ion channel blockade, ATPase inhibition, pH measurements and vesicle tracking

Ulf Bickmeyer[1]

[1]Alfred Wegener Institut , Ökologische Chemie, Am Handelshafen 12, 17570 Bremerhaven, Germany

Marine organisms have long been known to harbor secondary metabolites with specific cellular targets. Especially sponges are a rich source for bioactive substances. We investigated brominated pyrrole alkaloids and sesquiterpenes. Some brominated pyrole alkloids have been described to inhibit voltage operated calcium channels (Bickmeyer et al. 2004). Especially dimeric molecules containing several bromine and guanidine groups show half maxinal concentrations around 2- 5µM. One alkaloid out of this group is a potent pH sensitive cellular dye (Bickmeyer et al. 2008), which can be used for vesicle tracking of neuronal vesicles as well as labeling of acidic tissues in transparent animals. A sequiterpenehydrochinone shows similar effects as the Ca++ ATPase inhibitor thapsigargin (Bickmeyer et al.2010). The substance is in the mode of action not distinguishable from thapsigargin except slight differences in the concentration range.

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# Channel noise in models of single neurons

Dmitry Zarubin[1,2], Ekaterina Zhuchkova [1], Susanne Schreiber [1,2]

[1]Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Germany
[2]Bernstein Center for Computational Neuroscience, Berlin, Germany

Neural signaling is based on changes in neuronal membrane potential. Essentially, a neuron recruits a number of discrete elementary events – the opening and closing of ion channels in the cell's membrane – in order to process information. Opening and closing is, however, a stochastic process, and consequently the variance of the summed conductances for a given channel type depends on the total number of ion channels. Several studies have shown that ion channel noise can fundamentally limit precision, speed and accuracy of computation in the nervous system (see for example Schneidman et al., 1998; White et al., 2000), in particular, if ion channel numbers in individual neurons are only in the range of thousands, but not tens of thousands. Among the cell-intrinsic noise sources, ion channel noise was shown to be the most significant one, surpassing the effects of thermal noise (Manwani and Koch, 1999).
Here, we explore the effect of channel stochasticity on the membrane potential in simple Markov-type neuronal models. We analyze the effect of channel noise on the subthreshold as well as the spiking dynamics, comparing stochastic simulations to those of deterministic models with additive current noise of fixed statistics.

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# Characterization of Blockers and Modulators of Insect Odorant Receptors

Katharina Röllecke[1], Markus Werner[1], Hanns Hatt[1], Günter Gisselmann[1]

[1]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstr.150, 44780 Bochum, Germany

Insect odorant receptors are heteromultimeric proteins composed of a constant subunit, called OR83b in *Drosophila melanogaster* and a variable subunit which is responsible for the ligand specificity. Systematic screening attemps by several groups lead to the identification of ligands for most of these variable subunits in *Drosophila* and *Anopheles*. Information on the pharmacology of the OR83b subunit are sparse. Antagonists or modulators of this subunit are of potential interest as insect repellent as well as general pharmacological tools for the characterization of insect odorant signal transduction. Recently, odorant receptors were found to be ligand gated ion channels and the OR83b subunit is substantial for the ion channel function. Therefore, it was of interest to find general blockers for this ion channel function that were not interfering with ligand binding on the variable subunit.

For our pharmacological investigation, we expressed recombinant odorant receptor subunits from *Bombyx mori*, *Heliothis virescens* and *Drosophila melanogaster* in *Xenopus* oocytes and measured the odorant evoked currents by two-electrode voltage clamp. We screened a variety of substances for blocking or modulating effects and identified a group of amylorid derivates as effective blockers for all tested receptor combinations. We demonstrated that these substances inhibit odorant evoked current but not the ligand binding to the variable part of odorant receptors. The most active substance was MIA (5-(N-methyl-N-isobutyl)amiloride) showing $IC_{50}$ values for blocking of odorant evoked currents ranging from 2 to 10 μM depending on the subunit composition. We suggest that substances of this group act on the highly conserved OR83b-type subunit which is an integral part of all or most odorant receptors found in insects and are potential blockers of the ion channel pore.

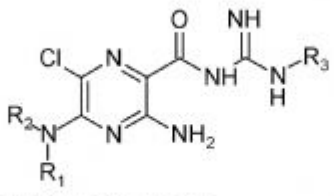


Fig. 1: Structure of the potent Blocker MIA (5-(N-methyl-N-isobutyl)amiloride, R1 = iBut, R2 = Me, R3 = H)

# Characterization of the interaction between TRPM8 ion channels and G proteins

Sven Zielke[1], Christian H. Wetzel[2]

[1]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstraße 150, 44780 Bochum, Germany
[2]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstraße 150, 44780 Bochum, Germany

TRPM8 is a member of the transient receptor potential (TRP) ion channel family. In mammalian there are 28 known TRP ion channels. On the one hand TRP channels can be activated by a huge variety of endogenous and exogenous chemical compounds and on the other hand they can be activated by physical stimuli like touch and temperature. Thus, they play a major role in sensing sensory signals and responding to touch, pain, temperature, taste, osmolarity and further stimuli. There are 6 thermosensitive TRP channels in mammals: TRPV1, TRPV2, TRPV3, TRPV4, TRPA1 and TRPM8. TRPM8 can be activated by temperatures below 25°C, and by natural as well as synthetic chemical compounds like menthol, eucalyptol, icilin or the second messenger PIP2. In general, the activation of TRPM8 leads to the opening of a pore that is permeable to monovalent cations, as well as Ca2+ and Mg2+. TRPM8 receptors are expressed in neurons of the trigeminal and dorsal root ganglia.
For a long time, it was believed that the TRPM8 receptor mediates signals only in a ionotropic manner, but there is compelling evidence for an agonist dependent activation of a heterotrimeric G protein. In our lab, Katharina Klasen could show that TRPM8 is able to interact with a heterotrimeric G protein of the Gq family (Klasen et al., in preparation). This interaction triggers the function of PLC which hydrolizes PIP2 to IP3 and DAG. Afterwards, Dominik Hollatz could localize the site of interaction to the C terminus of the TRPM8 protein (Klasen et al., in preparation).
FRET experiments with a couple of TRPM8 C-terminal fragments were used to narrow down the sequence of the C-terminal interaction site of TRPM8 with the Gq protein. These FRET experiments show that multiple domains contribute to the TRPM8/Gq protein interaction site.
Furthermore, I could demonstrate that the exchange of the TRPM8 C terminus with the corresponding sequence of TRPV1 in TRPM8/TRPV1 chimera resulted in the loss of metabotropic capacity of TRPM8.

# Characterizing the modulatory effect of odorants at GABA(A) receptors

Olaf Kletke[1], Olga A. Sergeeva[2], Anja Poppek[1], Stavros Manteniotis[1], Hanns Hatt[1], Guenter Gisselmann[1]

[1]Ruhr University Bochum, Dept. Cellphysiology, Bochum, Germany
[2]Heinrich Heine University, Dept. Neuro- und Sensory Physiology, Düsseldorf, Germany

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system of vertebrates. GABA is able to activate GABA(A) receptors which are composed out of five subunits and have more than ten distinct binding sites for different classes of modulators like benzodiazepines, barbiturates, neurosteroides and ethanol. Beside that, it was previously shown, that odorants like terpenes e.g. menthol and borneol were able to modulate recombinant GABA(A) receptors.
In our study we systematically characterized the modulatory effects of over 100 odorants from different chemical classes at recombinantly expressed GABA(A) receptors in oocytes. In addition to terpenes, we were able to identify new chemical classes of modulators. The best hits from this screening were taken to record full dose response curves at a1ß2γ2 GABA(A)receptors. By expressing different subunit combinations we could demonstrate that odorant modulation targets the beta subunit. To validate possible binding sites or mechanisms in detail, we used mutations previously described for distinct classes of modulators. We found that some odorants had an ambivalent mode of action and were blocking or potentiating depending on the concentration range. Both actions were independend and can be separated by the M286W or N265M mutation of the beta2 subunit.

# Chloride channels activity modulate phagocytosis in murine microglia

Barbara Harl[1], Judith Schmölzer[1,2], Martin Jakab[2], Markus Ritter[2], Hubert H. Kerschbaum[1]

[1]University of Salzburg, Department Cell Biology, Hellbrunnerstrasse 34, 5020 Salzburg, Austria
[2]Paracelsus Medical University, Institute of Physiology and Pathophysiology, 5020 Salzburg, Austria

Microglial cells, innate immune cells in the nervous system, phagocyte pathogenic invaders as well as endogenous cell debris. Phagocytosis is associated with local volume changes, including formation and extension of engulfment pseudopodia and, consequently, successful internalization of particles. Because volume regulation depends on the activity of Cl-channels, we quantified the impact on Cl-channel blockers on particle engulfment. Microglial cells were incubated for 15 minutes with microspheres (Ø 4µm) and particle uptake was evaluated using scanning electron microscopy and confocal laser scanning microscopy. The anion-channel inhibitors, 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), flufenamic acid (FFA) and 4-[(Butyl-6,7-dichloro-2-cyclopentyl-2,3-dihydro-1-oxo-1H-inden-5-yl)oxybutyric acid (DCPIB), the anion-channel inhibitor and K+Cl- -cotransport-inhibitor, [(dihydroindenyl)oxy]acetic acid (DIOA), or Cl - - free solution suppressed uptake of microspheres in primary murine microglia as well as the microglia cell line, BV-2. Furthermore, we characterized anion channels in BV-2 using whole cell patch clamp recordings. Ion currents were monitored during a voltage ramp from -100mV to +100mV. Anion channels in BV-2 cells were sensitive to the transmembrane osmotic gradient. Superfusion of the cell with a hypotonic solution or perfusion the cell with a hypertonic solution activated an outwardly rectifying anion current. This current was blocked by NPPB, FFA, DCPIB, or DIOA. Interestingly, incubation of BV-2 cells with microspheres for about three hours significantly increased anion current. In summary, our data suggest that cellular volume regulation via anion channels formation of engulfment pseudopodia and internalization of particles.

# μ-Conotoxin SIIIA discriminates between NaV channel subtypes by interacting with their pore loops in domain-2

Enrico Leipold[1], Rene Markgraf[1], Michael Kijas[1], Alesia Miloslavina[2], Diana Imhof[2], Stefan H. Heinemann[1]

[1]Center of Molecular Biomedicine, Friedrich Schiller University of Jena , Department of Biophysics, Hans Knoell St. 2, 07745 Jena, Germany

[2]Center of Molecular Biomedicine, Friedrich Schiller University of Jena , Department of Biochemistry, Hans Knoell St. 2, 07745 Jena, Germany

Voltage-gated sodium channels ($Na_V$ channels) play a pivotal role in neuronal excitability because they initiate and propagate action potentials. Therefore, various venomous animals target $Na_V$ channels with potent neurotoxins to disturb neuronal excitation for defense and hunting. Marine cone snails developed μ conotoxins that specifically inhibit NaV channels via a pore occlusion mechanism. They bind to receptor site-1 in the outer vestibule of the channel pore thereby reducing $Na_V$ channel conductance. μ-Conotoxins are subtype specific as they discriminate between different $Na_V$ channel types. Detailed information on their subtype specificity is only barely available because toxin binding potentially involves all four channel domains and therefore makes systematic studies difficult. Nevertheless, understanding the underlying mechanisms is of considerable importance because suppression of $Na_V$ channels, e.g. $Na_V1.7$, with subtype-specific probes is a promising analgesic strategy. Here we combine mutagenesis of $Na_V$ channels with whole-cell patch-clamp measurements to elucidate the subtype specificity of synthetic μ-conotoxin SIIIA (originally from Conus striatus) on a molecular level. Application of 10 μM SIIIA blocked rat-$Na_V1.2$, rat-$Na_V1.4$, human-$Na_V1.4$, and mouse-$Na_V1.6$ by 91.3 ± 1.3 % (n=7), 86.1 ± 1.3 % (n=6), 83.3 ± 0.8 % (n=6) and 82.5 ± 4.0 % (n=6), respectively. Human-$Na_V1.7$ channels were inhibited by 58.1 ± 5 % (n=8), whereas human-$Na_V1.5$ (n=6), rat-$Na_V1.8$ (n=5) and human-$Na_V1.8$ (n=8) were insensitive. By analyzing the SIIIA block on rat-$Na_V1.4$/human-$Na_V1.5$ chimeras we located the determinants for SIIIA specificity in the first half of the channels with a major contribution of domain-2 and a minor contribution of domain-1. We constructed an alternative set of channel mutants in the background of rat-$Na_V1.4$ where the domain-2 pore loops were exchanged against the pore loops of rat-$Na_V1.2$, human-$Na_V1.4$, human-$Na_V1.5$, mouse-$Na_V1.6$, human-$Na_V1.7$, rat-$Na_V1.8$, and human-$Na_V1.8$. All mutant channels showed SIIIA sensitivity comparable the pore loop donors, highlighting the importance of the pore loop in domain-2 as the major determinant for the subtype specificity of SIIIA. Further site-directed substitutions identified residue A728 in rat-$Na_V1.4$ as crucial for its high sensitivity of towards SIIIA. Likewise, N889 at the homologous position in $Na_V1.7$ is responsible for the channel's reduced SIIIA sensitivity. In conclusion, these results provide new information on the subtype specificity of μ-conotoxin SIIIA and may pave the way for the rational design of selective $Na_V$ channel antagonists for research and medical applications.

# ClC-2 constitutes the chloride leak conductance in neurons

Ilka Rinke[1], Valentin Stein[1]

[1]Max Planck Institute of Neurobiology, Synaptic Receptor Trafficking, Am Klopferspitz 18, 82152 Munich-Martinsried, Germany

Neurons exhibit a resting permeability for potassium, sodium and chloride. We found that the voltage-gated chloride channel ClC-2 provides the chloride leak conductance at resting conditions. The chloride conductance mediated by ClC -2 has been previously described in neurons and non- neuronal cells (Chesnoy-Marchais, 1983; Misgeld et al., 1986). ClC-2 is inwardly rectifying, with significant conductance at membrane potentials more negative than the chloride equilibrium potential ($E_{Cl}$). Further, ClC-2 stabilizes the relationship between $E_{Cl}$ and the resting membrane potential independent of electroneutral chloride transport (Staley, 1994; Staley et al., 1996). Due to these properties, it is not surprising that ClC-2 regulates neuronal chloride homeostasis by providing a fast chloride extrusion mechanism (Rinke et al., 2010). In addition, we recently provided evidence that ClC-2 influences neuronal excitability via affecting intrinsic membrane properties in hippocampal CA1 pyramidal neurons (Rinke et al., 2010). Because ClC -2 is active at resting membrane potential and contributes to the membrane resistance (Madison et al., 1986), we assumed that ClC -2 constitutes a significant part to the chloride leak conductance in this particular cell type. Here, we observed the chloride conductance of ClC -2 in principal neurons of hippocampus, cortex and cerebellum. The loss of ClC -2 in these neurons leads to a small shift of the resting membrane potential towards more depolarized values and to a remarkable increase of the membrane resistance in pyramidal cells of CA1 and cortex. As a result, the number of action potentials increases in all recorded neuron types lacking ClC-2. Thus, ClC-2 is proposed to provide the molecular basis for the chloride leak conductance regulating the excitability of neurons.

# Differential association of KCC2 and the $Na^+$-$K^+$-ATPase alpha-subunit during development

Kerstin Harms[1], Juanying Ye[2], Ole Nørregaard Jensen[2], Hans Gerd Nothwang[1], Jens Schindler[1]

[1]University of Oldenburg, Neurogenetics Group, Carl-von-Ossietzky-Str. 9-11, 26129 Oldenburg, Germany
[2]University of Southern Denmark, Protein Research Group, 5230 Odense M, Denmark

KCC2 is a neuron-specific isoform of the potassium chloride co-transporters. Its transport activity lowers the intracellular chloride concentration of neurons which is a prerequisite for the inhibitory action of GABA and glycine. However, KCC2 is already expressed at neonatal stages in transport-inactive state when GABA and glycine still act depolarizing. In addition to its transport activity, KCC2 seems to be involved in the morphology of dendritic spine.

To identify interacting proteins which are involved in the regulation of the different KCC2 functions throughout development, we performed immunoprecipitations of the KCC2 protein complex. Thereby immature (P0) as well as mature (P30) brains were analyzed.

In order to optimize the solubilizing conditions of the adult KCC2 protein complex, we used several detergents with various concentrations. The size of the native protein complex was analyzed on blue native gels. Independent of the detergent we used, we observed an immunoreactive band at ~550 kDa. This protein complex was immunoprecipitated and analyzed by mass spectrometry.

One protein which was associated with KCC2 in mature brain tissue was the alpha3-subunit of the $Na^+$-$K^+$-ATPase. We detected 9 different peptides from the alpha-subunit of the $Na^+$-$K^+$-ATPase, 2 of them were unique for the alpha3-subunit. Similar results were obtained when using Igepal CA-630 or DDM as detergent. At neonatal ages, the same experiments (DDM and Igepal CA-630) did not identify any peptides of the alpha-subunit of the $Na^+$-$K^+$-ATPase.

The data obtained by mass spectrometry were confirmed using immunohistochemical analysis. KCC2 and the alpha3-subunit of the $Na^+$-$K^+$-ATPase colocalized in various brain regions such as the hippocampus and the cerebellum of the adult brain.

Further immunoblot and mRNA analyses of the entire brain revealed the upregulation both of KCC2 and the alpha3-subunit of the $Na^+$-$K^+$-ATPase during development.

We currently investigate whether this interaction has functional consequence for the transport activity of KCC2.

# Differential regulation of NBCe1-A and NBCe1-B in mouse hippocampal neurons *in vitro*.

Oliver Oehlke[1], Eleni Roussa[1]

[1]Albert-Ludwigs-Universität Freiburg, Institut für Anatomie und Zellbiologie II, Albertstr. 17, 79104 Freiburg, Germany

Acid-base transporters are key molecules in the regulation of intracellular pH in many cell types. In the central nervous system, rapid pH shifts occur in the formation of action potentials. Additionally, changes in pH itself may affect normal neuronal activity and portray a common hallmark in many pathological conditions in the brain. Among the transporters expressed in neurons, the $NH_2$-terminal variants of the electrogenic sodium-bicarbonate-cotransporter 1 A and B (NBCe1-A and -B) are potential candidates to be involved in intracellular pH regulation due to the fact that these transporters have been shown to represent key molecules in warranting pH homeostasis in various tissues.

In the present study, we have investigated distribution and regulation of N-terminal variants of electrogenic sodium-bicarbonate-cotransporter 1 A and B (NBCe1-A and -B) under physiological and pathological conditions in primary hippocampal neurons *in vitro*.

A mouse E18.5 primary hippocampal cell culture served as the experimental model. After 12 days *in vitro* (DIV), transporter mRNA expression, protein abundance, and subcellular distribution was monitored under physiological (stimulation of normal neuronal activity via KCl) and pathological (acid- and alkali-load) conditions.

Primary hippocampal neurons at DIV12 expressed NBCe1-A and NBCe1-B, and they localized these transporters predominantly in a compartment near the nucleus. Acute extracellular acidosis resulted in the redistribution of NBCe1-A from pools in the cell soma towards the processes, compared to the controls. Transporter redistribution was quantified using the Pearson's correlation coefficient for NBCe1-A and distinct organelle markers along the trafficking route. Furthermore, upon redistribution, the plasma membrane fraction of NBCe1-A is significantly increased.

Localization of NBCe1-B is not altered after an acid-load, compared to the controls, whereas treatment of the cells with an alkali-load induced NBCe1-B trafficking away from the soma. Redistribution of NBCe1-B upon alkalosis increased the membrane-incorporated fraction, compared to controls and after induction of acidosis. Notably, neither the amount of whole NBCe1-A and -B protein nor transporter mRNA expression levels were altered.

Simulation of normal neuronal activity *in vitro* resulted in the initiation of NBCe1-B trafficking along axo-dendritic structures. Potential association of the redistributed transporter with distinct synaptic compartments has been examined by colocalization with pre- and postsynaptic markers, respectively. Subcellular distribution of NBCe1-A was comparable to the controls after KCl stimulation.

The results of the present study indicate that NBCe1-A and NBCe1-B trafficking in primary hippocampal neurons is differentially regulated under pathological (acid-base disturbances), as well as under physiological (normal neuronal activity) conditions, thus underlining the differential distribution and regulation of the $NH_2$-terminal variants of NBCe1 known from various other tissues.

# Downstream signalling of TRPM8

Dominik Hollatz[1], Katharina Klasen[1], Christian H. Wetzel[1]

[1]Ruhr-Universität Bochum, LS für Zellphysiologie, Universitätsstr. 150, 44801 Bochum, Germany

Members of the transient receptor potential (TRP) ion channel family serve as cellular sensors that are activated by a variety of physical and chemical stimuli. Activated TRP channels communicate their signal into the cell by gating ion fluxes through their intrinsic pores.
The cold and menthol receptor TRPM8 can be activated by several stimuli: chemical stimuli like menthol, eucalyptol or the synthetic drug icilin. TRPM8 can also be activated by temperature below ~25 °C. Upon activation, it allows cations to enter the cell through the intrinsic channel leading to depolarisation of the membrane potential.
Very recently, we found evidence for a functional and direct physical interaction of the cold and menthol receptor TRPM8 with Gaq protein (Klasen et al., in preparation). These results suggest that TRPM8 is not only an ion channel and target of metabotropic signalling, but also actively interferes with downstream metabotropic pathways. Thus, TRPM8 might function as both, an ion channel and G protein-coupling/activating receptor.
Performing $Ca^{2+}$ imaging experiments in HEK293 cells transiently expressing TRPM8, we observed that a population of cells is able to respond to application of menthol with a transient $Ca^{2+}$ signal, even in absence of extracellular $Ca^{2+}$. This observation leads to the assumption that internal $Ca^{2+}$ stores are involved in this mechanism. In order to investigate the signalling cascade, we accomplished blocking experiments with the phospholipase C (PLC) blocker U73122, and found evidence for the involvement of PLC in this metabotropic signalling cascade, since the menthol-induced $Ca^{2+}$ signal was abolished after treatment with U73122. In addition, $Ca^{2+}$ imaging experiments in HEK293 cells overexpressing TRPM8 and a dominant -negative variant of Gaq pointed to a vital role of Gq/PLC in this signalling pathway. Moreover, we performed Förster resonance energy transfer (FRET) experiments to find possible interaction sites of TRPM8 with Gaq. FRET could be detected for a CFP-tagged C-terminal fragment of TRPM8 and the YFP-tagged Gaq, indicating a direct physical interaction between Gaq and the C terminus of TRPM8.
This observation extends the operational range of the TRPM8 receptor from its function as a pure ion channel to a molecular switch with additional metabotropic capacity.

# Dysfunction of the voltage-gated sodium channel $Na_V1.1$ is associated with diminished inhibition in various brain regions

Ulrike Barbara Stefanie Hedrich[1], Melinda Martin[2], Camille Liautard[3], Massimo Mantegazza[3], Andrew Escayg[2], Holger Lerche[1]

[1]Hertie-Institute for Clinical Brain Research, Depts. of Neurology and Epileptology , Otfried-Mueller-Str. 27, D-72076 Tuebingen, Germany; [2]Department of Human Genetics, Emory University, 615 Michael Street, Whitehead Building, Suite 301, Atlanta, Georgia 30322;[3]IN2M-IPMC, Nice-Sophia Antipolis, 660 Route des Lucioles, 06560 Valbonne, France

Neuronal voltage-gated sodium channels ($Na_V$s) are essential for the generation and propagation of action potentials. Mutations of the *SCN1A* gene, encoding the a-subunit of the $Na_V1.1$ neuronal sodium channel, can lead to different neurological disorders, amongst others generalized epilepsy with febrile seizures plus (GEFS+).
One of the first identified human GEFS+ associated *SCN1A* mutation is R1648H, which is located in the voltage sensor of domain IV.
We used a knock-in mouse model carrying the human GEFS+ mutation *SCN1A*-R1648H (M.S. Martin et al.,*JBC*, 2010) to study the effects on the neuronal excitability.
Whole-cell patch clamp recordings were performed in acute brain slices to examine the firing properties of GABAergic interneurons. Both hippocampal stratum oriens and thalamic nucleus reticularis interneurons from heterozygous mice showed a decreased firing rate compared to those from wild-type littermates. As expected, membrane properties such as input resistance and resting membrane potential did not vary between heterozygous and wild-type interneurons. As a control we also studied excitatory pyramidal cells within the stratum pyramidale of the hippocampal CA1 region but no differences could be found between heterozygous and wild-type cells. Thus, our data suggest a decreased excitability of interneurons in various brain regions of the *SCN1A*-R1648H knock-in mice, which could explain the occurrence of epileptic seizures in mice and GEFS+ patients.

# Eag1 modulates synaptic transmission and firing rate of neurons in the cerebellar cortex

Lena Sünke Mortensen[1], Roser Ufartes[1], Takeshi Sakaba[3], Walter Stühmer[1], Luis A Pardo[2]

[1]Max Planck Institute of Experimental Medicine, Molecular Biology of Neuronal Signals, Hermann-Rein-Str. 3, 37075 Göttingen, Germany

[2]Max Planck Institute of Experimental Medicine, Oncophysiology Group, Göttingen, Germany

[3]Max Planck Institute of Biophysical Chemistry, Independent Junior Research Group Biophysics of Synaptic Transmission, Göttingen, Germany

[4]International Max Planck Research School Neurosciences, Göttingen, Germany

Ether-à-gogo 1 (Eag1) is a delayed rectifier potassium channel that is widely expressed in the mammalian brain. High levels of expression are seen in the hippocampus and in the cerebellum, but its physiological role remains undescribed. Mutant mice lacking a functional channel are viable and show no obvious abnormal behaviour. In this study, we confirmed synaptic localization of Eag1 and used whole-cell patch clamping in acute slices of the mouse cerebellum to investigate the effect of Eag1 gene knock-out on synaptic transmission at the parallel fiber (PF) – Purkinje cell (PC) synapse and on firing properties of granule- and Purkinje cells. The PF-PC synapse shows strengthening of the synapse in response to repetitive stimulation in the second to minute range (short-term facilitation). Our data show a significant increase in facilitation at the PF-PC synapse in Eag1-deficient mice in comparison to the wild type. Current clamp recordings in the cell body of granule cells revealed that neither the response to subthreshold current injection nor the evoked firing frequencies and properties of the action potential were altered in $Eag1^{-/-}$cells. This suggests that the events leading to the increased facilitation take place in the synapse itself. Current clamp recordings in the cell body of Eag1 deficient Purkinje cells showed that the membrane potential was less polarized than in the wild type. Upon stimulation by current injection, $Eag1^{-/-}$ cells fired at higher frequency while the action potential properties remained unchanged even at high frequencies. Our results indicate an involvement of Eag1 in synaptic transmission as well as in controlling Purkinje cell activity. Eventually, this could be important for regulation of both motor coordination and learning.

# Examination of the spontaneous activity of circadian pacemaker neurons of the accessory medulla of the cockroach *Leucophaea maderae* with calcium-imaging.

Hongying Wei[1], Monika Stengl[1]

[1]biology, neurobiology, Heinrich Plett Strasse 40, 34132 Kassel, Germany

The accessory medulla (aMe) of the brain´s optic lobes comprises the master circadian pacemaker in the cockroach *Leucophaea maderae*, which controls locomotor activity rhythms. The aMe neurons fire spontaneous action potentials in the absence of external signals, apparently due to endogenous membrane potential oscillations. Calcium ($Ca^{2+}$)-imaging of aMe neurons in primary cell culture measured spontaneous activity as transient rises ($Ca^{2+}$-spikes) of intracellular $Ca^{2+}$-concentrations, which depended on calcium inward currents. In 96% of the aMe neurons addition of the non-selective voltage activated $Ca^{2+}$ channel (VACC) blocker Mibefradil inhibited spontaneous $Ca^{2+}$-spike activity and reduced intracellular $Ca^{2+}$ levels (n=57). Spontaneous activity disappeared in 64% of all aMe cells, after blocking hyperpolarization-activated nonselective cation channels (Ih) with DK-AH 269 (n=39), while 50% lost $Ca^{2+}$-spike activity with addition of the L-type Ca2+ channel blocker nifedipine (n=105). The apamin-sensitive small-conductance calcium-activated potassium channels (SK) were active in about 93% of the neurons (n=41). In contrast to mibefradil, apamin elevated the frequency of $Ca^{2+}$ transients as well as the baseline Ca2+ concentration. The SKs also were necessary for restraining the excitability of the aMe neurons. The iberiotoxin-induced block of the large-conductance calcium-activated potassium channels (BKs) did not significantly affect spontaneous activity of aMe neurons. Increasing extracellular $Ca^{2+}$ concentration from 1µM to 1mM increased the intracellular baseline $Ca^{2+}$ levels. This increase resulted in 41% of the cells in an increase (n=116) and in 15% in a decrease (n=116) of $Ca^{2+}$ spike frequency. Our findings suggest that VACCs and SKs, but not BKs underly the spontaneous activity of the circadian pacemaker neurons in the cockroach, which strongly depends on the extracellular Ca2+ concentration.[Supported by DFG-grant STE531/18-1 and 21-1].

# Extracellular tagging of the voltage-dependent N-type calcium channel

Romy Schneider[1], Johannes Kohl[2], Ulrich Thomas[1], Martin Heine[1]

[1]Leibniz Institute for Neurobiology, Research Group Molecular Physiology, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Division of Neurobiology, MRC Laboratory of Molecular Biology, Cambridge CB2 0QH, UK

The release of neurotransmitter is one of the most important steps in synaptic transmission at chemical synapses. Voltage-dependent calcium channels (VDCCs) are key players in this process. They open in response to membrane depolarization and permit the influx of calcium ions that in turn mediate synaptic vesicle (SV) fusion and neurotransmitter release. Presynaptic VDCCs have to be in close proximity to docking sites for SV and changes of the channel kinetics can modulate synaptic transmission. Similarly, the channel position relative to the docking site may be varied to modulate release. To date there is only experimental evidence from very specialized synapses, like calyx of held, for this hypothesis. Extracellularly tagged VDCCs would allow high-resolution measurements, required to investigate this issue with optical methods. Till now, many attempts failed to create a functional, extracellularly tagged N-type or P/Q-type channel, which can be expressed in neurons and can be used to measure single molecule dynamics. Here we report a functional, extracellularly HA-tagged N-type calcium channel, which is surface expressed in HEK and neuronal cells. The electrophysiological properties of the tagged channel (current voltage relationship, activation and inactivation) were analyzed by using whole-cell patch-clamp recording in HEK cells, with the result that the kinetics of the tagged channel are almost unaffected. In addition, HA antibody binding had only minor effects on the function of the channel. We use this construct to investigate the sub cellular distribution of N-type channels by using immunocytochemistry and high-resolution optical methods.

# Functional characterization of Pannexin1 interaction domains

Nora Prochnow[1], Wiebke Reuter[2], Stefanie Wengel[3], Christina Gründken[4], Rolf Dermietzel[5], Georg Zoidl[6]

[1]Institute of Neuroanatomy and Molecular Brain Research, Department of Anatomy, MA6/147, Universitätsstr. 150, D-44780 Bochum, Germany

In 2003, a novel gene family related to the innexin genes, a family of gap junction forming proteins expressed in invertebrates, was discovered and termed pannexins. In contrast to connexins, the gap junction forming proteins of vertebrates, the prototype protein pannexin 1 (Panx1) forms almost exclusively hemichannels and has been described as a large conductance channel found on the surface of horizontal cell dendrites and in a multitude of mammalian central nervous system (CNS) and non-neuronal tissues.
Using whole cell voltage clamp recordings in transfected N2a cells, we demonstrated that the Panx1 isoform of the zebrafish (zf) forms voltage activated hemichannels with a large unitary conductance in vitro. The findings indicate that zfPanx1 displays properties similar to its mammalian homologues. Since functional studies addressing Panx1 channel properties in different laboratories using various voltage clamp and solution protocols have raised discussions about how to investigate this large conductance channel, and, as a second step, how such investigations can be transferred to native tissues without induction of local or generalized exocytotoxicity, we started to optimize whole cell patch clamp recordings of zfPanx1 hemichannels. Here, we describe the use of N2a cells, overexpressing wild type and mutants of zfPanx1 and compared protocols applied in different laboratories site by site. The outcome of this study is that we were able to narrow down experimental variables and describe a simple, optimized method to elicit Panx1 mediated membrane currents in both cells and native tissue. Furthermore, we used this method for investigation of putative zfPanx1 interaction domains in mutants lacking the kinase binding regions associated with membrane trafficking of the channel forming protein.

# Impaired Development of Auditory Brainstem Nuclei after Loss of $Ca_V1.3$ Calcium Channels: Emphasis on the Lateral Superior Olive

Desiree Griesemer[1], Jan Hirtz[1], Michael Boesen[1], Nadine Braun[1], Florian Kramer[1], Britta Müller[1], Hans Gerd Nothwang[1,2], Jörg Striessnig[3], Stefan Löhrke[1], Eckhard Friauf[1]

[1]University of Kaiserslautern, Animal Physiology Group, Department of Biology, D-67653 Kaiserslautern, Germany
[2]University of Oldenburg, Institute for Biology and Environmental Sciences, Department of Neurogenetics, D-26129 Oldenburg, Germany
[3]University of Innsbruck, Institute of Pharmacy, Pharmacology and Toxicology, A-6020 Innsbruck, Austria

Within the $Ca_V1$ family of voltage-gated calcium channels, $Ca_V1.2$ and $Ca_V1.3$ channels are the predominant subtypes in the central nervous system. Specific functions for each subtype were described in the adult brain, yet their role in the developing brain is still very poorly understood. Here we have addressed the issue of activity-dependent development in the auditory brainstem of $Ca_V1.3$ subunit-deficient ($Ca_V1.3^{-/-}$) mice. Our study was prompted by the fact that $Ca_V1.3^{-/-}$ mice lack cochlea-driven activity (Platzer et al., 2000, Cell). Thus their auditory centers are deprived from peripheral input. We started with a quantitative morphometric analysis and found a drastically reduced volume in all auditory brainstem centers (by 25-59%) which became manifest by postnatal day 12, i.e. before hearing onset. The degree of volume decreases was disproportionately high compared to the whole $Ca_V1.3^{-/-}$ brainstem which shrunk by 24%. The lateral superior olive (LSO), a key nucleus in the medullary brainstem, was strikingly malformed in $Ca_V1.3^{-/-}$ mice and had significantly fewer neurons (ca. 500 or 1/3 less). We analyzed the remaining LSO neurons by employing both morphological and electrophysiological means. These neurons displayed normal dendritic trees and received functional glutamatergic input, yet they fired action potentials predominantly with a multiple pattern upon depolarization with rectangular current pulses, in contrast to the single firing pattern prevalent in controls. The latter finding appears to be due to a reduction of low-threshold, dendrodotoxin-sensitive potassium conductances, which we observed in $Ca_V1.3^{-/-}$ LSO neurons. Taken together, our results imply that functional $Ca_V1.3$ channels are indispensable for brainstem development and particularly necessary in the auditory system. We propose that the unique LSO phenotype in $Ca_V1.3^{-/-}$ mice, which hitherto was not described in other hereditary deafness models (e.g. dn/dn mice, otoferlin mice, prosaposin mice) is caused by the synergistic contribution of two factors: on-site loss of $Ca_V1.3$ channels in the neurons plus lack of peripheral input.

# Input-Resistance Dependent Switch in Spiking Precision of Neocortical Pyramidal Cells

Clemens Boucsein[1], Julian Ammer[2], Ad Aertsen[1], Jan Benda[2]

[1]University of Freiburg, Faculty of Biology, Neurobiology and Biophysics, Schaenzlestrasse 1, 79104 Freiburg, Germany[2]Ludwig-Maximilians-University Munich, Department Biology II, Division of Neurobiology, Munich, Germany

The temporal precision with which action potentials are generated strongly influences network activity dynamics and has far-reaching implications for coding schemes utilized in the brain. From their spiking dynamics, the majority of neurons can be assigned to one of two classes: fast spiking cells that precisely follow synaptic input and show properties of a resonator, and regular spiking cells which respond with much less temporal precision to incoming signals, but faithfully translate the integrated amount of input into a wide range of firing rates.

Here, we tested principal cells of the neocortex for their ability to respond precisely to slightly supra-threshold, transient inputs. In contrast to their counterparts in the hippocampus, neocortical pyramidal cells locked precisely to short current pulses, even though they are generally classified as regular spiking cells or integrators. Surprisingly, however, most neocorical pyramidal cells switched to temporally imprecise spiking dynamics when depolarized towards the spike threshold. Pharmacological blocking experiments and artificial changes of leak conductance via dynamic clamp revealed that this switch in spiking dynamics can be explained by a change in input resistance, rather than by properties of specific voltage gated channels. Simulations and phase-plane analysis of neuron models revealed that neocortical pyramidal cells are readily switched from resonators to integrators by membrane resistance changes well within the physiologically plausible range.

Taken together, our findings implicate that neocortical cells can operate in any one of two distinct working regimes, with qualitatively different spiking dynamics. A switch between these two regimes may occur through any slow modulatory mechanism that causes moderate changes in input resistance.

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# Ionic current modulations of honeybee mushroom body and antennal lobe neurons

Sophie Ziegler-Himmelreich[1], Bernd Grünewald[1]

[1]Goethe University Frankfurt, Institut of Cell- and Neurobiology, Siesmayer 70, 60322 Frankfurt, Germany

Two neuropil structures in the insect brain are known to be involved in odor learning and memory formation - the antennal lobes (AL) and the mushroom bodies (MB). Pharmacological and electrophysiological investigations in several species showed that MB intrinsic Kenyon cells (KC) and antennal lobe (AL) neurons carry ionotropic, excitatory acetylcholine (ACh) and inhibitory γ-aminobutyric acid (GABA) receptors. Furthermore, a metabotropic octopamine receptor is known as a membrane compartment of these cells from the honeybee brain. Coincident activations of these receptors are supposed to be one mechanisms of cellular plasticity underlying classical conditioning.

To investigate the consequences of coincident activations of these receptors we performed patch-clamp recordings to measure transmitter-evoked $Na^+$, $Ca^{2+}$ and $Cl^-$ currents in cultured pupal KC and AL cells and their modulation by biogenic amines. Using $Ca^{2+}$-imaging techniques we investigated the interactions and modulatory effects of ACh, GABA and different biogenic amines like octopamine on the intracellular $Ca^{2+}$-signal. During imaging experiments, $Ca^{2+}$ transients were evoked in AL neurons and KC by pressure applications of 100-500µM acetylcholine.

Coincident applications of GABA (500µM-1mM) as well as forskoline (10µM) reduced the amplitudes of the ACh-induced $Ca^{2+}$-signals. By contrast octopamine (10µM) almost completely abolished $Ca^{2+}$ transients in AL cells and KC. During patch-clamp recordings ionic currents were induced by applications of ACh or GABA. Co-applications of ACh and GABA modulated transmitter -induced cationic or $Cl^-$ currents. Applications of octopamine or other biogenic amines also modulated ACh- and GABA-evoked membrane currents. These findings indicated that learning-related plasticity occur both in AL neurons and MB KCs. In further experiments we plan to pharmacologically verify our present results using blockers and agonists of ACh and GABA receptors.

# Knockdown of the 18 kDa translocator protein (TSPO) in glial cell lines inhibits cell death induced by glutamate, Abeta(1-42) and nitric oxide (NO). Implications for neurodegeneration.

Leo Veenman[1], Julia Bode[2], Lena Fridkin[1], Sivan Zeno[1], Luba Shargorodsky[1], Michal Gaitner[1], Evgeny Levin[1], Avraham Weizman[3], Silke KIetz[2], Max Lakomek[2], Moshe Gavish[1]

[1]Technion - Israel Institute of Technology, Faculty of Medicine, Ephron Street, P.O.B. 9649, Haifa 31096 Bat Galim, Israel
[2]Abteilung Pädiatrie I, Zentrum Kinderheilkunde und Jugendmedizin, Universitätsmedizin Göttingen, D-37075 Göttingen, Germany
[3]Laboratory of Biological Psychiatry, Felsenstein Medical Center, Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv 69978, Israel.

Various studies have suggested that the 18 kDa mitochondrial Translocator Protein (TSPO) may take part in cell death associated with brain damage, as for example occuring with disease and injury. In the brain, TSPO is primarily present in glial cells, including astroglia. We have found that TSPO knockdown by genetic manipulation prevents apoptosis otherwise induced by the synthetic TSPO ligand FGIN -1-27, in cells of astrocytic origin (rat C6 glioblastoma cell line). Other synthetic TSPO ligands (PK 11195 and Ro5 4864) can prevent cell death of C6 cells and also of human cells of astrocytic origin (the U118MG cell line). TSPO knockdown in C6 cells also prevents collapse of the mitochondrial membrane potential, an initiating step for the mitochondrial apoptosis pathway, and the same is true for the U118MG cell line. Generation of reactive oxygen species (ROS) and depletion of ATP appears to be part of apoptosis induction by activated TSPO, implicating ATP(synth)ase, as found for the U118MG cell line. Our data also show that astrocytic cell death appears to be a major component of kainic acid induced brain damage in rats. The brain damage is further characterized by enhanced TSPO expression and microglia activation. In cell culture, TSPO knockdown can prevent cell death of C6 glioblastoma cells of rat astrocytic origin otherwise induced by glutamate. This is also true for U118MG cells. Nitric oxide (NO) is thought to take part in glutamate induced cell death. In cell culture of U118MG, we found that TSPO knockdown by genetic manipulation prevents cell death induced by NO. TSPO knockdown in U118MG cells also prevents astrocytic cell death otherwise induced by Abeta(1-42), an active agent in Alzheimer's disease. Furthermore, with microarray assays we showed that TSPO knockdown affects gene expression of glutamate receptors, glumate transporters, and enzymes of glutamate metabolism. Interestingly, TSPO knockdown appeared to allow for accelerated metabolism of glutamate. Thus, our various experiments show that the TSPO appears to be involved in various mechanisms potentially related to glutamate induced cell death. We chose to investigate astrocytic type cells, as astrocytes are important for the maintenance of normal brain functions and their impairment negatively affects neuronal survival. Our data suggest that astrocytic TSPO may be involved in astrocytic cell death, which may have implications for our understanding of neurodegenerative disease and brain trauma.

# Modulation of recombinant P/Q-type calcium currents by Aß globulomer - an automated analysis using the Patchliner

David Hermann[1], Ali Haythornthwaite[2], Mario Mezler[1], Gerhard Gross[1], Hans Schoemaker[1], Ulrich Ebert[1], Heinz Hillen[1], Stefan Barghorn[1], Karsten Wicke[1], Niels Fertig[2], Andreas Draguhn[3], Volker Nimmrich[1]

[1]Abbott GmbH & Co KG, Pharmacology, Knollstr, 67061 Ludwigshafen, Germany
[2]Nanion Technologies GmbH, Germany
[3]Institute for Physiology and Pathophysiology, University of Heidelberg, Germany

Oligomeric ß-amyloid (Aß) has been shown to impair synaptic transmission and is therefore thought to contribute to cognitive malfunctioning in Alzheimer's disease. We recently introduced a stable oligomer preparation, $Aß_{1-42}$ globulomer, which suppresses synaptic vesicle release by modulation of the P/Q -type calcium current in hippocampal neurons. $Aß_{1-42}$ globulomer also alters those currents in HEK293 cells as well as Xenopus oocytes recombinantly expressing the P/Q-type calcium channel. A search for compounds reversing such pathology in a high-content fashion requires automated patch clamp analysis for the determination of state - and frequency dependence. This was so far hampered by the fact that stable calcium currents in recombinant cell lines are notoriously difficult to obtain in automated systems. In addition, the automated processing of Aß oligomers has not yet been established.

Here we present the development of an automated analysis of P/Q currents and the modulation thereof by $Aß_{1-42}$ globulomer. The inducible HEK293 P/Q cell line was tested on a 4-channel Patchliner capable of recording from 4 cells simultaneously. Cells could be captured to the patch clamp aperture with high success and GO seals were routinely achieved. Introducing BAPTA into the internal solution almost completely abolished current rundown, enabeling the analysis of compounds. The concentration-response curve of ω-agatoxin IVA, a selective blocker of P and Q-type calcium channels, revealed an estimated $IC_{50}$ of 800 nM, comparable to conventional patch clamp analysis. Roscovitine, an enhancer of P/Q calcium currents, prolonged the tail current response, which is in good agreement with data from conventional recordings in literature. $Aß_{1-42}$ globulomer (820 nM) shifted the I/V curve and increased the amplitude of P/Q responses at a voltage step to -10 mV. Aß monomer had no effect and also did not shift the I/V curve.

In summary, the inducible P/Q cell line could be stably recorded in an automated patch system, and exhibited pharmacological responses comparable to conventional recordings. The possibility to investigate the effect of toxic Aß oligomers on modulation of P/Q channels was demonstrated. Such automated analysis of Aß-induced P/Q channel modulation will provide the basis for a large-scale characterization of compounds interfering with the Aß pathology, enabeling the development of novel therapeutics.

# $Na_V1.9$ regulates axon growth in cultured embryonic motoneurons

Robert Blum[1], Narayan Subramanian[1], Benjamin Dombert[1], Steven Havlicek[1], Andrea Wetzel[1], Sibylle Jablonka[1], Michael Sendtner[1]

[1]Universitätsklinikum der Julius-Maximilians-Universität, Institut für Klinische Neurobiologie, Würzburg, Germany

Spontaneous $Ca^{2+}$ transients in embryonic neurons have a critical function in neuronal growth and differentiation. In animal models of spinal muscular atrophy (SMA), motoneurons exhibit reduced axon elongation. Studies from our group showed that local spontaneous excitability in axonal growth cones of cultured embryonic motoneurons correlates with axon elongation (Jablonka et al., 2007). Motoneurons deficient in the Survival motoneuron (Smn)–gene exhibit severe defects in clustering $Ca_V2.2$ channels in axonal growth cones. These defects correlate with reduced frequency of local $Ca^{2+}$ transients (Jablonka et al., 2007) in axons and axon terminals. However, the upstream mechanisms underlying activity-dependent axon growth are not well understood.
Here we asked whether local spontaneous activity of sodium channels is the source of activity-dependent axon growth and spontaneous neuronal excitability. Indeed, low concentrations of the sodium channel antagonist saxitoxin (STX) caused a significant reduction in axon length of cultured motoneurons. STX was 5 to 10-fold more potent than the sodium channel blocker tetrodotoxin (TTX). Both toxins did neither affect motoneuron survival nor dendritic elongation. In addition, low concentrations of STX reduced the rate and amplitude of local $Ca^{2+}$ transients seen in axons of motoneurons. Motoneurons prepared form TrkB kinase knockout mice showed reduced axon elongation and treatment with STX had no additional growth inhibiting effect. This observation was reminiscent of data by Blum et al. (2002). These authors described that TrkB-dependent activation of $Na_V1.9$, a tetrodotoxin (TTX)-resistant member of the family of voltage-gated sodium channels, is involved in activity-dependent growth of young hippocampal neurons. This led us assume that the tetrodotoxin-resistant voltage-gated sodium channel $Na_V1.9$ is involved in spontaneous excitation during axonal growth. qRT-PCR experiments verified the abundant expression of $Na_V1.9$ transcripts in motoneurons. $Na_V1.9$ protein was detected in axonal swellings and axonal growth cones. In these regions, $Na_V1.9$ and TrkB receptors were in close proximity. This localization correlates with the spatial origin of local spontaneous activity. The genetic targeting of $Na_V1.9$ with lentivirally expressed shRNA caused reduced axon growth while axon-length of motoneurons expressing control constructs was not affected. This identifies $Na_V1.9$ as an upstream factor of growth-mediating spontaneous $Ca^{2+}$ transients in embryonic motoneurons before synapse formation. $Na_V1.9$ might serve as an attractive activator for axon regeneration under pathophysiological conditions. The functional base of $Na_V1.9$ activation remains to be studied. Here, activated TrkB receptors might form a functional link.

Jablonka, S. et al. J Cell Biol 179 (2007).
Blum, R. et al. Nature 419 (2002).

# Protein expression of the chloride transporters NKCC1, KCC2 and KCC4 in the auditory brainstem of chicken during embryonic development

Tobias Ackels[1], Hermann Wagner[1], Marcus J. Wirth[1]

[1]RWTH Aachen University, Department of Zoology and Animal Physiology, Mies-van-der-Rohe-Straße 15, 52056 Aachen, Germany

In most adult neurons the intracellular chloride concentration is lower than outside of the cell. Therefore, activation of $GABA_A$ receptors causes chloride ions to enter the cell and hyperpolarize the cell membrane. In many developing neurons, however, the reversal potential for chloride is higher than the membrane potential. This leads to a GABA-induced efflux of chloride and depolarization of the cell membrane, which is crucial for cell growth and development. Important regulators of chloride homeostasis are the transporters of the SLC12 family. Presumably, the outward directed potassium-chloride-cotransporter 2 (KCC2) is responsible for low intracellular chloride levels. The inward directed sodium-potassium-chloride-cotransporter 1 (NKCC1) accumulates chloride ions in the cell.

Neurons in the auditory brainstem of birds maintain an elevated intracellular chloride concentration even after hatching and show a depolarising effect of GABA. The GABAergic input to Nucleus laminaris (NL) and Nucleus magnocellularis (NM) from the Nucleus olivaris superior (SON) elicits a depolarisation, but is nevertheless inhibitory due to shunting inhibition. The physiological relevance of this effect is to increase the accuracy of sound localization.

First, we analyzed the mRNA expression of several members of the SLC12 family during development in the chick auditory brainstem and compared it with the chick optic tectum. These experiments showed that the expression of NKCC1, KCC2 and KCC4 is highly regulated throughout embryonic development. NKCC1 showed a stronger upregulation in the auditory brainstem than in the tectum. The strongest upregulation was found for KCC2. An increase in mRNA-level occurred in both brain areas with the highest expression in tectum at stage P1 (post natal day 1). For KCC4 a moderate upregulation was found in both tissue samples. The analysis of other transporters showed no significant changes in their expression pattern.

In a second step we studied protein expression in the auditory brainstem. Protein lysates of embryonic stages E7 (embryonic day 7), E9, E12, E15, E18 and P1 were separated with SDS-PAGE and analyzed with immunoblotting. For the detection of specific chloride transporters in the chick different antibodies had to be tested, and their specificity was assured by pre-absorption with immunogenic peptides of the chloride transporters. In this poster we present developmental expression profiles of the three transporters NKCC1, KCC2 and KCC4.

# Quantification of mRNA expression of chloride transporters in auditory brainstem of developing chicken

Marcus J. Wirth[1], Andreas Kriebel[1], Jörg Mey[1], Hermann Wagner[1]

[1]RWTH Aachen University, Department of Zoology and Animal Physiology, Mies-van-der-Rohe-Straße 15, 52056 Aachen, Germany

In most brain regions of adult vertebrates GABAergic inhibition is based on hyperpolarisation. Chloride ions enter the neurons via $GABA_A$ receptor channels following the electrochemical gradient, caused by the potassium-chloride-cotransporter 2 (KCC2) a member of SLC12 ion transporter family. Early in development, however, the electrochemical gradient for chloride is reversed in many neurons. Thus, the activation of $GABA_A$ receptors induces a depolarisation because $Cl^-$ ions leave the neurons. The sodium -potassium-chloride-cotransporter 1 (NKCC1) can cause high intracellular $Cl^-$ levels. In addition to NKCC1 and KCC2, other SLC12 $Cl^-$-transporters as well as anion exchangers like AE3 can take part in $Cl^-$homeostasis.
In the auditory brainstem of chicken, the depolarising effect of GABA persists after hatching. Nucleus laminaris and Nucleus magnocellularis receive GABAergic input from the Nucleus olivaris superior. This input is depolarising but inhibitory due to a shunting inhibition, which is relevant for sound localization based on the processing of binaural synaptic input. Neurons in the Nuclei laminaris and magnocellularis therefore seem to have increased intracellular $Cl^-$ levels compared to other brain regions. While the molecular basis for this difference is still unknown, a differential activity of $Cl^-$ transporters is likely. We therefore analyzed the expression patterns of several members of the SLC12 family (NKCC1, NKCC2, KCC1, KCC2, KCC4, NCC, CCC6, CCC9) and AE3 at developmental stages E7, E10, E12, E15, E17, and P1 with real-time PCR in the auditory brainstem. The optic tectum was investigated as a control tissue.
Results can be divided in three developmental patterns: 1) Absence of expression: Transcripts of NCC, NKCC2 and CCC9 were not detected in auditory brainstem or tectum, whereas expression could be found in kidney. No homological sequence was found in chicken for the mammalian KCC3 gene, indicating that this transporter may be absent in chicken. 2) Constant expression: KCC1, CCC6 and AE3 were expressed in auditory brainstem and optic tectum, but no significant regulation during development was found. 3) Developmental increase of expression: NKCC1, KCC2 and KCC4 were significantly regulated during development. With time KCC4 expression showed a moderate upregulation in both brain areas. NKCC1 expression increased as well, and the upregulation was higher in auditory brainstem than in tectum. The strongest upregulation of expression was found for KCC2 in the tectum shortly after hatching. While no time-dependent decrease in expression was measured for any of the $Cl^-$-transporters, the net effect of outward- versus inward-transporting activities may be inferred from the relative KCC2/NKCC1 expression ratios. A comparison of both brain areas revealed a 2-fold higher expression of the KCC2 at all times in the auditory brainstem, whereas in the tectum, the KCC2/NKCC1 ratio increased to 8 at the time after hatching. We suggest that a strong excess of KCC2 mRNA expression is necessary to reach a $Cl^-$ reversal potential lower than the membrane resting potential.

# Regional differences in regulation of glutamergic signaling during Cuprizone induced demyelination

Abolfazl Azami Tameh[1], Tim Clarner[2], Cordian Beyer[2], Markus Kipp [2]

[1]Kashan University of Medical Sciences, Anatomy, 5th of Qotb –e Ravandi Blvd, 87155.111 Kashan, Iran
[2]Institute of Neuroanatomy, Faculty of Medicine, RWTH Aachen University, Wendlingweg 2, D-52074 Aachen, Germany

Glutamate induced excitotoxicity has been associated with a wide range of acute and chronic neurodegenerative disorders. This phenomenon seems to be also involved in the pathology and repair of demyelinating disorders, such as multiple sclerosis (MS). Cuprizone toxic demyelination model represents a reversible demyelination and remyelination system which resembles characteristics of MS. In this study, regulation of glutamergic signaling during cuprizone-induced demyelination in the white and grey matter of mouse brain was analyzed.
Demyelination was induced by feeding 8-week-old male C57BL/6 mice a diet containing 0.2% cuprizone. Complex regulation of ionotropic and metabotropic receptor subunits as well as glutamate transporters was analyzed by Affymetrix gen-chip array analysis and followed up by real-time PCR and western-blot analysis. Cellular localization of glutamate transporters was analyzed by fluorescence double-labeling.
Among ionotropic glutamate receptors the expression of N -methyl-d-aspartate NMDA -receptor subunits were differentially regulated in white and gray matter. Glutamate receptor ionotropic N-methyl-d-aspartate subunit 2A (GRIN2A) was up regulated in both areas while GRIN2B and GRIN3B were up regulated in white and gray matter respectively ($p<0.05$). Regulation of glutamate receptor metabotropic 1 (GRM1) and GRM2 subunits was region specific. They were down regulated only in gray matter ($p<0.01$). Among glutamate transporters, solute carrier family 1 member 3 (SLC1A3) was highly expressed in white matter both on mRNA and protein level and was co-localized mostly with GFAP+ astrocytes.
The presented data indicate that different glutamate receptors and transporters are altered differently in gray and white matter during cuprizone -induced demyelination which could help pharmacological intervention with glutamate signaling in order to improve MS symptoms and outcome.

# Regulation of HCN1 subcellular trafficking may involve N-terminal interaction with sorting nexins

Wiebke Wilkars[1], Evita Mohr[1], Roland Bender[1]

[1]Universitätsklinikum Hamburg-Eppendorf, Institut für Anatomie I, Martinistraße 52, 20246 Hamburg, Germany

Rationale: HCN1 channels are important regulators of the neuronal membrane potential in cortical neurons. Depending on the type of neuron expressing these channels, they can be active at either dendritic, somatic or axonal locations. The mechanisms that govern this neuron-type-specific trafficking to different compartments are currently unknown. Here we examined whether - similar to beta-subunits of related potassium channels - specific binding proteins associated with the HCN1 N-terminus could play a role in the regulation of HCN channel trafficking.

Methods: To search for N-terminal interaction partners of HCN1, we performed a yeast-two-hybrid (Y2H) analysis using a human brain cDNA library as "prey" and the N-terminal 133 aa of HCN1 as "bait". Positive clones were further analysed using biochemical and molecular biological methods.

Results: The Y2H-analysis revealed ca. 30 cDNAs that interacted with the HCN1 N-terminus. Several of these cDNAs coded for proteins which are part of the sorting machinery of the cell. We chose sorting nexin 3 (SNX3) for further analysis. Our data so far suggest that SNX3 is robustly expressed in cortex and hippocampus, and that it is capable to interact with HCN1.

Conclusions: Interaction with sorting nexins is increasingly recognized to be an important determinant of subcellular localization and fate of membrane-bound proteins. Our data indicate that a specific sorting nexin - SNX3 - may play such a role for HCN1 channels.

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# Role of axonal $Na_V1.6$ $Na^+$ channels in action potential generation in Layer 5 neocortical neurons

Ilya A. Fleidervish[1], Efrat Katz[2], Anja Scheller[3], Miriam Meisler[7], Sandra Göbbels[3], Michael J. Gutnick[2], Frank Kirchhoff[3,4], Fred Wolf[5,6]

[1]Dept. of Physiology, Faculty of Health Sci., Ben-Gurion Univ., Beer-Sheva 84105, Israel
[2]Koret Sch. of Vet. Med., Hebrew Univ., Rehovot 76100, Israel
[3]Dept. of Neurogen., Max Planck Inst. of Exp. Med., Gottingen, Germany
[4]Dept. of Mol. Physiol., Univ. of Saarland, Homburg, Germany
[5]Res. Group Theor. Neurophys., Max Planck Inst. for Dynamics, Gottingen, Germany
[6]Bernstein Cntr. for Comput. Neurosci., Gottingen, Germany
[7]Dept. of Human Genetics, Univ. of Michigan, Ann Arbor, United States

In the axon initial segment (AIS) of the neocortical pyramidal cell, the dominant subtype of Na+ channel is Nav1.6, which has been implicated in generation of persistent Na+ current (INaP), is suspected to be essential for setting action potential (AP) threshold and may define the site of AP initiation in the AIS. If Nav1.6 is indeed responsible for INaP, this current should be reduced in Nav1.6 null animals. Moreover, if it is critically important for positioning AP initiation in the AIS, cortical spikes in such animals should lack signatures of axonal AP initiation: rapid onset and biphasic wave form.
Conclusive testing of these hypotheses has been impeded because Nav1.6 null mice do not live more than 3 weeks. We therefore generated a Cre/loxP mouse with selective ablation of the encoding SCNA8a gene in excitatory neurons of the forebrain, including cortical pyramidal cells. We crossbred heterozygous NEX-Cre mice with homozygously floxed SCNA8a mice. Absence of Nav1.6 from pyramidal neuron AIS was verified by immunostaining; the mutant mice were viable through adulthood.
Current clamp recordings from Layer 5 pyramidal cells in cortical slices from P20-P30 mice revealed a significant rise in current and voltage thresholds for AP generation in the NEX-Cre+/-xSCNA8afl/fl mutants as compared to wild type littermates. Nav1.6 deletion also significantly compromised the ability to spike repetitively during prolonged depolarization. High-speed SBFI fluorescence Na+ imaging revealed a significant reduction in AIS Na+ flux associated with AP generation and with prolonged subthreshold somatic depolarization. In voltage clamp recordings, the magnitude of INaP generated by a slow ramp was smaller in the AIS of mutant mice than in wild type animals. However, the activation was still leftward shifted as compare with the persistent current in the soma. We conclude that Nav1.6 is, in fact, critically important for setting AP threshold and underlies the generation of a substantial fraction of INaP. Interestingly, rapid onset and biphasic waveform of pyramidal neuron APs were unaffected by Nav1.6 ablation, indicating either that these features are not valid indicators of axonal AP initiation, or that Nav1.6 is not critically important for positioning the AP initiation site in the AIS.
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# Somatic sodium channels account for second phase of action potential upstroke in soma of layer 5 pyramidal cells

Andreas Neef[1], Fred Wolf[1,2], Michael J Gutnick[3], Ilya A Fleidervish[4]

[1]MPI Dynamics and Self-Organization, Nonlinear Dynamics, Bunsenstr. 10, 37073 Goettingen, Germany
[2]BCCN Goettingen
[3]Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot, Israel
[4]Department of Physiology, Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel

Mechanisms of action potential (AP) generation in neocortical pyramidal cells have been the focus of intense experimental and theoretical research over the last several decades. It has proven very difficult, however, to arrive at a consensus model which can satisfactorily account for all of its features. One of the still unresolved issues is lack of accurate description of $Na^+$ channel kinetics in different neuronal compartments. Here, we measured kinetics of somatic $Na^+$ channels using high temporal resolution (5-10 kHz, -3dB, low pass four-pole Bessel filter) cell-attached recordings from layer 5 pyramidal neurons in neocortical slices. The data were described by fitting different Markov models with differential evolution fit algorithms. The limited speed of voltage steps and the effect of current filtering were accounted for in the fit procedure. Activation kinetics was best described by Markov models with two activating gates, while inactivation was best described as a process that runs in parallel to activation. The best model described the channel data well enough to allow quantitative prediction of the somatic $Na^+$ current during the somatic spike. To this end the AP waveform recorded in current clamp in the same preparation, was used to drive Na+ channels in the model. The resulting simulated current matched the second phase of the AP upstroke in the phase plot (dV/dt vs V). This is consistent with the long standing idea that somatic $Na^+$ channels are the main current sink during this second phase of the AP upstroke but contribute little to its initial phase.

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# The life time of the desensitized state of glutamate receptors

Anna Lisa Carbone[1], Andrew J. Plested[1,2]

[1]FMP Berlin, Molecular Neuroscience and Biophysics, Berlin, Germany

[2]Neurocure, Charité Universitätsmedezin, Berlin, Germany

Glutamate receptor ion channels (GluRs) mediate excitatory neurotransmission at fast chemical synapses in the brain. Glutamate receptor subtypes are named for their selective agonists: NMDA, AMPA and Kainate. Although GluRs share a common architecture, the different subtypes have distinct kinetic properties. For example, the recovery of AMPA receptors from desensitization is two orders of magnitude faster than that of kainate receptors. Despite careful study (e.g. Weston et al., 2006), the molecular determinants of these differences remain unclear.

We generated two chimeras with reciprocal exchanges between the ligand binding domains of an AMPA receptor subunit, GluA2 (Q, flip), and that of a kainate receptor subunit, GluK2. The chimera containing the binding domain of the GluA2 receptor in a GluK2 background was named B2P6 (Binding 2, Pore 6); the reverse chimera was named B6P2. We expressed the chimeras in HEK-293 cells and studied their properties in outside-out patches using a fast perfusion system at 23°C.

Both chimeras exhibited rapid activation and desensitization like that of wild-type channels. The B2P6 chimera had $k_{des}$ = 74 ± 3 $s^{-1}$ (n = 7 patches), slightly slower than wild-type GluA2 ($k_{des}$ =148 ± 12 $s^{-1}$; Plested and Mayer, 2009). The reverse chimera, B6P2, desensitized faster, with $k_{des}$ = 350 ± 10 $s^{-1}$ (n = 5), similar to the reported data for GluK2 (170 ± 20 $s^{-1}$; Plested and Mayer, 2007).

Strikingly, rates of recovery from desensitization were fully exchanged between the two chimeras, being entirely determined by the identity of the ligand binding core. The B2P6 chimera recovered rapidly, $k_{rec}$ = 64 ± 6 $s^{-1}$ (n = 4 patches), even faster than wild-type GluA2 (28 ± 3 $s^{-1}$; Plested and Mayer, 2009), and nearly 200-fold faster than for GluK2 (krec = 0.38 ± 0.07 $s^{-1}$ Plested and Mayer, 2007). The B6P2 chimera recovered very slowly, with $k_{rec}$ = 0.7 ± 0.1 $s^{-1}$. This rate was very similar to the recovery of wild-type GluK2, and 40-fold slower than that of wild-type GluA2.

The apparent affinity for glutamate at the B2P6 chimera was 350 ± 70 µM (n = 3), the same as for GluA2 wild-type channels when desensitization is blocked (300 µM; Zhang et al., 2006). The glutamate EC50 for the B6P2 chimera was 680 ± 140 µM (n = 3), similar to reported values for GluK2 (500 µM; Traynelis and Wahl, 1997). Activation by selective agonists and ion sensitivity were also exchanged between the chimeras, suggesting that the neurotransmitter binding sites and the active dimer arrangement were preserved in chimeras.

These results show that the kinetic differences between AMPA and kainate receptors arise mainly from the ligand binding cores.

# The role of KCNQ channels in the thalamus

Manuela Cerina[1], Philippe Coulon[2], Hans-Christian Pape[3], Thomas Budde[4]

[1]Westfälische Wilhelms-Universität Münster, Instiut für Physiologie I, Robert-koch-Straße 27a, 48149 Münster, Germany
[2]Westfälische Wilhelms-Universität Münster, Instiut für Physiologie I, Robert-koch-Straße 27a, 48149 Münster, Germany
[3]Westfälische Wilhelms-Universität Münster, Instiut für Physiologie I, Robert-koch-Straße 27a, 48149 Münster, Germany
[4]Westfälische Wilhelms-Universität Münster, Instiut für Physiologie I, Robert-koch-Straße 27a, 48149 Münster, Germany

KCNQ channels are low-threshold potassium channels which are known to be composed of subunits of the Kv7 K+channel family (Kv7.1-Kv7.5). Four of these (Kv7.2-Kv7.5) are expressed in the nervous system, and Kv7.2 and Kv7.3 are the principal molecular components of the slow voltage-gated M-channels. Since they do not inactivate, they generate a steady voltage-dependent outward current called the M-current. It stabilizes the membrane potential in the presence of depolarizing inputs and may have a key role in regulating the excitability of various central and peripheral neurons.
Previous evidence shows that a mutation in M-channels causes neonatal epilepsy, which emphasizes the importance of this channel in regulating excitability. Given that the dorsal thalamus seems to be a "pacemaker" of certain forms of slow oscillations and it was proposed to be a key determinant of the hypersynchronus activity during absence seizures, the M-channel could be expected to be an important regulator of thalamic oscillations. However, it was widely assumed that M-channels do not play a role in the thalamocortical relay and thus little is known about thalamic M-channels. In addition to their role in absence seizures, the centro-basal thalamic nuclei are also involved in a variety of physiological functions, such as sensory discrimination or transmission and modulation of pain signals.
The present study aims at elucidating the role of M-channels in thalamocortical signalling, in the regulation of the sleep/wake cycle, and in absence seizures. Moreover, it will be investigated whether they are capable of modulating signals along the pain pathway.
M-channels are closed by receptors coupled to Gq, such as M1 and M3 muscarinic receptors, and several endogenous and exogenous compounds can modulate their gating properites. Here, a modulation of these channels is obtained pharmacologically by using the M-channel blocker XE991 which decreases M-current amplitude, and the enhancer Retigabine, a broad-spectrum anticonvulsant and an effective analgesic, which increases this current. Interestingly, this effect seems to be age-dependent, with the current amplitude reaching its peak at P20. Moreover, also muscarinic receptor agonists and antagonists effectively modulate these channels in the thalamus.

# ß4-subunit dependent $Ca^{2+}$ gating of large-conductance voltage- and $Ca^{2+}$- dependent $K^+$ (BKCa) channels

Henrike Berkefeld[1], Bernd Fakler[1]

[1]University of Freiburg, Department of Physiology, Engesserstr.4, 79108 Freiburg, Germany

Cellular excitability is uniquely controlled by large conductance voltage and $Ca^{2+}$ activated $K^+$ (BKCa) channels. Their dependence on voltage as well as $Ca^{2+}$ translates membrane potential and intracellular $Ca^{2+}$ concentration into one hyperpolarising $K^+$ current.

BKCa channels are allosterically gated by both stimuli, meaning that each cellular parameter can trigger activation on its own. Biophysically, voltage-gating has been thoroughly investigated; the influence of constant $[Ca^{2+}]_i$ has been analysed. Reversely, $Ca^{2+}$-gating by fast changes of $[Ca^{2+}]_i$ in the presence of different voltages has not yet been studied systematically.

Here we analyse the $Ca^{2+}$-gating of hetereologously expressed BKCa channels with and without ß4-subunit in inside-out patches of CHO cells by application of varying $Ca^{2+}$ concentrations via a piezo-driven fast application device. $Ca^{2+}$ driven opening shows a concentration dependent delay that slows the opening relative to voltage dependent gating. The ß4 subunit slows activation and deactivation time constants in the voltage as well as in the $Ca^{2+}$ dependent opening regime.

# Poster Topic

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# „Role of the spine apparatus in synaptic transmission – Two-photon Ca2+ imaging combined with glutamate uncaging at individual synapses in organotypic slice cultures”

Anja Tippmann[1], Alexander Drakew[1], Michael Frotscher[1]

[1]Anatomy and Cell Biology, Neuroanatomy, Hansastr. 9a, 79104 Freiburg, Germany

Transient changes in the concentration of free cytoplasmic Ca2+ are assumed to link synaptic excitation to intracellular mechanisms underlying synaptic plasticity. Postsynaptic Ca2+ stores potentially regulate the kinetics of these Ca2+ transients. It has been suggested that the so called spine apparatus represents such a Ca2+ store in dendritic spines. The spine apparatus consists of flat cisterns of endoplasmic reticulum with intervening electron dense plates. The large complex spines of hilar mossy cells and CA3 pyramidal neurons contacted by the mossy fibers typically contain this organelle. We found previously that mutant mice lacking the actin-binding protein Synaptopodin fail to develop spine apparatuses in otherwise unchanged dendritic spines. The absence of Synaptopodin is accompanied by a reduced long term potentiation at the hippocampal CA3-CA1 synapse and an impaired spatial learning performance (Deller et al. 2003).

To test the hypothesis that the spine apparatus contributes to Ca2+ kinetics in synaptic transmission, we compared Ca2+ transients in large complex spines in hippocampal organotypic slice cultures of wild-type mice and Synaptopodin knockout mice. We combined the patch-clamp technique with two-photon Ca2+ imaging and two-photon glutamate uncaging. Local photolysis of the caged compound MNI (4-methoxy-7-nitroindolinyl)-glutamate allowed us to correlate induced excitatory postsynaptic events and the corresponding Ca2+ changes at individual synapses of hilar mossy cells.

We found a remarkable heterogeneity among the elicited signals in complex spines in both wild-type tissue and Synaptopodin knockout tissue. The amplitudes of EPSCs as well as Ca2+ transients varied largely from synapse to synapse. However, induced Ca2+ transients were blocked by the N-methyl D-aspartate (NMDA) receptor antagonist D-AP5, indicating that Ca2+ influx through the NMDA receptor is essential for synaptically evoked Ca2+ transients in large complex spines. We currently analyze to what extent these Ca2+ transients are altered in complex spines of Synaptopodin mutant mice.

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# A role of STIM1 for slow glutamatergic synaptic transmission in cerebellar Purkinje cells

Jana Hartmann[1], Rosa M. Karl[1], Horst A. Henning[1], Anna Ansel[1], Kenji Sakimura[2], Yoshihiro Baba[3], Tomohiro Kurosaki[3], Arthur Konnerth[1]

[1]TU München, Institut für Neurowissenschaften
[2]Niigata University, Brain Research Institute, Japan
[3]RIKEN Research Center for Allergy and Immunology, Yokohama, Japan

Glutamatergic synaptic transmission at parallel fiber synapses of Purkinje cells is mediated by AMPA receptors and the metabotropic glutamate receptor type 1 (mGluR1). In Purkinje cells, mGluR1 is expressed at high levels and is crucial for cerebellar function. Glutamate binding to mGluR1 activates two distinct Gprotein-dependent pathways: calcium release from endoplasmic reticulum (ER) calcium stores and a slow excitatory postsynaptic current (sEPSC). We have previously demonstrated that the *canonical transient receptor potential* (TRPC) cation channel TRPC3 is required for the mGluR1-sEPSP[1]. TRPC3 is located in Purkinje cell spines and contributes to postsynaptic spine calcium signaling by providing a calcium entry pathway downstream of mGluR1 activation (Henning, Hartmann & Konnerth, unpublished). The second messenger activating TRPC3 is unknown. Furthermore, the mechanism by which the ER calcium content is maintained throughout many cycles of synaptic excitation is still a matter of debate for central neurons, including Purkinje cells. In non-excitable cells TRPC channels have been shown to closely associate and interact with the *stromal interaction molecule* 1 (STIM1) and Orai proteins that together are responsible for the replenishment of calcium stores.
We performed a quantitative RT-PCR analysis of STIM1,2 and Orai1-3 expression in the cerebellum and single Purkinje cells. In contrast to other brain regions in which STIM2 is the main STIM[2], we found that in the cerebellum STIM1 is more abundantly expressed than STIM2. In Purkinje cells, the expression level of STIM1 is ten times higher than that of STIM2. Single-cell RT-PCR analysis of Purkinje cells also showed that Orai2 is more abundant in these cells than the other two Orai isoforms. On the protein level, the presence of STIM1 and Orai2 in Purkinje cells was demonstrated by means of immunohistochemistry. To analyze the functions of STIM1, we generated a Purkinje cell-specific STIM1-deficient ($STIM1^{pko}$) mouse line by employing the Cre/*loxP* gene targeting strategy. $STIM1^{pko}$ mice exhibit distinct alterations in their motor coordination when walking on an elevated beam. We studied glutamatergic synaptic signaling at parallel fiber-Purkinje cell synapses in $STIM1^{pko}$ mice by using whole-cell recordings and calcium imaging in acute cerebellar slices. We found that both synaptically-evoked signals downstream of mGluR1 (calcium release and sEPSC) are largely abolished in the absence of STIM1. Similarly, calcium signals and inward currents evoked by the mGluR-specific agonist DHPG were nearly absent in $STIM1^{pko}$ mice. By contrast, the amplitude of AMPA receptor-mediated fast EPSCs in $STIM1^{pko}$ mice in comparison to control mice on average is increased by 30% when similar stimulation parameters are used. Finally, paired-pulse facilitation at the parallel fiber synapse was not affected by the deletion of STIM1 in Purkinje cells indicating a postsynaptic origin of the observed alterations in synaptic responses. Together, our results demonstrate that STIM1 has an essential role in synaptic transmission at parallel fiber synapses of Purkinje cells, by forming a critical link between synaptic activation of mGluR1 and opening of TRPC3 channels.

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# A special form of $Ca^{2+}$-regulated exocytosis: Spontaneous acrosomal secretion is prevented by a CaMKIIa-MUPP1 complex in mammalian spermatozoa

Nele Zitranski[1], Frauke Ackermann[2], Laura Vieweg[1], Heike Borth[1], Thomas Gudermann[1], Ingrid Boekhoff[1]

[1]Ludwig-Maximilians-Universität München, Walther-Straub-Institut für Pharmakologie und Toxikologie, Goethestr. 33, 80336 München, Germany

[2]Karolinska Institute, Department of Neuroscience, SE-171 77 Stockholm, Sweden

$Ca^{2+}$-triggered, regulated exocytosis of vesicles plays a basic role in many signalling processes including hormone secretion and neurotransmitter release. A special form of regulated exocytosis is the sperm acrosome reaction, which bears characteristic functional parallels to $Ca^{2+}$-regulated exocytosis in neurons. It fulfils two key functions ensuring success in mammalian fertilization: first, the release of hydrolyzing enzymes is necessary for the penetration of the egg`s glycoprotein matrix, and second, the externalization of membrane components is required for gamete fusion. We have recently demonstrated that the Multi-PDZ-domain protein MUPP1, which contains 13 PDZ motifs, is involved in the initial tethering and/or docking between the acrosomal vesicle and the target plasma membrane. We now investigated whether cognate neuronal binding partners of MUPP1 are also expressed in spermatozoa, and whether they contribute to the regulation of acrosomal secretion. Our results show that a CaMKIIa co-localizes with MUPP1 in the acrosomal region of epididymal spermatozoa and comigrates with MUPP1 in cortex- and sperm-derived detergent resistant membrane fractions. Pulldown experiments with GST-constructs of MUPP1 covering all PDZ domains confirmed an interaction of testicular and sperm-derived CaMKIIa with PDZ 10/11, which was abolished by adding $Ca^{2+}$/calmodulin. Furthermore, we found that inhibiting the catalytically active kinase in epididymal spermatozoa by pretreating mouse sperm with the CaMKII inhibitor AIPII, as well as a competitive displacement of CaMKIIa from PDZ domains 10/11 led to a significant increase in spontaneous acrosomal exocytosis rate. These results imply that a CaMKIIa/MUPP1 complex is involved in arresting the acrosomal fusion machinery, hence preserving an intact acrosome until the sperm contacts the oocyte. In this scenario, MUPP1 might represent a central signaling platform for regulating the assembly/disassembly of binding partners pertinent for acrosomal secretion, thereby precisely adjusting the increase in $Ca^{2+}$ to synchronized fusion pore formation.

# AKAP79/150 and Caldenrin – a new relationship in the synapse

Xenia Gorny[1], Marina Mikhaylova[2], Björn Schott[1], Michael Kreutz[2], Constanze Seidenbecher[3]

[1]Leibniz Institute for Neurobiology, Dept. Behavioural Neurology, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Leibniz Institute for Neurobiology, PG Neuroplasticity, Brenneckestr. 6, 39118 Magdeburg, Germany
[3]Leibniz Institute for Neurobiology, Dept. Neurochemistry, Brenneckestr. 6, 39118 Magdeburg, Germany

AKAP79/150 is a multi-adaptor protein in the postsynaptic density (PSD). Due to its binding to the kinases PKA and PKC, the phosphatase PP2B/CaN, scaffolding proteins such as SAP97 and PSD-95, as well as the beta2-adrenergic receptor, and the calcium sensor calmodulin (CaM), AKAP79/150 is likely to play a key role as an important molecular interface in signal transduction.
Calcium is critically involved in synaptic signalling, which is mediated not only by CaM but also by the calcium-binding protein caldendrin.
Using purified proteins in GST pulldown assays we found caldendrin as a second calcium-binding protein that interacts directly with AKAP79/150. Furthermore, selective colocalization of recombinant AKAP79 and caldendrin in Cos7 cells and endogenous AKAP150 and caldendrin in rat primary neurons could be shown.
Unlike the ubiquitously expressed CaM, caldendrin is almost exclusively expressed in the nervous system and highly enriched in the PSD. Caldendrin shares large similarity with CaM in its C-terminal part containing four EF-hand motifs. It differs from CaM in having a larger hinge region between both EF hand pairs and a distinct molecular surface. Furthermore, the N-terminal part of caldendrin has no similarity to other known proteins.
One example of caldendrin functioning in signal transduction is its calcium-dependent binding to Jacob, a neuronal protein triggering the cAMP response element-binding protein (CREB) shut-off pathway upon translocation to the nucleus after activation of NMDA-type glutamate receptors.
Although the CaM binding sequence in AKAP79 is not sufficient for binding to caldendrin, it overlaps with the caldendrin binding region so that both calcium sensor proteins compete for the bindung of AKAP79/150 under calcium conditions. However, caldendrin interaction with AKAP79/150 is less calcium-dependent than binding of CaM to AKAP79/150. Therefore both interactions might play distinct roles in signal transduction in the synapse.

# An extracellular stimulation protocol to detect barrel vs. septal regions in acute slice preparations of the rat barrel cortex

Rembrandt Bakker[1], Martijn Selten[1], Moritz Negwer[1], Dirk Schubert[1]

[1]Radboud UMC Nijmegen, Donders Inst. for Brain, Cognition and Behaviour, P.O. Box 9101, 6500 HB Nijmegen, Netherlands

*Background/Aims*

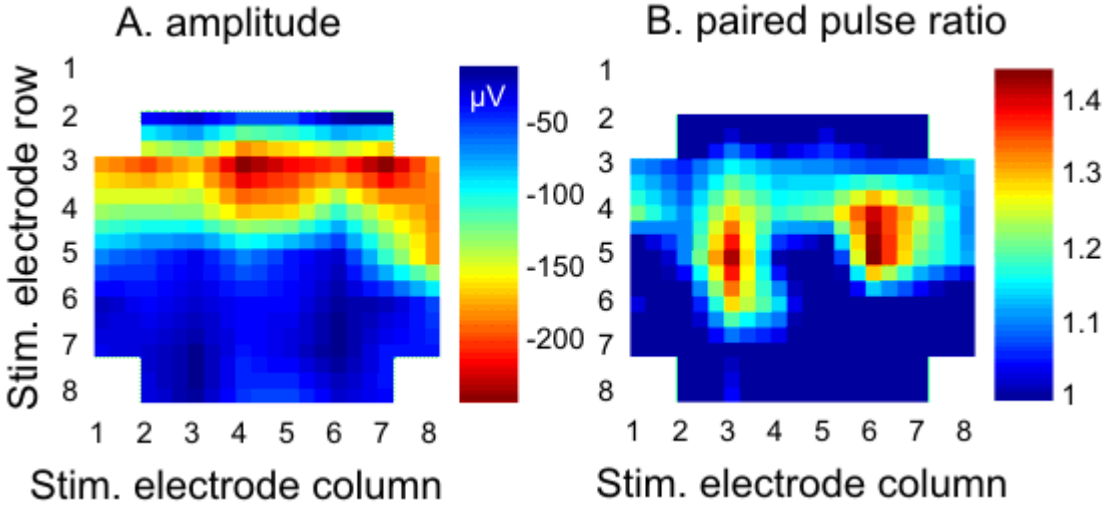
The stimulus-response behavior of acute slice preparations of the rat barrel cortex provides valuable insights in the organization, plasticity and fault-tolerance of the primary somatosensory (barrel) cortex. With commercially available micro-electrode-arrays (MEAs), this response behavior can be measured at a resolution of 50-400 µm. If the MEA is used both for recording and stimulation, the same resolution applies to the stimulus location. Our aim is to develop a stimulation and data analysis strategy which achieves maximum separation between the barrel fields and the septal regions in between.

*Methods & Analysis*

We use a 60 electrode chip (8x8, empty corners; MultiChannelSystems, Reutlingen, Germany). We stimulate all 60 electrodes one by one, in a sequence which maximizes the physical distance between subsequent stimulations. All stimuli are applied as pulse pairs with 50 ms intervals, to obtain a measure for short-termm plasticity. The stimulus sequence is fully automated, applied with 10s intervals. The stimulus shape varied between experiments, good results were obtained with a -800mV/+400mV 2x 300 µs bipolar square pulses. This stimulus protocol results in 60 datasets, each containing 1 stimulus channel and 59 response channels. To reduce this vast dataset to an 8x8 matrix revealing barrels, we consider only the response channel just above the stimulated electrode, and compute two statistical measures: (1) The 10th-percentile of the first 50 ms after stimulus, as a robust estimate of the peak depth (Amplitude). (2) The ratio between the peak depths of the paired stimuli (Paired Pulse Ratio or PPR).

*Results and conclusion*

Fig. 1 shows the results for an example slice. It is not a surprise that the signal amplitude (Fig. 1A) responds more strongly to stimuli within a barrel column than outside of it. The yellow-red patches in Fig. 1A do not highlight the barrels themselves, but rather synaptic activity in layers II/III of the same functional column. This is in agreement with Bakker et. al. [Neural Networks, 2009 22(8):1159-68] who report that strong responses in layers II/III follow stimulation of deeper layers. A very interesting result of our analysis is Fig. 1B, which shows that short-term plasticity is particularly prominent in the septal regions. Further analysis is under way to establish the robustness and underlying cause of this finding.



# Architecture of the extracellular matrix at the nanoscale

Gilbert Werner Walter Franken[1], Oliver Kobler[1], Renato Frischknecht[1], Constanze Seidenbecher[1]

[1]IfN, Neurochemistry, Brenneckestr. 6, 39118 Magdeburg, Germany

Neurons of the central nervous system (CNS) are embedded in an extracellular matrix (ECM). Specializations of this matrix, the perineuronal nets (PNNs), surround the soma and proximal dendrites of subpopulations of neurons. Previous studies have shown that PNNs consist of chondroitin sulfate proteoglycans (CSPGs), glycoproteins and hyaluronan. Many of studies could show molecular interactions between brain extracellular matrix molecules which led to a model how they might be structured. So far it was not possible to visualize the distribution of the matrix proteins in living cells or tissue beyond the spatial resolution limit of commonly used lens-based (far-field) fluorescence microscopy, which is limited by diffraction about half of the wavelength of light: $\lambda/2 \sim 200$ nm. To overcome this problem we used stimulated emission depletion (STED) microscopy. Two-color STED allows us to observe the spatial relationship between two matrix components like brevican or aggrecan, or the matrix and the synaptic or glial subcompartments at the nanoscale. Accordingly it is also possible to observe developmental changes of the ECM during its maturation.

# Aspartate decarboxylase Black: expression and co-localization with Ebony in *Drosophila* brain

Bernhard Hovemann[1], Anna Ziegler[1]

[1]Ruhr-Universität Bochum, Fakultät für Chemie und Biochemie, Universitätsstrasse 150, 44780 Bochum, Germany[1]

The visual neurotransmitter histamine is formed by Histidine Decarboxylase in photoreceptor cells. It cycles between photoreceptor axons and associated glia. Released histamine is taken up into glia and converted by Ebony into the inactive derivative ß-alanyl histamine (carcinine) [1]. Carcinine shuttles back into axonal endings where it is split into its components ß-alanine and histamine by Tan. Histamine is taken up by synaptic vesicles whereas ß-alanine might shuttle back into glia. Black, which forms ß-alanine by decarboxylation of aspartate, is the putative source of ß-alanine supply [2].
A similar interaction between Ebony and Tan is evident in cuticle. It gives rise to a stable proportion between dopamine and ß-alanyl dopamine. The amount of free dopamine has a direct impact on cuticle melanization. Enhanced cuticle melanization of mutations in both, ebony and black, disclose a defect in ß-alanyl dopamine formation because accumulating dopamine is fed into extensive melanin production [3].
On the contrary, a comparable co-operation between Ebony and Black in carcinine formation in the eye could until now not be demonstrated. Whereas ebony and tan mutations reveal a visual phenotype in electroretinogram (ERG) recordings by missing "on"- and "off"-transients, black flies show wildtype ERGs [2,4]. This raised the question how the supply of ß-alanine for Ebony activity in the eye is facilitated and in which way, if at all, Black function is involved visual signal transduction.
Previously, two protein (DGAD2) variants had been predicted by Philips et al. from the black gene, which when mutated revealed reduction of aspartate decarboxylase activity [2]. Transcripts of black were localized to lamina glia which also expresses Ebony.
Our aim was to investigate in detail substrate specificity and biochemical activity of the Black protein. We also determined the expression pattern of alternative ß-alanine forming enzymes in the brain. Because a co-expression pattern of Black and Ebony beyond lamina glia could give hints as to a putative co-operation we performed immunocytochemical localization of Black vs. Ebony. The obtained evidence provides clues towards the function of Black in the nervous system and particularly in visual signal transduction of Drosophila.

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# Can beta-alanyl histamine synthase Ebony keep up co-operatively with fast neurotransmitter transport into glia in the Drosophila eye?

Silvia Hartwig[1], Bernhard T. Hovemann[1]

[1]Ruhr-Universität Bochum, AG Hovemann, Universitätsstr. 150, 44780 Bochum, Germany

In the visual system of arthropods histamine is the neurotransmitter for stimulus transmission from photoreceptor cells to postsynaptic neurons. In Drosophila melanogaster exists a recycling pathway for histamine in which the enzymes Ebony and Tan and unidentified transporters co-operate: Released histamine is sequestered into glia where it is inactivated by Ebony -catalyzed fusion with ß-alanine to yield beta-alanyl histamine (carcinine). After transfer into photoreceptor axons carcinine is cleaved by Tan into histamine and beta-alanine. Histamine is taken up into synaptic vesicles whereas beta-alanine might shuttle back into glia.
Histamine release at the synaptic cleft is tonic. To convert tiny changes of photoreceptor depolarization into a postsynaptic signal, removal and inactivation of histamine must be fast. We anticipate that histamine transport into glia by an unidentified transporter is a fast process. To avoid accumulation of histamine at the glial side Ebony inactivates it by conversion into carcinine. We have recently shown that cleavage of carcinine after transport into photoreceptor axon is a process whose reaction velocity is adjusted by carcinine substrate concentration. An unresolved key stone of our working model is that Ebony is able to catalyze the inactivation of histamine with the required speed.
Ebony is related to Nonribosomal Peptide Syntases (NRPS), which operate in a two-step reaction mechanism: 1.) ATP dependent activation and concomitant binding of the first substrate as 4'phosphonpantetheinyl thioesther followed by 2.) Nucleophilic attack by the second substrate and peptide bond formation. The first step would correspond to beta-alanine activation and binding as thioesther by Ebony. The kinetic parameters of this reaction have been determined by us and others. They appeared to be slow and are not appropriate to co-operate in fast histamine conversion.
To validate the co-operative function of Ebony and a histamine transporter in a fast inactivation process we propose the following model: Ebony is loaded with beta -alanine in glia in a slow process leading to a concentration of activated enzyme that is sufficient to react with peak amounts of histamine. The second step, formation of carcinine, is a fast reaction that must keep up with transport velocity.
Investigations are hampered by instability of Ebony and the difficulty in preparing the required amounts of 4'phosphopantetheinylated holo-Ebony. Here, we show expression and purification of this protein in Baculovirus system and preliminary kinetic data.

# Comparative functional characterization of putative Synaptotagmin-binding interfaces in SNAP-25

Ralf Mohrmann[1], Heidi de Wit[2], Emma Connell[3], Bazbek Davletov[3], Matthijs Verhage[2], Jakob B Sørensen[4]

[1]Universität des Saarlandes, Abt. Physiology, Geb. 59, 2. OG, 66421 Homburg, Germany

[2]Center for Neurogenomics and Cognitive Research, Dept. of Functional Genomics, Vrije Universiteit Amsterdam and VU medical Center, Amsterdam, The Netherlands

[3]MRC Laboratory of Molecular Biology, Hills Road, Cambridge, UK

[4]University of Copenhagen, Dept. of Neuroscience and Pharmacology, Faculty of Health Sciences, Copenhagen, Denmark

SNARE complexes involved in exocytosis possess multiple negative surface charges that possibly allow for an attachment of regulatory factors. In line with this idea, it has been proposed that SNARE-complex interaction with synaptotagmin-1 (syt- 1) could be mediated by negatively charged residues in SNAP-25. Confusingly, several distinct groups of amino acids in SNAP-25 were reported to be essential for Syt-1 binding. One proposed group exists within the C-terminal SNARE-domain (D172/D179/D186/D193) of SNAP-25 (Zhang et al. Neuron. 2002, 3:599-611), while another site extends around Layer 0 (D51/E52/E55) in the first SNARE-domain (Rickman et al., Mol Biol Cell 2006, 17:283-294). A third potential group may consist of D166/E170 (slightly N-terminal to Layer 0), as we have previously shown that E170 is required for fast release. To investigate the nature of the Syt-1 binding interface, we introduced alanine-substitutions at all three putative binding sites and comparatively characterized the phenotypes of the resulting mutant variants. SNAP-25 variants were expressed in Snap-25 deficient chromaffin cells, and their ability to support secretion induced by photolysis of caged-$Ca^{2+}$ was assayed by capacitance measurements and amperometric recordings. We found substantial differences in the secretion phenotypes of the three mutant variants, which widely excludes that all proposed sites cooperate in exactly the same mode of syt-1 interaction. Although no variant fully phenocopied the changes seen in syt-1 -/- cells, SNAP-25A D51A/E52A/E55A caused a slowdown of secretion most reminiscent of the syt-1 -/- phenotype. In contrast, SNAP-25A D166A/E170A slowed down release by abolishing the fast burst component, but also dramatically decreased total release. SNAP-25A D172A/D179A/D186A/D193A had no effect on release kinetics and only slightly decreased the fast burst component. Interestingly, the severity of the phenotypes was also dependent on the SNAP-25 isoform (A/B) that the mutations were introduced into. Pull-down assays demonstrated that mutation of the two groups around Layer 0 indeed restricted syt-1 binding, while mutation of the C-terminal site did not decrease affinity towards syt-1. We also analyzed vesicle pool integrity on the ultrastructural level and found that all mutations significantly affected docking of vesicles, which depends on SNAP-25 and syt-1 (De Wit et al., 2009, Cell 138:935-946). This implies that SNAP-25/Syt-1 assisted docking might rely on another mode of interaction between SNAP-25 and syt-1 than fusion triggering. In summary, our preliminary data suggests that negative charges around Layer 0 most likely function as a syt-1 binding interface. Nevertheless, SNARE-syt-1 interactions are potentially more complex and possibly involve multiple interaction modes depending on the specific mechanistic step and the available SNAP-25 isoforms.

# Differential dendritic and somatic input mapping in layer V pyramidal neurons

Mihael Zohar[1], Philipp Schnepel[1,2], Ad Aertsen[1,2], Clemens Boucsein[1,2]

[1]Neurobiology and Biophysics, Faculty of Biology, University of Freiburg, Schänzlestrasse 1, 79104 Freiburg, Germany
[2]Bernstein Center Freiburg, University of Freiburg, Hansastrasse 9a, 79104 Freiburg, Germany

Layer V pyramidal neurons of the rat neocortex play an important role in signal processing, constituting the major output of the cortical volume. They show a very specific morphology including a prominent apical dendrite spanning all layers and terminating in an extended tuft. EPSPs arising from contacts at distal dendrites are strongly attenuated while they propagate to the soma, where they are often hardly detectable. However, recent studies showed that the dendrites of pyramidal neurons are capable of regenerative potential generation like calcium and NMDA spikes, even though the physiological conditions under which they occur and the neuronal populations driving them remain speculative. In contrast to the connectivity of the somata examined with paired recordings, photo stimulation and anatomy, very little is known about the projection patterns onto the different dendrites of the neurons and the contribution of the presynaptic neurons from different layers to the signal integration within these structures.

In the present study, simultaneous dendritic and somatic whole-cell patch-clamp recordings in combination with presynaptic photo stimulation via glutamate un-caging were used to assess the properties and layer dependency of synaptic inputs onto different compartments of layer V pyramidal neurons in acute slices of the rat somatosensory cortex.

With recordings from the apical dendrite it was possible to detect connected presynaptic neurons with horizontal distances of up to 1mm eliciting only small EPSPs which were not detectable at the soma. The obtained functional input maps from the distal dendrite and those obtained from the soma show differences concerning the layer dependency of the connected presynaptic neurons. In addition to the reported input populations onto the somata of layer V pyramidal neurons from other studies located in layer II/III, V and VI, we found the supragranular layers constituting the most prominent input to the distal apical dendrites of layer V neurons. The specific projection pattern of presynaptic neuron populations onto different postsynaptic compartments of layer V pyramidal cells points to a distributed integration of inputs from different layers, which may also have an impact on the generation of regenerative potentials in dendrites. Our findings could help to further understand the effect of dendritic integration, in particular nonlinear mechanisms and the involved neuron populations, on signal integration in layer V neurons.

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# Direct activation of glutamate receptors by local photolysis of caged glutamate in presubicular pyramidal cells and interneurons

Desdemona Fricker[1], Jean Simonnet[1], Michael Bendels[2], Emmanuel Eugene[1], Ivan Cohen[1], Richard Miles[1]

[1]CRICM, INSERM/CNRS/Univ. Paris 6, Pitié-Salpêtrière Hospital, Paris
[2]Charité, Berlin

The presubiculum is a region situated between the subiculum and the entorhinal cortex which appears to have a specific role in the coding of space. Some presubicular cells are sensitive to head direction (Taube et al., 1990), others signal a combination of place and direction of movement (Boccara et al., 2010). While head direction cells have been recorded in the behaving animal, the presubicular microcircuit is less explored on a cellular level. We investigated the morphological and electrophysiological properties of single presubicular neurons (P21 - 35) in the in vitro slice preparation. Cells were recorded in current clamp whole-cell configuration, and filled with biocytin contained in the potassium gluconate based intracellular solution. Neurons were chosen in the superficial layers of presubiculum (n = 58), with somata located in layer II or III. The dendritic arborization of filled neurons could be visualized and 14 cells were completely reconstructed using Neurolucida. Most cells were pyramidal shaped neurons with triangular somata and a principal apical dendrite extending towards the pial surface, while other neurons had stellate or fanned dendrites. The mean resting membrane potential was - 66 ± 8 mV and input resistance was 421 ± 210 MOhms. Action potential threshold was -41 ± 3 mV, and the amplitude was 96 ± 6 mV. The action potential half-width ranged from 0.23 to 1.62 ms, and may be used to distinguish between principal cells and interneurons. Pyramidal cells fired at frequencies up to 60 Hz, while putative interneurons could fire at frequencies up to 162 Hz upon positive current injection. In order to signal a stable head position, presubicular neurons must be able to sustain persistent firing with little adaptation. Low frequency repetitive firing close to threshold was rather irregular and timed by spontaneous EPSPs. Adaptation varied little for different levels of depolarization, and the adaptation index was never smaller than 0.8.

Spot laser photolysis of caged MNI-glutamate was used to study direct activation of glutamate receptors on presubicular neurons. A 2 ms light pulse from a 405 nm laser (~ 5 µm spot size) evoked gluEPSPs at somato-dendritic sites. Responses could be blocked pharmacologically using APV and NBQX. GluEPSPs were amplified and prolonged by plateau potentials when holding membrane potential was depolarized. A grid like stimulation pattern was used to determine the spatial response profile for direct activation of glutamate receptors in different cell types, typically over a radius of 100 µm around the soma. In presubicular layer II and III pyramidal neurons, evoked gluEPSPs were small and always subthreshold. Action potentials could be initiated from gluEPSPs when neurons were depolarized by current injection. In contrast, pyramidal cells in CA1 were easily excited, and action potentials could be initiated from resting membrane potential. Photolytic activation of glutamate receptors in interneurons in both hippocampus and presubiculum led to spike discharge from resting membrane potential for stimulation sites close to soma and proximal dendrites.

# Effects of neurofascin on the scaffolding protein gephyrin at inhibitory synapses

Jennifer Metzger[1], Martin Kriebel[1], Sabine Trinks[1], Hansjürgen Volkmer[1]

[1]Natural and Medical Institute at the University of Tuebingen, Molecular Neurobiology, Markwiesenstr.55, 72770 Reutlingen, Germany

Inhibitory synapses as well as GABAA receptor subunits found at the axon initial segment are involved in different psychiatric disorders like depression and schizophrenia. Therefore, elucidation of mechanisms underlying GABAA receptor, clustering and the implication of the postsynaptic scaffolding component gephyrin is of pivotal importance. We developed a Hela cell based assay for genome-wide siRNA screens to identify novel signaling pathways implicated in the control of gephyrin expression/organization.

Hits obtained suggest a contribution of FGFR1 signaling to the neurofascin dependent gephyrin clustering. The cell adhesion molecule neurofascin is known to interact with the FGFR1 and to also signal via FGFR1 in its other functions like for example neurite outgrowth. Neurofascin is localized at the axon initial segment (AIS) of hippocampal neurons, which are targeted by gabaergic axo-axonic presynaptic input. In addition to FGFR1-specific siRNA, our screen also revealed further downstream components of FGFR-dependent signaling cascades including PI3K as well as MAP kinases. Our results are further confirmed by the finding that neurofascin dependent gephyrin clustering is sensitive to the application of pharmacological inhibitors of the FGFR1 pathway or the overexpression of a dominant-negative mutant of FGFR1.

The relevance of the impact of neurofascin on synaptic structures was further corroborated by lentiviral knockdown of neurofascin in adult rat dentate gyrus which results in a specific reduction of gephyrin clusters at the AIS in vivo. Furthermore there was also a concomitant loss of axo-axonic input represented by GAD65 staining of inhibitory presynaptic structures.

# Effects of SynCAM mediated synapse formation

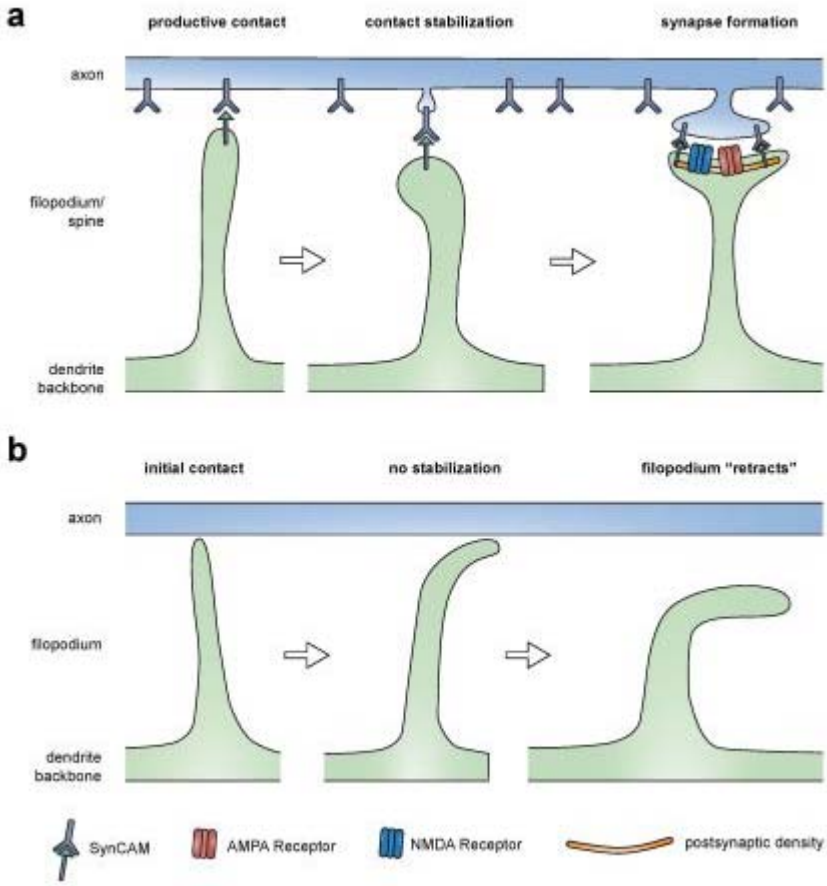
Alexander J. Krupp[1], Elissa M. Robbins[2], Karen Perez de Arce[2], Ananda K. Ghosh[2], Adam I. Fogel[2], Antony Boucard[3], Thomas C. Südhof[3], Thomas Biederer[2], Valentin Stein[1]

[1]Max Planck Institut für Neurobiologie, Synaptic Receptor Trafficking, Am Klopferspitz 18, 82152 Martinsried, Germany
[2]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520, USA
[3]Department of Molecular and Cellular Physiology, Stanford University, Palo Alto, California 94304, USA

Synaptogenesis is required for wiring neuronal circuits in the developing brain, and continues to remodel adult networks. However, the components organizing synapse development in vivo remain incompletely understood. We now demonstrate that the immunoglobulin adhesion molecule SynCAM 1 first drives excitatory synapse formation, then maintains synapse number, and alters synaptic plasticity. Analysis of an inducible transgenic mouse line and knock-out mice demonstrates that elevated SynCAM 1 increases excitatory synapse number, while its loss results in fewer synapses. SynCAM 1 acts subsequent to promoting synapses to maintain this increase in synapse number. Moreover, SynCAM 1 alters synaptic function by regulating long-term depression, a measure of synaptic plasticity. This organization of synapses by SynCAM 1 affects spatial learning, with knock-out mice learning better. The reciprocal effects of SynCAM 1 increase and loss reveal that this adhesion molecule contributes to the regulation of synapse number and plasticity, and impacts how neuronal networks undergo activity-dependent changes.

Despite the significant molecular insights into the synapse-organizing roles of trans-synaptic interactions, three decisive aspects remain insufficiently understood. First, to which extent are synapse numbers regulated by synaptic adhesion in the brain? Second, at which steps during the lifetime of synapses are individual synaptic adhesion molecules engaged? Third, do these synapse-organizing proteins alter synaptic physiology and affect neuronal network functions?

We now demonstrate that SynCAM 1 significantly impacts these three aspects. Combining electrophysiology, electron microscopy, and Golgi staining, we demonstrate that elevated expression of SynCAM 1 in a transgenic mouse model increases functional excitatory synapse number. This activity corresponds to its endogenous role, as SynCAM 1 knock-out (KO) mice exhibit fewer excitatory synapses. Moreover, SynCAM 1 functions dynamically at developing synapses: Using the temporal control afforded by the design of our transgenic model, we show that continued SynCAM 1 elevation is required to maintain the increase in synapse number it drives in the first place. Unexpectedly, SynCAM 1 additionally alters the plasticity of synapses once they are formed, with its overexpression abrogating long-term depression (LTD) and its loss increasing LTD. These complementary changes in synapse number and plasticity correlate with altered cognitive functions, and SynCAM 1 KO mice exhibit improved spatial learning. Our results reveal important contributions of SynCAM 1 to synapse number and function in the developing and adult brain. This supports a model that synaptic adhesion can promote synapse formation and restrict synaptic plasticity to regulate the formation and remodeling of neuronal circuits.


a
productive contact
contact stabilization
synapse formation
axon
filopodium/
spine
dendrite
backbone
b
initial contact
no stabilization
filopodium "retracts"
axon
filopodium
dendrite
backbone
SynCAM
AMPA Receptor
NMDA Receptor
postsynaptic density

# Electrodiffusion in mossy fiber - cerebellar granule cell synapses

Sergiy Sylantyev[1], Leonid SAVTCHENKO[1], Dmitri Rusakov[1]

[1]Institute of Neurology, University College London, DCEE, Queen Square, WC1N 3BG London, United Kingdom[1]

The waveform of rapid synaptic responses determines signal transmission and integration properties in the neural networks. It has long been predicted that intra-cleft diffusion of electrically charged neurotransmitter molecules such as glutamate or acetylcholine should be sensitive to the local electric fields exerted by receptor currents. We have previously shown that this phenomenon may affect the time course of EPSCs at Schaffer collateral - CA1 pyramidal cell synapses in the hippocampus (Sylantyev et al, Science, 2008, 319: 1845). However, how common this phenomenon is amongst central synapses and to what extent it affects integration of synaptic inputs remains poorly understood.

To address these issues in more favorable voltage-clamp conditions, we carried out experiments in cerebellar granule cells, the smallest (and therefore highly electrically compact) neurons in the brain. Firstly, we applied rapid (1 ms) pulses of glutamate to semi-intact granule cells pulled out of the acute cerebellar slice in whole-cell mode and found that the kinetics of the cell AMPA receptors were voltage-independent. In contrast, AMPA receptor mediated EPSCs evoked in these cells in situ by stimulation of mossy fibres were decelerated at positive compared to negative holding voltages, consistent with glutamate electrodiffusion in the cleft. This was also in line with the observation that the fast-dissociating AMPA receptor antagonist γ-DGG suppressed AMPA receptor mediated EPSCs to a greater extent at negative compared to positive voltages.

Next, we investigated summation of synaptic inputs in granule cells evoked by bursts of repetitive stimuli applied to mossy fibers. We found that the time course of summation was affected by the local AMPA receptor current density, in the opposite direction at positive versus negative holding voltages.

Our numerical simulations predict that in synapses equipped with NMDA receptors single action potentials boost mGluRs activation; in turn, there are numerous data proving mGluRs to be a regulator of NMDARs response. Thus, to test experimentally whether electrodiffusion affects mGluRs' response kinetics as it was predicted in our modelling, we monitored conditioning of NMDARs response by mGluRs ligand. First, we evoked NMDAR-mediated responses at positive and negative holding voltage. Subsequent application of MCPG and deduction of an averaged response curve after MCPG treatment from control curve allowed us to separate the mGluRs response. Next, to model the electrodiffusion impact on mGluRs effect we applied a 2 ms voltage step to +50 mV at -70 mV holding voltage and to -50 mV at +40 mV holding voltage 0.5 ms before the electrical stimulation of a neural tissue. As it was predicted in our simulation, the positive voltage step at a negative holding voltage enhanced response of mGluRs, whereas the negative voltage step decreases an mGluRs response amplitude, as it was predicted by our model of electrodiffusion.

Our preliminary data suggest therefore that the influence of electric fields (due to synaptic currents) on the glutamate dwell time inside the synaptic cleft may affect integration properties of synaptic circuits in different areas of the brain.

# Essential cooperation of N-cadherin and neuroligin 1 at glutamatergic synapses

Bernd van Stegen[1], Adriana Stan[1], Kurt Gottmann[1]

[1]Heinrich Heine Universität Düsseldorf, Neuro- und Sinnesphysiologie, Universitätsstraße 1, 40225 Düsseldorf, Germany

Synaptic adhesion molecules such as N-cadherin and Neuroligin1 play major roles in regulating synapse structure and function. At the molecular level, several different families of synaptic adhesion molecules have been described to mediate trans -synaptic signaling. This led to the proposal of a complex cooperation of different types of adhesion molecules that together might control synapse formation.
Using N-cadherin knockout neurons derived from mouse ES cells, we found by immunocytochemistry and live-cell imaging of individual vesicle clusters, that N-cadherin is strongly involved in the accumulation of synaptic vesicles at nascent synapses. Intriguingly, N-cadherin appears to control vesicle accumulation indirectly, because N-cadherin expression alone does not induce presynaptic vesicle clusters. To further address this, we studied the accumulation of presynaptic vesicles that is induced by Neuroligin1 expression in cultured N-cadherin knockout neurons. Strikingly, in the absence of N-cadherin expression Neuroligin1 did not induce additional vesicle clustering. Also blocking N-cadherin function abolished the vesicle clustering activity of transiently expressed Neuroligin1. In addition to its vesicle clustering activity also the synaptic targeting of expressed Neuroligin1 was impaired in the absence of N-cadherin. Most importantly, also the synaptic clustering of endogeneous Neuroligin was reduced in N-cadherin knockout neurons. The N-cadherin and Neuroligin adhesion systems appeared to be linked by postsynaptic scaffolding proteins as indicated by experiments impeding S-SCAM function.
In addition to vesicle accumulation at nascent synapses, the cycling of synaptic vesicles at mature synapses may depend on a cooperation of N-cadherin and Neuroligin1. In line with this hypothesis, expression of a dominant-negative N-cadherin mutant protein impaired the uptake of FM dyes. This indicates an important role of N-cadherin in synaptic vesicle cycling.
In summary, our data support a molecular cooperation model, in which N-cadherin might indirectly control presynaptic vesicle accumulation by targeting and activating postsynaptic Neuroligin1, which ultimately induces presynaptic vesicle clustering via neurexin binding.

# Examining the molecular basis of light adaptation at the photoreceptor ribbon synapse

Michaela Fuchs[1], Johann Helmut Brandstätter[1]

[1]Department of Biology, Animal Physiology, University of Erlangen-Nuremberg, Erlangen, Germany

Photoreceptor ribbon synapses transmit light signals over a wide dynamic range and continuously adjust their synaptic output to changes in stimulus intensity. Such a high level of performance requires a specialized presynapse and complex adaptive mechanisms. From the literature it is well known that the synaptic ribbon is a highly dynamic organelle, which changes its size and shape depending on illumination and thus activity [1,2]. However, the molecular basis of these adaptational changes remains to be elucidated. The aim of this study is to identify the molecules at the photoreceptor ribbon synapse which are involved in the light-dependent, adaptational processes. Using immunocytochemistry, high resolution light and electron microscopy, and laser microdissection in combination with quantitative real-time PCR, we have started to study the expression and localization of presynaptic proteins during the regular 24 h cycle and under various light and dark regimes in the pigmented retina of the C57BL/6 mouse.

Comparing the structure of the ribbon during the regular diurnal cycle – 12/12 light/dark with an average illumination of 200 lux – we found no obvious differences in ribbon length and shape. Even more surprising was the finding that neither a dark nor a light stimulus (200 lux) following a 3 h period of light or dark adaptation, respectively, triggered any significant structural ribbon changes. Only when the retina was exposed to bright light (1000 lux), the outer plexiform layer (OPL) appeared disorganized and the photoreceptor ribbon structure less compact. Electron microscopy confirmed the formation of club-shaped ribbons and spherical ribbon material.

Most of our knowledge about the dynamic changes of the ribbon structure, following different light and dark regimes, originates from studies of the albinotic retina [1,2]. To trigger comparable structural changes at the photoreceptor ribbon synapse of the pigmented C57BL/6 mouse retina, much higher light intensities are required. Thus, it remains to be shown whether the structural changes are indeed physiologically relevant or whether other mechanisms contribute to light adaptation at the photoreceptor ribbon synapse.

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# Functional characterization of the dendritic spines of spiny interneurons

Volker Scheuss[1], Bonhoeffer Tobias[1]

[1]Max Planck Institute of Neurobiology, Cellular and Systems Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

A classical doctrine of neuroscience claims that pyramidal cells are spiny and inhibitory interneurons are aspiny. Nevertheless, spiny inhibitory interneurons have been described e.g. in the hippocampus and cortex. In pyramidal cells, dendritic spines serve both structural and functional roles, which provide the cells with special functionality in neuronal signaling and plasticity. Functionally, spines compartmentalize biochemical signals and contain molecular signaling complexes, which regulate synaptic strength in an activity dependent manner. Structurally, spines serve as substrate for excitatory synapse formation and by growing out from the dendrite by up to ~2 µm increase the choice of passing by axons that synaptic contacts can be formed with. So far, spines of spiny interneurons have been described morphologically but not functionally and it remains to be shown if they have similar characteristics as pyramidal cell spines and thus provide spiny interneurons with similar functionality.
In acute hippocampal slices from transgenic mice expressing eGFP under control of the GAD65 promotor (Lopez-Bendito et al., Cereb Cortex 14:1122-1133, 2004), we identified spiny GFP positive inhibitory interneurons by 2-photon microscopy to characterize their dendritic spines. Mapping the distribution of AMPAR type glutamate receptors on the dendrites of spiny interneurons with focal 2-photon glutamate uncaging yielded a similar distribution of hotspots of glutamate sensitivity as in pyramidal cells located on spines as well as dendritic shafts. This suggests that spines of spiny interneurons receive excitatory synapses, in line with published ultrastructural data showing asymmetric synapses on these spines. GFP fluorescence recovery after photobleaching (FRAP) recordings in interneuron spines gave significantly lower recovery time constants (151 ± 14 ms, mean ± SEM, n = 40 spines, 9 cells) compared to pyramidal cell spines (211 ± 13 ms, mean ± SEM, n = 42 spines, 4 cells). Thus, spines on interneurons can compartmentalize biochemical signals however on average less efficiently than pyramidal cell spines. Recording currents evoked by focal 2-photon glutamate uncaging at individual spines at -70 mV and +40 mV holding potential showed that spiny interneurons express both AMPA and NMDA type glutamate receptors. Furthermore, the current voltage relationship of AMPA type currents recorded with spermine in the patch pipette showed that the expressed AMPA receptors are non- rectifying and thus $Ca^{2+}$ impermeable. Therefore, spiny interneuron spines are likely to display NMDA receptor-dependent plasticity similar to pyramidal cell spines. Preliminary data from perforated patch recordings, suggests that interneuron spines can display functional and morphological plasticity in response to an induction protocol paring 2-photon glutamate uncaging with postsynaptic action potentials.
In summary, dendritic spines of spiny interneurons appear to have similar functional characteristics as pyramidal cell spines. Thus by integrating into neural circuits via dendritic spines like pyramidal cells, spiny interneurons might serve a distinct role in neural function and plasticity compared to aspiny interneurons.

# Heterologous expression of synaptic vesicle membrane protein SV31 generates a putative novel compartment in PC12 cells

Joern Barth[1], Walter Volknandt[1]

[1]Institute for Cell Biology and Neuroscience, Department of Neurochemistry, Max-von-Laue-Str. 9, 60438 Frankfurt, Germany

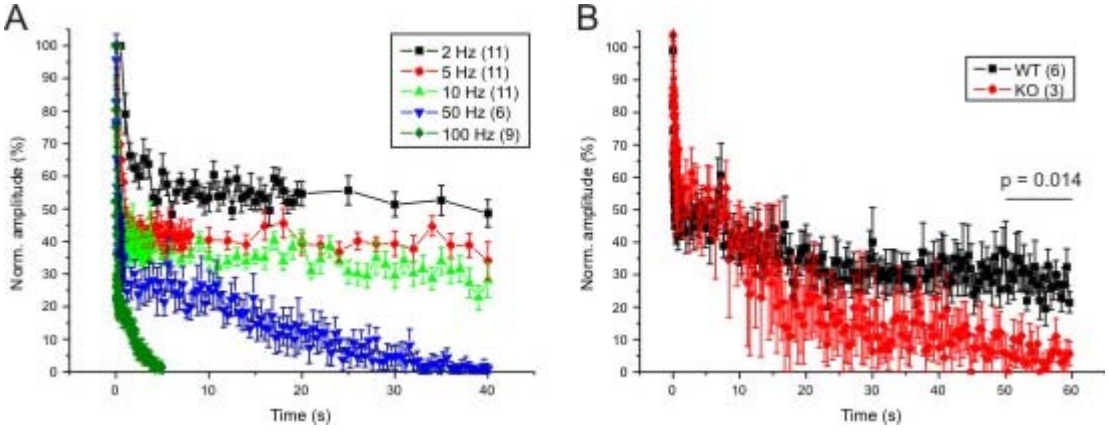
Synaptic vesicle proteins that govern all relevant functions of the synaptic vesicle life cycle like vesicle transport, migration to the presynaptic plasma membrane, uptake and storage of neurotransmitters and regulated endocytosis and exocytosis, have been identified in the last decades by different labs. However, the analysis of the proteome of synaptic vesicles leads to the detection of proteins, which have not been previously assigned to the synaptic vesicle compartment, including several novel protein candidates. One of those is SV31, a synaptic vesicle membrane protein of 31 kDa with six putative transmembrane helices that, according to its membrane topology and phylogenetic relation, may function as a vesicular transporter. Based on amino acid sequence similarities of SV31 to a procariotic $Zn^{2+}$ transporter, we analyzied its metal-ion binding properties and could show that SV31 is capable to bind the divalent cations $Zn^{2+}$ and $Ni^{2+}$, but not $Fe^{2+}$, $Co^{2+}$, $Mn^{2+}$, and $Ca^{2+}$.
Analysis of the subcellular distribution of recombinant red fluorescent SV31 fusion protein in NGF-differentiated PC12 cells indicates a sorting to a putative novel compartment. These data suggest that the integral synaptic vesicle protein SV31 is present in subpopulations of synaptic vesicles and represents a novel cation binding protein and a putative transporter.

# High fidelity transmission at inhibitory auditory synapses and the role of the glycine uptake transporter GlyT2

Florian Kramer[1], Désirée Griesemer[1], Eckhard Friauf[1]

[1]University of Kaiserslautern, Animal Physiology Group, Erwin Schroedinger Str.13, 67663 Kaiserslautern, Germany

Inhibitory synaptic activity, mediated by glycine, is crucial for many aspects of acoustic information processing in the mammalian auditory brainstem. Neurons forming the inhibitory projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) can follow high frequency inputs for computation of sound localization. It is known that the MNTB can follow glutamate-mediated input rates of up to 800 Hz (Wu and Kelly, Hear Res, 1993; Taschenberger, J Neurosci, 2000). This system is ideally suited to analyze high fidelity transmission in inhibitory synapses, which, in contrast to excitatory inputs, have not been investigated so far. Here, we have assessed the fidelity of the MNTB-LSO connection in acute brain slices from mice at P10-12. Focal electrical stimulation in the MNTB was combined with patch-clamp recordings of LSO neurons at room temperature (22-25°C), and stimulation frequencies were increased from 1 Hz to 100 Hz. Up to a frequency of 10 Hz, stimulation was transmitted with 100% fidelity, i.e. each presynaptic stimulus resulted in an IPSC. However, the IPSC amplitudes decreased during a stimulus train, reaching different steady state levels after 5 s (2 Hz: 55%; 5 Hz: 40%; 10 Hz: 30% of the initial amplitude; Fig. 1A). During 50 Hz and 100 Hz stimulus trains, the IPSC amplitudes decreased to zero within 40 s and 5 s, respectively. The complete breakdown, which is equivalent to 0% fidelity, is in striking contrast to the high fidelity value described for the inputs to the MNTB (at 34 °C). To assess the temperature dependency in the glycinergic transmission, we performed subsequent experiments at 37°C. At 50 Hz, IPSC amplitudes also decreased, yet never declined below 20% within 60 s. The mean value, occurring between 50-60 s, was significantly higher than the value obtained at room temperature (21.4±4.4%, n = 16 vs. 0.5±0.2% n = 6; $p < 0.001$). These results suggested that the amount of glycine that is continually released upon high frequency stimulation is temperature-dependent and that an enzyme-based mechanism participates in the supply. We hypothesized that GlyT2, the neuronal glycine transporter which removes the transmitter molecules from the synaptic cleft (Gomeza et al., 2003), is involved and helps to replenish the transmitter supply. To test this hypothesis, we recorded MNTB-LSO IPSCs in GlyT2-/- mice (50 Hz, 37°C, 60 s). They declined with time, more rapidly than in the wild-type controls, and tended to reach significantly lower steady state levels after 50-60 s (Fig. 1B; 5.2±12.5%, n = 3 vs. 28.4±4.6%, n = 6, p = 0.014). We are currently complementing our genetic approach with pharmacological experiments, in which the selective GlyT2 antagonist ALX-1393 is applied to the bath solution. In summary, our results obtained so far indicate that the fidelity of inhibitory transmission from the MNTB to the LSO appears to be of lower quality than the excitatory transmission to the MNTB. However, a final conclusion awaits a thorough analysis of the stimulation experiments at 37°C and at higher frequencies than 50 Hz. Concerning the role of GlyT2, it appears that its absence results in a lower glycine amount in the presynaptic MNTB vesicles. The supply of glycine, however, appears to be independent of GlyT2 during the initial phase of stimulus trains (ca. 15 s), and GlyT2 transport activity becomes crucially important for glycine homeostasis only when continuous stimulation exceeds 30 s.



# How does proteoglycan deficiency affect the protein composition of ECM and synapses in the mouse brain?

Nora John[1], Karl-Heinz Smalla[1], Eckart D. Gundelfinger[1], Constanze I. Seidenbecher[1]

[1]Leibniz Institute for Neurobiology, Neurochemistry & Molecular Biology, Brenneckestraße 6, 39118 Magdeburg, Germany

Chondroitin sulfate proteoglycans like brevican and neurocan are important constituents of brain extracellular matrix. Together with link proteins, tenascins and hyaluronic acid they form a macromolecular meshwork of perineuronal nets, which surround and insulate nerve cells and synaptic contact sites. Investigations with knock out mice deficient for one or both proteins show multiple changes in physiological and morphological features. For example the loss of neurocan leads to a dysplastic phenotype of the CA1 pyramidal cell layer in the early development of single and double knock out mice. The loss of brevican and / or neurocan changes the amount and distribution of extracellular matrix components and affects proteins, which are influenced by the extracellular matrix. We conduct biochemical studies with defined subcellular fractions to study changes in protein patterns. Our investigations lead to a complex interplay of proteins in the extracellular matrix environment of mouse forebrain synapses.

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# Impact of Pannexin1 activity on single cell postsynaptic response properties in the CA1 region of the mouse

Julian Hanske[1], Amr Abdulazim[1], Nora Prochnow[1], Georg Zoidl[1]

[1]Institute of Anatomy, Departement of Neuroanatomy and Molecular Brain Research, Universitätsstr. 150, D-44780 Bochum, Germany

Panx1 channels have been revealed to be large-pore ion channels broadly distributed in the central nervous system (CNS).
The expression of Panx1 at postsynaptic sites in the hippocampus in co-localization to the postsynaptic density protein 95 (PSD-95) initially indicated that Panx1 may contribute to hippocampal synaptic transmission. Previously it has been shown that Panx1 modulates field excitatory postsynaptic potentials (fEPSP) and early long-term potentiation (LTP) (0-5 min) of hippocampal CA1 neurons in the mouse.
These findings are in accordance to the insights that Panx1 channels are opened by prior activation of N-methyl-D-aspartate receptors (NMDARs) and the capability of Panx1 channels to conduct adenosine triphosphate (ATP) which is putatively involved in retrograde presynaptical inhibitory and interneuronal signaling.
The aim of this study was to verifiy the previously described role of Panx1 in modulation of hippocampal excitatory postsynaptic response on a single cell level.
Therefore, we used a transgenic Panx1 knock out (KO) mouse for whole-cell patch clamp recordings in hippocampal CA1 neurons of acutely dissected mouse hippocampal slices in vitro. Different pharmacological conditions were induced to characterize the hippocampal postsynaptic response properties of Panx1.
Inhibition of Panx1 by Mefloquine lead to a decrease in membrane currents. This decrease was lacking in Panx1 KO mice, indicating the Panx1 specifity of the current.
Evoked inhibitory postsynaptic currents (IPSCs) could be blocked by the application of either Suramin or Mefloquine.
These results confirm the previously described putative inhibitory modulation of excitatory postsynaptic hippocampal potentials by Panx1 channels and emphazises on the potential brain protective property of Panx1 channels against excitotxicity.

# Impaired transmission at corticothalamic excitatory inputs and intrathalamic GABAergic synapses in the thalamus of heterozygous BDNF knockout mice

Thomas Laudes[1], Thomas Munsch[1], Volkmar Leßmann[1]

[1]Otto-von-Guericke Universität Magdeburg, Institut für Physiologie, Leipziger Str. 44, 39120 Magdeburg, Germany

Beside its role in development and maturation of synapses, brain-derived neurotrophic factor (BDNF) is suggested to play a critical role in modulation and plasticity of glutamatergic as well as GABAergic synaptic transmission. Acute or chronic application of BDNF has been shown to enhance glutamatergic transmission in in vitro slice preparations, whereas GABAergic transmission is mostly impaired under these conditions. In heterozygous BDNF knockout (BDNF+/-) mice which chronically lack BDNF, an impairment of glutamatergic and GABAergic transmission was found in the visual cortex (Abidin et al., 2006, 2008). More recently, a BDNF-dependent modulation of glutamatergic synaptic transmission in anterior thalamic nuclei has been demonstrated (Tsanov et al., 2009).

Since corticothalamic inputs are of critical importance for rhythmic activity of the thalamocortical network, we started investigating the role of BDNF in excitatory transmission at glutamatergic synapses onto relay (TC) neurones of the ventrobasal complex (VB) of the thalamus. Intrathalamic inhibitory transmission was characterized at GABAergic synapses between neurones of the reticular thalamic nucleus (RTN) and TC neurones in BDNF+/- mice. Horizontal slices of wild-typ (WT) and BDNF+/- mice were cut at 250 µm thickness. Spontaneous and miniature synaptic responses were studied in whole-cell voltage-clamp mode.

Recordings in TC neurones revealed a strong reduction in the frequency of mEPSCs from 0.95 ± 0.24 Hz, n = 12 in WT to 0.3 ± 0.05 Hz, n = 19 in BDNF+/- mice, whereas the amplitude (WT: 28.46 ± 2.03 pA; BDNF+/-: 25.24 ± 0.81 pA) was not significantly different between genotypes. For mIPSCs recorded in TC neurones, both, frequency (WT: 6.06 ± 0.6 Hz, n = 28; BDNF+/-: 4.13 ± 0.54 Hz, n = 17) as well as amplitude (WT: 24.43 ± 0.75 pA; BDNF+/-: 21.75 ± 0.54 pA) showed a significant reduction in knockout animals. Recordings of sEPSCs in TC cells confirmed a significant reduction in the frequency (WT: 0.79 ± 0.12 Hz, n = 15; BDNF+/-: 0.46 ± 0.07 Hz, n = 13) and the amplitude (WT: 24.45 ± 1.22 pA; BDNF+/-: 20.01 ± 0.38 pA) of synaptic events in BDNF+/- mice, as compared to WT littermates. Consistently, sIPSCs of TC neurones also showed a significant reduction in frequency (WT: 3.91 ± 0.51 Hz, n = 18; BDNF+/-: 2.71 ± 0.28 Hz, n = 19) but not in amplitude (WT: 29.53 ± 1.61 pA; BDNF+/ -: 29.29 ± 1.33 pA) between genotypes. Preliminary results of IPSCs evoked by stimulation of intrathalamic GABAergic inputs onto TC neurones (eIPSCs) indicated a reduced amplitude (WT: 1231 ± 150.9 pA, n = 11; BDNF+/-: 800.3 ± 123.8 pA, n = 10), but an unchanged decay time constant (WT: 38.62 ± 4.25 ms; BDNF+/-: 29.06 ± 3.36 ms) of eIPSCs in knockout animals.

The present findings suggest that a chronically reduced level of BDNF to ~50 % in heterozygous knock-out animals, strongly affects glutamatergic and GABAergic synaptic transmission in thalamic circuits. We hypothesise that this impairment of excitatory and inhibitory transmission may have profound consequences for the generation of rhythmical activity in the thalamocortical network.

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# Inactivation of ADF and n-cofilin enhances neuronal excitability

Andreas Görlich[1], Anika-Maria Zimmermann[1], Michael Wolf[1], Marco Sassoé-Pognetto[2], Eckhard Friauf[3], Walter Witke[4], Marco Rust[1]

[1]Neurobiology/Neurophysiology Group, University of Kaiserslautern, Germany
[2]Department of Anatomy, Pharmacology and Forensic Medicine and National Institute of Neuroscience-Italy
[3]Animal Physiology Group, University of Kaiserslautern, Germany
[4]Institute of Genetics, University of Bonn, Germany

We have previously shown that n-cofilin, a member of the ADF/cofilin family of actin depolymerizing proteins, is critical for a number of postsynaptic mechanisms in CA1 pyramidal cells such as the control of dendritic spine density and morphology, long-term potentiation (LTP) and depression (LTD) or AMPA receptor mobility. As a consequence, mice with a forebrain-specific inactivation of n-cofilin performed significantly weaker in several paradigms of associative learning. While n-cofilin activity is highly important for postsynaptic physiology, presynaptic mechanisms were unchanged in the absence of n-cofilin. Interestingly, the expression of the close n-cofilin-homolog ADF (actin depolymerizing factor) was found up-regulated in n-cofilin-deficient tissue – a finding that suggests functional redundancy of both ADF/cofilin proteins. To test this hypothesis and to further elucidate the synaptic function of ADF and n-cofilin, we analyzed CA1 pyramidal cells of mutant mice that lack either ADF alone or both ADF/cofilin proteins by means of electrophysiological, morphological and biochemical approaches. While the defects in ADF mutant mice were only minor, we found drastically impaired presynaptic function in double mutant mice.

# Information coding via action potentials and graded signals in the fly's visual system.

Diana Rien[1], Rafael Kurtz[1]

[1]Bielefeld University, Neurobiology, 100131, 33501 Bielefeld, Germany

There are several possible mechanisms that allow sensory information to be propagated in neuronal networks. The conventional view of signal processing is that graded dendritic changes of membrane potential are converted into the digital pattern of spikes. However, from the study of invertebrates it is known that graded voltage changes can also serve as neuronal output signals. Recent research on cortical neurons provides evidence that graded shifts in the membrane potential as well as the action potential waveform might play a larger role than previously thought in modifying the neuronal output information.
A good model system to study synaptic integration in vivo is the visual system of the blowfly Calliphora vicina. In their third visual neuropile 60 so-called Lobula plate tangential cells (LPTCs) can be distinguished. These LPTCs are sensitive to motion in a directional selective way. This allows us to investigate directly how sensory information input from the fly's eyes is processed and transmitted to other brain areas.
In this study we recorded extracellularly from an identified motion-sensitive neuron and simultaneously intracellularly from elements of its presynaptic cell ensemble. Both graded voltage changes and action potentials have been shown to contribute to signal transmission from one and the same presynaptic neuron to its postsynaptic target. To distinguish between action potentials and graded changes in membrane potential in synaptic transmission we applied the voltage-clamp-controlled current-clamp (VCcCC) technique to the presynaptic cell. This technique suppresses the slow membrane potential changes without affecting faster voltage responses.
Our results revealed that the overall activity of the postsynaptic cell is controlled both by the graded voltage and by the spikes of a presynaptic neuron. Background voltage also influences the timing between pre- and postsynaptic spikes. This effect is most probably indirect because hyperpolarisation increased the amplitude of presynaptic spikes, which led to a shortening in the latency for spike initiation in the postsynaptic neuron.

# Localization of GFP-tagged synaptic proteins by photooxidation electron microscopy

Thomas Dresbach[1], Sabine Angermüller[2], Joachim Kirsch[2], Nina Wittenmayer[1,2]

[1]University of Goettingen Medical School, Center of Anatomy, Dept of Anatomy and Embryology, Kreuzbergring 36, D-37075 Goettingen, Germany

[2]University of Heidelberg, Institute for Anatomy and Cell Biology, Im Neuenheimer Feld 307, D-69120 Heidelberg, Germany

In this work, we describe a protocol for the localization of GFP-tagged synaptic proteins via photooxidation driven generation of electron dense diaminobenzidine (DAB)-deposits which are detectable by electron microscopy. To this end, we transfected primary cultured hippocampal neurons with CFP-tagged versions of Bassoon or Synaptophysin. Bassoon is an active zone cytomatrix protein which is transported from the Golgi apparatus to synapses via so-called Piccolo-Bassoon-transport vesicles (PTV's), while Synaptophysin is one of the most abundant integral membrane proteins of synaptic vesicles. Our data indicate that CFP-Bassoon photoconversion results in a dark, fuzzy coat surrounding vesicles which accumulate in clusters in the soma and in neurites. Photoconversion of CFP-Synaptophysin reveals DAB-deposits at membranes of tubular structures within the soma and neurites. This method allows for the correlation of light microscopical signals with the respective subcellular structures and provides information about the transport and localization of recombinant synaptic proteins at the ultrastructural level.

# LOCALIZATION OF THE ORPHAN CARRIER SLC10A4 IN THE PERIPHERAL NERVOUS SYSTEM AND ITS CO-EXPRESSION WITH VMAT2 AND VAChT

Simone Burger[1], Marcela Moncada[1], Stephanie Schmidt[1], Rüdiger Gerstberger[2], Joachim Geyer[1]

[1]Institute of Pharmacology and Toxicology, Justus-Liebig-University of Giessen, Frankfurter Str. 107, 35392 Giessen, Germany
[2]Institute of Veterinary Physiology, Justus Liebig University of Giessen, Frankfurter Str. 100, 35392 Giessen, Germany

The solute carrier family SLC10 comprises two well established sodium-dependent bile acid transporters, the Na+/taurocholate cotransporting polypeptide (NTCP; SLC10A1) and the apical sodium-dependent bile acid transporter (ASBT; SLC10A2). These carriers maintain the enterohepatic circulation of bile acids in the liver (NTCP) and the intestine (ASBT). A third member of the SLC10 family was recently identified and was termed sodium-dependent organic anion transporter (SOAT; SLC10A6). SOAT does not transport bile acids but sulfoconjugated steroid hormones in a sodium-dependent manner. In 2004, a further new member of this carrier family was cloned from rat adrenal gland and is referred to as SLC10A4. Although SLC10A4 shows the highest phylogenetic relationship to NTCP, it showed no transport activity for bile acids and sulfoconjugated steroid hormones when expressed in Xenopus laevis oocytes or HEK293 cells.
Gene expression analysis by real-time quantitative PCR revealed that SLC10A4 expression is highest in the brain. Immunofluorescence co-localization studies with antibodies directed against a C-terminal epitope of the rat SLC10A4 protein, against the cholinergic marker protein VAChT, and against the monoaminergic marker protein VMAT-2, revealed, that SLC10A4 is expressed in cholinergic and monoaminergic neurons and innervations of the rat central and peripheral nervous system as well as in the epithelium of the urinary bladder. Additionally, SLC10A4 is expressed in granules of rat peritoneal and tissue associated mast cells, what has been verified by immunofluorescence and electron microscopic examinations. Western blot and immunoprecipitation experiments revealed that SLC10A4 is expressed in synaptic vesicles of the rat brain. This vesicular expression pattern can also be seen in rat PC12 cells, human SH-SY5Y cells and in stably transfected SLC10A4-HEK293 cells. Transport studies were performed with [3H]choline and [3H]dopamine, but in contrast to the high affinity choline transporter CHT1 or the dopamine transporter DAT, respectively, SLC10A4 showed no transport activity when expressed in HEK293 cells. Due to its expression pattern in the neuronal and non-neuronal cholinergic system, in monoaminergic neurons and granules of mast cells as well as in neuronal cell cultures, we assume a specialized function of SLC10A4 in the regulation, release or storage of neuronal components like neurotransmitters or neuropeptides or in the exocytosis and retrieval of vesicles and granules.

# Miniaturisation effects on the sensory and CNS structures of the wasp *Encarsia formosa*.

Reinhold Hustert[1]

[1]Georg-August-Universität Göttingen, JFB-Institut f. Zoologie u. Anthropologie, Neurobiologie, Berliner Str. 28, 37073 Göttingen, Germany

Spatial restrictions in very small insects could lead to reduced size and number of neurons within the CNS. That is obvious for the low number of cuticular sensilla present on their surface, which in turn may require less integration of their afferent input by fewer interneurons in the CNS. As to neuronal size reductions, biophysical considerations indicate that axons with diameters below 0.1µm can impede the normal information transfer by means of action potentials (spikes). That concerns both axons in peripheral nerves and spike propagation within the CNS.
Light and TEM sections of the parasitic 0.6mm long wasp Encarsia formosa show size reductions for neurons but no obvious reduction of total neuron numbers if compared to the CNS of larger wasp species. Soma diameters average at about 2µm and they lie densely packed in several layers around the neuropiles of the CNS, which partly lies squeezed into spaces left by the musculature, sclerites and other tissues of the body cavity. The smallest axon diameters of neurons connecting between ganglia of the ventral nerve chord are in the range of 0.05µm. That is where normal information transfer by spikes is thought to collide with opening probability of the ion channels of the axonal membrane.
In contrast, there is also a "giant fiber" system of several axons (dia. 1.2-1.6µm) from the abdominal to the thoracic CNS, which are thought to transfer relatively rapid information from a set of long (100µm) wind-sensitive hairs located near the end of the abdomen. Wind puffs that move these hairs elicit short and rapid escape flights of the wasps. Escape flights are also elicited by visual stimuli, and correspondingly, "large" axons are seen to descend from the brain to the thoracic ganglia.
Presently it looks as if this miniature wasp has a "normal" CNS, although the extremely low diameters of subsets of axons could have restricted axonal spike conduction.

# Molecular mechanisms of BDNF-TrkB signalling in dendritic spine plasticity at hippocampal neurons

Yves Kellner[1], Marta Zagrebelsky[1], Martin Korte[1]

[1]Zoological Institute, Div. Cellular Neurobiology, Spielmannstr. 7, 38106 Braunschweig, Germany

The fine tuning of neural networks during development and learning implies both functional and structural plastic processes. While it is known that spine number and morphology can change during activity-dependent plasticity the mechanisms and intracellular molecules mediating these processes are largely unknown. Interestingly, plasticity processes at spines demonstrated to depend on the actin cytoskeleton. Neurotrophins and their receptors have been shown to mediate functional and structural plasticity at synapses. Specifically, BDNF (Brain-derived neurotrophic factor), through its receptor TrkB, is a crucial component of activity-dependent strengthening of hippocampal synapses. Moreover, several studies suggested a role of BDNF as a positive modulator of the morphology of pre- and postsynaptic structures.

However, the cellular and molecular mechanism by which BDNF-TrkB signalling modulates structural plasticity of pre- and postsynaptic structures are still largely unexplored or controversial. Specifically, we would like to address how changes in the actin cytoskeleton and in f-actin dynamics may mediate the BDNF-induced structural changes. Previous studies, describing the role of BDNF on spine morphology were performed in different animal species: ferret (Horch et al., 1999); mouse (Gao et al., 2009); rat (Ji et al., 2005 and 2010; Tyler and Pozzo-Miller, 2001) and under not comparable experimental conditions (age and culture conditions) making it difficult to draw general conclusions. We therefore started by reproducing and expanding (under strictly controlled experimental conditions) a series of previously published data. In a loss-of-function approach (24 h treatment with a specific BDNF blocking antibody in 21 DIV primary hippocampal cultures) we observed a significant reduction in dendritic spine density, associated with an increase in spine length and a decrease in head width. On the other hand surprisingly in a gain-of-function approach, the applying exogenous BDNF for 24 h, did not induce changes in spine density or morphology previously described (Ji et al., 2005 and 2010). Immunostaining of the transcription factor c-Fos and also calcium imaging demonstrate that the BDNF we used is indeed active and that in our cell culture conditions the hippocampal neurons respond to the exogenous application of BDNF. Taken together, our results so far, indicate that indeed endogenous BDNF positively modulates the shape of dendritic spines. In ongoing experiments, we use Lifeact as an actin maker (Riedel et al., 2008) and fluorescence recovery after photobleaching (FRAP) to clarify the role that actin dynamics play in mediating the structural plasticity effects of BDNF on the structure of mature dendritic spines in the living tissue.

# Monitoring lipid recycling in synapses of the central nervous system

Martin Kahms[1], Cora S. Thiel[1], Jürgen Klingauf[1]

[1]University Muenster, Institute for Medical Physics and Biophysics, Robert-Koch-Str. 31, 48149 Muenster, Germany

During synaptic transmission, neurotransmitters stored in presynaptic vesicles are released by exocytosis through fusion of vesicles with the plasma membrane. In a subsequent step, membranes and proteins at the synapse are reinternalized by a reverse process, endocytosis. Synaptic vesicles (SVs) are the key organelles in chemical neurotransmission characterized by a defined stoichiometry of protein and lipid constituents that has to be maintained during repetitive rounds of exo-endocytosis.

In the analysis of the synaptic vesicle cycle genetically encoded pH-sensitive fluorescent proteins like pHluorin have become indispensable tools. Fused to luminal domains of synaptic proteins like e.g. synaptobrevin/Vamp2, these probes are capable of detecting changes in pH that accompany exocytosis and subsequent reacidification of endocytosed vesicles

Here we describe a new class of fluorescent lipid probes, based on pH-sensitive organic dyes coupled to phospholipids, as a promising complementary assay to genetically encoded fluorescent proteins for studying the resorting and recycling of specific lipids. In hippocampal neurons, cell membranes can be stained, and SV lipid recycling can be monitored yielding fluorescence transients with kinetics mirroring those of the well characterized pHluorin signal.

Furthermore, this approach can be used to study vesicle recycling in acute preparations like bipolar cells of the retina that are not accessible to transfection methods.

Taking advantage of the optical properties of the fluorescent dye we are able to analyze the fate of exocytosed lipid molecules and found that lipid constituents of exocytosed vesicles are not immediately retrieved by compensatory endocytosis. Instead, endocytosed vesicles recruit lipid molecules mainly from the surface pool and this non-identity of exo- and endocytosed lipid material was found even for strong stimulation up to 600 action potentials at 20 Hz. By quantification of vesicle incorporation of different dye-labeled lipids relative to the endocytic marker FM 1-43 in dual-colour ratiometric measurements we mapped the relative amount of lipids incorporated into SVs and observed an almost two-fold enrichment of a putative raft-lipid compared to a non-raft lipid.

This way we shed light on a presynaptic mechanism neglected so far, namely lipid recycling, and hope to unravel for the first time the possible role of lipids in protein resorting and SV recycling.

# N-cadherin mis-match expression results in impaired synaptic function, synapse elimination, and axon retraction

Kurt Gottmann[1], Kay Jüngling[1], Kim Pielarski[1]

[1]University Düsseldorf, Institute of Neuro- and Sensory Physiology, Universitätsstr. 1 Gebäude 22.03, 40225 Düsseldorf, Germany

Formation of specific neuronal circuits during development involves both synapse formation and elimination of inappropriate synapses. While the role of transsynaptically interacting cell adhesion molecules in synapse formation is intensively studied, very little is known about their role in synapse elimination. N-cadherin is among the most important synaptic adhesion molecules and has been demonstrated to play a role in synaptic vesicle accumulation, spine morphogenesis and even in regulating synaptic function. A characteristic molecular feature of N-cadherin is its homophilic binding across the synaptic cleft, which has been proposed to form the basis of molecular target recognition. In addition to homophilic transsynaptic binding, N-cadherin appears to be also expressed in mis-match situations with N-cadherin present only on one side of a synaptic contact. However, the consequences of such a N-cadherin mis-match expression for synapse function and stability have not been studied.

To create a well controlled N-cadherin mis-match expression in cultured neurons, N-cadherin knockout neurons (derived from N-cad-/- mouse ES cells) were transfected with a N-cadherin vector by the Lipofectamine technique. Because of a very low transfection efficiency, single (postsynaptic) neurons expressed N-cadherin, which were synaptically innervated by non- transfected, N-cadherin knockout (presynaptic) neurons. N-cadherin expression was confirmed immunocytochemically. 2 days after transfection, patch-clamp recordings from transfected neurons revealed a strongly reduced frequency on AMPA receptor-mediated miniature EPSCs, whereas mini amplitude was unaltered. In line with this result, paired recordings in cocultures of knockout and wildtype neurons revealed a reduced amplitude and an increased failure rate of evoked AMPA receptor-mediated EPSCs.

Together, these findings indicate an impairment of presynaptic function, whereas no change in the number of synapses was detectable 2 days after transfection with VAMP2 immunostaining. To further study whether synapse elimination occurs in succession to impaired synaptic function, immunocytochemical stainings were performed 8 days after transfection. With prolonged N-cadherin mis-match, a significant reduction in synapse density on dendrites of transfected neurons was observed indicating synapse elimination.

To address, whether a N-cadherin mis-match expression leads to axon retraction, cultured cortical neurons with floxed N-cadherin genes were cotransfected with Cre and EGFP to induce a conditional N-cadherin knockout in single neurons. 8 days after transfection a retraction of axonal processes was detectable.

In summary, our results suggest that a N-cadherin mis-match expression induces a sequence of impaired synaptic transmission, synapse elimination and axon retraction. This N-cadherin mis-match mechanism might contribute to the formation of neuronal circuits by elimination of non-matching connections.

# Overexpression of Synaptopodin rescues the formation of spine apparatuses in hippocampal neurons

Mercedes Küffner[1], Yun-Chung Nam-Apostolopoulos[1], Michael K Schäfer[1], Michael Frotscher[1]

[1]Universität Freiburg, Institut für Neuroanatomie und Zellbiologie, Albertstr. 17, 79104 Freiburg, Germany

The spine apparatus, an extension of the dendritic smooth endoplasmic reticulum, is located in mature dendritic spines of cortical and hippocampal neurons. Thus, this neuronal organelle is mainly found in large mushroom spines (Spacek, 1985). Its involvement in the functional properties of the spine is still enigmatic. Synaptopodin, an actin-associated protein, plays a critical role in the formation of the spine apparatus, and Synaptopodin-knockout mice are deficient in spine apparatuses (Deller et al., 2003). Synaptopodin has been shown to bind actin directly (Mundel et al., 1997; Kremerskothen et al., 2005) which may contribute to the maintainance of spine structure. Here we demonstrate, using a combination of single cell electroporation in hippocampal organotypic slice cultures and immunoelectron microscopy, that overexpression of GFP -tagged Synaptopodin rescues spine apparatus formation in CA3 pyramidal neurons. However, transfecting the actin binding domain alone is not sufficient to rescue the defect. Moreover, when overexpressing full length Synaptopodin, we also find aberrant spine apparatus-like structures in the soma. Surprisingly, pharmacological inhibition of small Rho-GTPases (RhoA, Rac1, cdc42), which are important for the regulation of F-actin assembly, leads to an increased number and size of rescued spine apparatuses. These findings show that formation, size, and distribution of the spine apparatus strongly depend on Synaptopodin expression levels. Our preliminary pharmacological experiments further implicate that F-actin assembly is involved in this process.

# Presynaptic $Ca^{2+}$ influx and vesicle exocytosis at the endbulb of Held synapse

Holger Taschenberger[1], Kun-Han Lin[1]

[1]Max Planck Institute for Biophysical Chemistry, Dept. of Membrane Biophysics, Am Fassberg 11, D-37077 Göttingen, Germany

The release of neurotransmitters is triggered by $Ca^{2+}$ entering the cytoplasm through voltage-gated channels that open when an action potential (AP) invades the presynaptic terminal. Our knowledge about the functional properties of voltage-gated $Ca^{2+}$ channels expressed in presynaptic nerve endings is still spare because most terminals are not accessible to electrophysiological recordings. To optimize speed and temporal fidelity of transmission, the mammalian auditory system makes use of specialized presynaptic endings of unusually large size. In this study, we characterized voltage-gated $Ca^{2+}$ channels expressed in one of these presynaptic terminals, the endbulb of Held, formed by the endings of auditory nerve fibers that contact bushy cells located in the anterior ventral cochlear nucleus. Whole-cell patch-clamp recordings were obtained from endbulb terminals of mouse brainstem slices between postnatal day 9 and 12. The size of the synaptic terminals was quite variable. Whole-cell capacitance values ranged from 1 to 8 pF (mean 4.3 pF). The threshold for activation of pharmacologically isolated presynaptic $Ca^{2+}$ currents ($I_{Ca(V)}$) was around -40 mV. Peak amplitudes of ICa(V) elicited by step depolarizations (10 ms, -10 mV) ranged from 0.2 to 0.7 nA (mean 0.41 nA). Assuming a single channel current of ~0.15 pA and a pO of ~0.75 (Li et al., 2007), the estimated mean number of channels per terminal was ~3700. The mean $Ca^{2+}$ current density of ~0.11 nA/pF was remarkably similar to values obtained previously for another large terminal, the calyx of Held. To estimate the $Ca^{2+}$ influx during a single presynaptic AP we derived a HH-type $m^2$ model, based on measured maximal conductance, activation and deactivation kinetics of $I_{Ca(V)}$, and recorded AP waveforms to drive that model. Presynaptic APs had peak amplitudes of ~130 mV and a mean half-width of ~250 µs. Simulations suggested that such AP waveforms open nearly 60% of all activatable channels. The simulated $I_{Ca(V)}$ had a half-width of 490 µs and a peak amplitude of 0.53 nA, corresponding to the opening of ~2200 channels. Because of the large number of expressed $Ca^{2+}$ channels and their effective opening by presynaptic APs, it is likely that multiple $Ca^{2+}$ channels control the release of a single transmitter vesicle at the endbulb of Held synapse.

# Presynaptic NMDA receptors mediate an increased glutamate release in the vicinity of a focal laser lesion in rat visual cortex

Barbara Imbrosci[1], Li Yan[2], Ute Neubacher[2], Ulf T. Eysel[2], Thomas Mittmann[1]

[1]Physiology & Pathophysiology, Medicine, Duesbergweg 6, 55128 Mainz, Germany
[2]Neurophysiology, Medicine, Universitaet Str. 150, 44780 Bochum, Germany

Focal cortical lesions are accompanied by a functional reorganization of the cortical networks in the neighbouring brain areas. On the cellular level changes in synaptic plasticity seem to be, at least in part, responsible for this reorganization. In particular, an increased long-term potentiation (LTP) has been observed in neuronal networks surrounding focal cortical lesions.
Here, we investigated lesion-induced alterations of synaptic transmission in an ex vivo - in vitro model of focal lesions in rat visual cortex which might underlie the already described enhanced LTP.
Focal lesions were induced by use of an infrared laser in anaesthetized rats in vivo.
Whole-cell patch clamp recordings were performed from pyramidal neurons in cortical layers 2/3 in acute slices at 2 to 6 days following the induction of a focal lesion in the visual cortex.
We observed an increased frequency of AMPAR-mediated miniature excitatory postsynaptic currents (mEPSCs) post-lesion. Interestingly, bath application of D-AP5, a specific blocker of NMDARs, reduced the mEPSCs frequency of lesion-treated rats to the level of sham-operated animals. In contrast, no changes were observed in sham-operated animals. Since the experiments were performed at -80 mV in presence of intracellularly applied MK-801 (an antagonist of NMDARs) we excluded that this reduction was due to a block of postsynaptic NMDARs.
These results indicated the presence of tonically activated non-postsynaptic, likely presynaptic, NMDARs which could increase the release of glutamate post-lesion. Bath application of Ro25–6981, a specific antagonist of NMDARs containing the NR2B subunit led to a similar result further suggesting the involvement of NR2B-containing NMDARs in the control of glutamate release post-lesion.
To disclose whether these functional non-postsynaptic NMDARs expressed post-lesion might also modulate action-potential dependent glutamate release, AMPAR-mediated currents were evoked by stimulation of ascending fibres projecting to layer 2/3. Application of ifenprodil, another specific blocker of NMDARs containing the NR2B subunit, reduced the amplitude of AMPAR-mediated responses in all recorded neurons post-lesion however it failed to induce any changes in sham-operated rats.
Taken together these findings revealed a lesion-induced expression of functional, most likely presynaptic, NMDARs which might underlie the processes of facilitated synaptic plasticity by boosting glutamatergic synaptic transmission.

# Properties of synaptic transmission at a trigeminothalamic giant synapse

Francisco Urra[1], Thomas Kuner[1]

[1]Universität Heidelberg, Institute of Anatomy and Cell Biology, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

The thalamus relays sensory information from the periphery to the cortex. A trisynaptic pathway connects the whiskers with the somatosensory cortex. The principal nucleus of the trigeminal nerve (Pr5) in the brainstem receives sensory information from the whiskers and sends them to the ventral posteromedial nucleus of the thalamus (VPM), which in turn projects to the somatosensory cortex. Synaptic transmission between Pr5 and relay cells of the VPM is mediated by giant synapses, which just recently started to be studied using direct patch-clamp recordings. In this study we labeled trigeminothalamic (Pr5 -VPM) giant terminals by stereotaxic delivery of adeno-associated virus particles encoding synaptophysin-EGFP into the Pr5 nucleus of rats (age P12). Pr5-VPM giant terminals were identified in the VPM and directly stimulated with a double-barrel electrode after establishing whole-cell patch-clamp recordings from the postsynaptic relay neuron (rats age p24-p30). This allowed us to study synaptic transmission in identified Pr5-VPM giant synapses.

Voltage-clamp recordings of VPM relay cells at a membrane potential of –60 mV revealed postsynaptic currents with a mean amplitude of 1 ± 0.25 nA (n=15) in response to the stimulation of a single terminal. The EPSC rise time was 0.41 ± 0.03 ms and the decay time was 1.92 ± 0.24 ms, consistent with a fast AMPA receptor-mediated current. Spontaneous responses of relay cells of the VPM have mean amplitude of 22.31 ± 0.75 pA (n=22), giving rise to a quantal content of approximately 52. Upon high-frequency stimulation, the EPSCs showed a strong frequency-dependent short-term depression. This may result in a low-pass filtering of incoming signals thereby limiting the bandwidth of information transfer. Current-clamp recordings show that a single action potential elicited in a Pr5 -VPM terminal suffices to generate several action potentials in the relay cell, establishing a synaptic amplifier.

In conclusion, a single Pr5-VPM synapse can act as a driver synapse passing sensory information to the cortex, but the strong short-term depression limits the bandwidth of information transfer across this synapse. These properties are similar to synaptic transmission at another thalamic giant synapse relaying information from the somatosensory cortex to thalamus (L5B-POm synapse).

# Remodeling of the $Ca^{2+}$-sensing machinery for transmitter release during early development and maturation of the calyx of Held

Olexiy Kochubey[1], Ralf Schneggenburger[1]

[1]École Polytechnique Fédérale de Lausanne (EPFL), Brain Mind Institute, Laboratory of Synaptic Mechanisms, Station 19, CH-1015 Lausanne, Switzerland

The calyx of Held, a giant axo-somatic synapse in auditory brainstem, allows detailed characterization of the presynaptic intracellular $Ca^{2+}$-regulation of transmitter release. Transmitter release is triggered by $Ca^{2+}$ sensing molecules like Synaptotagmins which accelerate SNARE-dependent synaptic vesicle fusion. When calyces of Held are initially formed at ~ postnatal day 3 (P3), their transmitter release mechanisms are dominated by asynchronous release, which is then rapidly transformed into highly synchronous release (Chuhma 2001). An important $Ca^{2+}$ sensor for release at the calyx of Held is Synaptotagmin2 (Syt2), because k.o. of Syt2 leads to a strong decrease of $Ca^{2+}$-dependent release (Sun et al., 2007). In the absence of Syt2, some $Ca^{2+}$ evoked release with a lower $Ca^{2+}$ cooperativity persists, indicating the presence of remaining non-Syt2 $Ca^{2+}$ sensors that can drive residual release.
Here we investigated the development of the release phenotype at the calyx of Held synapse of Syt2 k.o. mice using presynaptic $Ca^{2+}$ uncaging, with the aim to uncover a possible developmental regulation of non-Syt2 $Ca^{2+}$ sensors. At P12 - P15, Syt2 k.o. calyces showed a more than 100-fold reduction of peak transmitter release rates relative to their wild-type littermates, and the remaining $Ca^{2+}$-evoked release had a low $Ca^{2+}$ cooperativity (slope in double logarithmic coordinates, ~ 1). At P5 - P6, however, significantly more $Ca^{2+}$ evoked release remained in Syt2 k.o. synapses, with an apparent cooperativity of ~ 2, similarly as reported before (Sun et al., 2007). Finally, at P2 - P3, short before calyx formation, we investigated synapse function with afferent fiber stimulation, and found that EPSCs were not largely changed with respect to those of wild-type control synapses. Immunohistochemistry showed that Syt2 was clearly present in calyx synapses at P4 - P14 and Syt1 was absent. Interestingly, however, Syt2 was absent before ~ P2 - P3, with Syt1 - positive small bouton-like nerve terminals dominating at these early ages. Thus, our results show that pre-calyceal synapses use a non-Syt2 $Ca^{2+}$ sensor, and that Syt2 expression comes up at the same time as calyx of Held formation. Overall, our findings suggest that $Ca^{2+}$-binding molecules other than Syt2 (e.g. other Syt isoforms or Doc2 family proteins) function early in development of the calyx of Held and are down-regulated during maturation and replaced by Syt2. In the Syt2 k.o. synapses, these molecules can (partially) substitute for the missing Syt2 up to ~ P10. This developmental down-regulation of non-Syt2 $Ca^{2+}$ sensors might also partially explain the reduction of asynchronous release which occurs early postnatally. When extrapolated to the entire hindbrain and spinal cord where Syt2 is expressed, our findings offer an explanation for the survival of Syt2 k.o. mice (Pang et al., 2006), and for the absence of detectable deficits in Syt2 k.o. mice younger than one week.

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# Role of neurofascin in inhibitory synapse organization at the axon initial segment

Sabine Trinks[1], Martin Kriebel[1], Jennifer Metzger[1], Hansjürgen Volkmer[1]

[1]Natural and Medical Institute at the University of Tuebingen , Molecular Neurobiology, Markwiesenstr.55, 72770 Reutlingen, Germany

The pathophysiology of mood disorders like depression and posttraumatic stress disorder is discussed to result from an imbalance of excitatatory and inhibititory signal transmission in the CNS due to molecular alterations. While mechanisms controlling excitatory synapse architecture and function are widely understood, little is known about inhibitory synapse formation and stabilization.

Two dynamically regulated neuronal isoforms of cell adhesion molecule neurofascin (186 kDa and 166kDa, respectively) are expressed at the AIS (axon initial segment) of principal neurons. While neurofascin 166 is involved in the control of neuronal connectivity such as neurite outgrowth and postsynaptic organization, neurofascin 186 may be implicated in AIS stabilization assigning different functions for these two isoforms.

Physiologically manifested stress in juvenility is accompanied by specific regulation of neurofascin expression both at the level of total mRNA levels and alternative splicing in terms of upregulated neurofascin 166. To elucidate a potential role for neurofascin in juvenile stress, we therefore analyzed the impact of shRNA-mediated neurofascin knockdown and neurofascin 166 overexpression in cultured neurons and in juvenile animals in vivo. Interference with neurofascin expression in juvenile rats after lentiviral knockdown reveals a specific impairment of gabaergic synaptic structures of axo-axonic chandelier neurons as exemplified by a loss of presynaptic GAD65 and postsynaptic gephyrin at the AIS. Moreover we have found enlarged gephyrin clusters at the AIS after overexpression of 166 kDa neurofascin in cultured neurons suggesting a role of neurofascin in postsynaptic organization.

Our findings suggest that neurofascin 166 expression induced by juvenile stress may lead to aberrant inhibitory synapse organization at the AIS.

# Roles of the protein Post Synaptic Density-95 in basal synaptic transmission

Stéphanie A. D. Bonnet[1], Oliver M. Schlüter[1]

[1]European Neuroscience Institute-Göttingen, Molecular Neurobiologie, Grisebachstrasse 5, 37077 Göttingen, Germany

PSD-95 is a signalling scaffold protein belonging to the DLG subfamily of MAGUKs (Membrane Associated Guanylate Kinases) which is enriched at the postsynaptic density of excitatory synapses. PSD-95 is involved in different processes, ranging from synapse maturation, over regulation of synaptic AMPA receptor (AMPAR) function to long-term synaptic plasticity. PSD-95 contains three consecutive PDZ domains, an SH3 domain and an enzymatically inactive GK domain. The role of each domain in the different functions of PSD-95 is still elusive. It has been shown that both the N-terminal, PDZ-1-2 domains and the SH3/GK domain is important for regulating the strength of AMPAR synaptic transmission. In this study, we dissected the role of each individual domain in basal synaptic transmission with the goal to identify the minimal requirement of PSD-95 in modulating AMPAR function.

We used a molecular replacement approach combined with electrophysiological recordings of CA1 pyramidal cells in rat organotypic hippocampal slices. Importantly, this approach allowed us to investigate the mutant constructs in a null PSD-95 background.

Loss of PSD-95 decreased AMPAR mediated synaptic transmission by 50%. We identified, that neither the PDZ3, nor the SH3 domain were required for PSD-95 to regulate the strength of AMPAR function, as deletion constructs enhanced AMPAR function two-fold, similar to the wild-type replacement construct, which served as reference point in the analysis. A mutant lacking the GK domain was clearly impaired in its function to enhance AMPAR function. However, this construct was still able to rescue the strength of AMPAR-mediated synaptic transmission to control cell levels.

In conclusion, we show that the first two PDZ domains of PSD-95 are sufficient to enhance AMPAR function, when they pair with full length endogenous PSD-95, but the GK domain is additionally required in a minimal PSD-95 to modulate the strength of AMPAR-mediated synaptic transmission.

# Serotonergic modulation of oriens-lacunosum moleculare interneurons in CA1

Claudia Böhm[1], Jochen Winterer[1], Dietmar Schmitz[1,2,3]

[1]NWFZ, Charité Universiätsmedizin Berlin
[2]NeuroCure Berlin, Excellence Cluster
[3]Bernstein Center for Computational Neuroscience Berlin

Midbrain raphe nuclei provide a strong serotonergic projection to the hippocampus, where serotonin (5-HT) exerts multiple, partially opposite effects onto its targets. Some of this diversity in serotonergic signalling can be related to various receptor subtypes. In the hippocampal area CA1 pyramidal cells and interneurons show different expression of 5-HT receptor subtypes, resulting in a differential modulation of intrinsic and synaptic properties.
Here, we investigate the effect of 5-HT on distal targeting oriens-lacunosum moleculare (O-LM) interneurons, which are innnervated by CA1 pyramidal cells. We show that serotonin decreases excitation of these interneurons *in vitro*. The reduced glutamatergic transmission is of presynaptic origin and is partially mediated by the $5\text{-HT}_{1B}$ receptor subtype. Calcium measurements revealed that 5-HT reduces presynaptic calcium influx, which is likely to account for the reduced glutamate release onto O-LM interneurons. The reduction of glutamatergic transmission on distal targeting interneurons could serve as a modulatory mechanism of pyramidal cell input during different behavioural states.

# Stability of active zone components at the photoreceptor ribbon complex

Hanna Regus-Leidig[1], Dana Specht[1], Susanne tom Dieck[2], Johann Helmut Brandstätter[1]

[1]University of Erlangen-Nuremberg, Department of Biology, Animal Physiology, Staudtstr. 5, 91058 Erlangen, Germany
[2]Max Planck Institute for Brain Research, Department of Synaptic Plasticity, Max-v.-Laue-Str. 3, 60438 Frankfurt/Main, Germany

Photoreceptor ribbon synapses translate light-dependent changes of membrane potential into graded transmitter release over several orders of magnitude in intensity. A specialized organelle at the active zone – the synaptic ribbon – is a key player in this process, and it is well known that the ribbon undergoes illumination and thus activity-dependent structural changes. However, the molecular basis for these changes is unknown. The aim of this study was to correlate the known ultrastructural ribbon changes to the distribution of proteins of the presynaptic ribbon complex.

In an in vitro assay distinct structural ribbon states – club -shaped and spherical-shaped – were enriched and the distribution of presynaptic proteins at the rod photoreceptor ribbon complex was analyzed with immunocytochemistry and light and electron microscopy.

We show that structural changes of the ribbon correlate with the redistribution of selected presynaptic proteins. The disassembly of the ribbon complex seems to be a multistep process. It starts with the removal of spherical ribbon and ribbon associated material composed of RIBEYE, Piccolo, RIM1, CtBP1, and KIF3A, while arciform density and active zone plasma membrane proteins, i.e. RIM2, Munc13, CAST1, and the L-type Ca2+ channel subunit Cacna1f remain largely unchanged at their synaptic location. Only later, in a second phase following the removal of ribbon material, the arciform density and plasma membrane proteins are redistributed from their synaptic localization and active zones disappear.

The results of our study show that photoreceptor ribbon and arciform density/plasma membrane components might be influenced differentially by activity driven processes, thus providing a molecular basis for further investigation of regulatory and adaptive processes in photoreceptor ribbon synaptic transmission.

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# Stonin2-dependent endocytic sorting of synaptotagmin 1 provides evidence for loss of synaptic vesicle identity during exo-endocytosis

Natalia L. Kononenko[1,2], Tanja Maritzen[2], Seong Joo Koo[2], M. Kasim Diril[2], Nadja Jung[2], Volker Haucke[1,2]

[1]Institute of Chemistry and Biochemistry, Department of Membrane Biochemistry, Freie Universität Berlin, 14195 Berlin, Germany

[2]Charite University Medicine Berlin, Neurocure, Germany

Synaptic transmission depends on the regulated exo -endocytosis of presynaptic vesicles (SVs), specialized organelles that store and secrete non-peptide neurotransmitters. How precisely SV identity is maintained over multiple rounds of vesicle cycling has been debated controversially. Whereas some studies have suggested that SVs maintain their identity during exo-endocytosis others have suggested intermixing of newly exocytosed SV proteins with a surface-stranded pool on the presynaptic plasmalemma. Moreover, endocytic mutants analyzed so far have displayed generalized defects in the endocytic retrieval of SV proteins, suggesting that recycling may involve clustered SV proteins, in line with data obtained by STED microscopy. We report here on the phenotypic characterization of knockout mice, lacking the synaptotagmin-specific endocytic sorting adaptor stonin 2. Stonin2 KO mice, although viable and fertile, display signs of progressive degeneration including an abnormal dilatation of the lateral ventricle and a partial loss of the lateral septum. These defects appear to be caused by the partial and selective loss of the stonin 2 binding partners AP2 and synaptotagmin 1 from the CA3 region of the hippocampus of KO animals. These alterations correlate with a strong accumulation of plasma membrane-stranded synaptotagmin 1 in brain slices of stonin2 KO mice and impaired endocytic retrieval of synaptotagmin-pHluorin but not synaptobrevin -pHluorin from mutant terminals of postnatal hippocampal neurons in culture. We will discuss the implications of these and other data for the mechanism of SV retrieval at central synapses in the mammalian brain

# Synapsins control short-term synaptic plasticity in the mouse calyx of Held

Mariya Vasileva[1], Daniel Gitler[2], Thomas Kuner[1]

Institute for Anatomy and Cell Biology, University of Heidelberg, In Neuenheimer Feld 307, 69120 Germany [1]
Department of Physiology, Faculty of Health Sciences, Ben Gurion of the Negev, Beer-Sheva 84105, Israel [2 2]

Synapsins are neuron-specific phosphoproteins, which are associated with the cytoplasmic surface of synaptic vesicles (SV). Three differentially spliced mammalian genes yield at least five synapsin isoforms, which have similar domain structures with variable C -terminal parts. Synapsins might anchor SV to the cytoskeleton associated with an active zone, thereby controlling SV replenishment to the release sites. Previous work has shown that deletion of synapsins decreases the total number of SV within presynaptic terminals without altering vesicle docking and clustering close to the active zones. However, it is still unclear how synapsins contribute to the functional organization of SV pools within synaptic terminals and how SVs are trafficked in order to maintain normal synaptic function.

We investigated synaptic transmission at the mature (P14-P18) calyx of Held, a giant glutamatergic terminal in the auditory brain stem, of mice lacking all three synapsin genes (synapsin triple knock out (SynTKO)). Whole-cell voltage clamp recordings from MNTB principal cells were made and the properties of spontaneous release were examined. spEPSC amliptude, rise and decay kinetics were not altered in comparison to age-matched wild type littermates (SynTWT). Then, EPSCs in response to isolated action potentials were measured. Amplitude and decay time of evoked EPSCs were similar to SynTWT. The EPSC rise time was, however, increased, suggesting a stronger contribution of asynchronous release in calyces lacking synapsins.

Short-term depression in response to trains of high-frequency stimuli was strongly increased. Calyces lacking synapsins depressed faster and to a larger extend than wild type. The increased depression might be due to a decrease in the fraction of the RRP mediating release late in the stimulus train. Furthermore, the fast component of recovery from depression was significantly slowed down, while the slow component remained unchanged. These results suggest that a subset of SVs in the calyx of Held may be recruited to the RRP with a mechanism dependent on synapsins. These might correspond to low-release probability, rapidly recovering SV.

Our results indicate that synapsins maintain use-dependent synaptic plasticity at a central nervous system synapse via controlling SV pools without interfering with the basal release mechanism.

# Synaptic targeting and secretion of BDNF-GFP and NT-4-GFP from CA1 pyramidal neurons in mouse hippocampal slices

Petra Lichtenecker[1], Tanja Brigadski[1], Volkmar Lessmann[1]

[1]Institute of Physiology, Faculty of Medicine, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany

Neurotrophins are important neuronal growth factors which play a role in a multitude of cellular processes, including neuronal differentiation, survival and synaptic plasticity. These proteins are released either via the regulated or the constitutive secretory pathway. While the secretion of neurotrophins in response to high-frequency electrical stimulation is thought to be responsible for activity- dependent synaptic plasticity processes, the constitutive secretion of neurotrophins may be important for maintaining synaptic function. Neurotrophins are targeted to the regulated secretory pathway in large secretory granules while constitutively released neurotrophins show a homogeneous distribution in neuronal cells. The neurotrophins BDNF and NT-4 are both expressed in the hippocampal formation and mediate their biological function via TrkB or p75 receptors. Whereas targeting and secretion of BDNF and NT-4 have been analysed in dissociated neuronal cultures, cell-specific targeting patterns of BDNF and NT-4 in CA pyramidal cells in intact brain slices are unknown.

In order to analyse the distribution of BDNF and NT-4 and the synaptic release of these neurotrophins in CA1 pyramidal cells, we have transfected single CA1 pyramidal neurons in organotypic hippocampal slice cultures with GFP-tagged neurotrophins. Hippocampal slices from newborn (P3 -P5) mice were prepared according to the Stoppini method. Using single cell electroporation in the loose patch configuration of the patch clamp technique, selected single postsynaptic CA1 pyramidal neurons were transfected with BDNF-GFP or NT-4-GFP cDNA at 9 DIV, respectively. Green-fluorescent BDNF- or NT-4-containing vesicles were observed in the somatodendritic compartment of these CA1 pyramidal neurons. BDNF-GFP-containing secretory granules were also targeted to distal dendrites. The targeting to dendritic regions was more efficient for BDNF-GFP-containing secretory granules than for those containing NT-4-GFP. Once targeted to dendritic secretory granules the neurotrophins colocalized with the postsynaptic protein PSD95, suggesting postsynaptic targeting of the neurotrophins to synapses of CA1 pyramidal cells. Furthermore, using an HA-tagged BDNF-GFP plasmid, we could show that the prodomain of BDNF is colocalised with the mature domain of BDNF in these secretory granules.

Fusion pore opening of BDNF-containing secretory granules and release of BDNF from these secretory granules was analysed after high frequency stimulation of Schaffer collaterals capable of inducing LTP in these organotypic hippocampal slice cultures. These data suggest postsynaptic secretion of NT-4 and BDNF from CA1 pyramidal neurons upon appropriate synaptic stimulation.

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# Synaptic transmission and postsynaptic integration of large synapses in the ventral nucleus of the lateral leminsucs of mongolian gerbils

Felix Felmy[1], Elisabeth M.M. Meyer[1], Benedikt Grothe[1,2]

[1]Ludwig-Maximilians Universität, Division of Neurobiology, Munich
[2] Bernstein Center for Computational Neuroscience Munich

In the auditory brainstem large excitatory calyceal synaptic terminals are present. The auditory nerve fibre gives rise to the endbulb of Held in the cochlear nucleus (CN), the bushy cells to the calyx of Held in the medial nucleus of the trapezoid body (MNTB) and the octopus cells to large terminals in the ventral nucleus of the lateral lemniscus (VNLL). In general such large synapses are believed to allow for a faithful information transfer. Synaptic transmission has been well studied at the synapses in the CN and the MNTB, but presynaptically, only the calyx of Held has been investigated. The features of synaptic transmission of large terminals in the VNLL are largely unknown, and thus no physiological comparison of auditory presynaptic terminals is available so far.

To compare the membrane physiology, the synaptic transmission and the postsynaptic integration of calyx of Held synapses and terminals in the VNLL we used pre - and postsynaptic whole -cell current - and voltage-clamp recordings and ratiometric fura $Ca^{2+}$-measurements from visually identified terminals of brain slices of Mongolian gerbils of postnatal day 9-11 at 34°C.

We find that both terminals have resting membrane potentials of -68 mV that the input resistance is lower and the membrane time constant faster in terminals in the VNLL. Action potential (AP) half width is not different, but its size was ~20 mV smaller in terminals in the VNLL. The resting $Ca^{2+}$ concentration and the endogenous calcium binding ratio are slightly larger in the VNLL. The VNLL synapse generates AMPA and NMDA currents (3.1 nA and 2.3 nA respectively) of about half the amplitude of clayx of Held synapses. Both AMPA and NMDA currents are faster in their kinetic profile in VNLL. Despite the large synaptic currents a single terminal in the VNLL is not able to trigger a postsynaptic AP. However, depolarising the postsynaptic cell with holding currents allowed for the generation of a fast onset AP. Under control conditions a fast transient potassium channel present in the postsynaptic VNLL somata appears responsible for the suppressing the AP generation by a single terminal in the VNLL.

In contrast to the calyx of Held information transfer in the VNLL is based on coincidence detection or temporal summation instead of a reliably one-to-one transmission. Thus, also at large, fast glutatamtergic synapses, the postsynaptic integration contributes substantially to the pattern of information transfer.

# The Multi-PDZ domain Protein (MUPP) 1: A neuronal scaffold involved in sperm acrosomal exocytosis

Heike Borth[1], Frauke Ackermann[2], Nele Zitranski[1], Laura Vieweg[1], Thomas Gudermann[1], Ingrid Boekhoff[1]

[11]Ludwig-Maximilians-University , Walther-Straub-Institute of Pharmacology and Toxicology, Goethestr. 33, 80336 Munich, Germany
[2]Karolinska Institute, Department of Neuroscience, SE-171 77 Stockholm, Sweden

Sperm undergo complex sequences of precisely timed events during the process of fertilization. One of these events is the acrosome reaction, a calcium -regulated exocytosis of hydrolyzing enzymes from the acrosomal vesicle, which is necessary to digest the egg's zona pellucida. Despite the difference in the size and the number of vesicles in neurons and spermatozoa, recent findings indicate some parallels between neurotransmitter release and acrosomal secretion, e. g. SNARE -mediated vesicle docking and priming which is mediated by a functional network of multi protein signalling complexes. Since adapter proteins responsible to control subcellular organization of such "signalosomes" have not yet been described in spermatozoa, attempts were made to identify scaffolding proteins expressed in the acrosomal region of different mammalian sperm. Using immunohistochemical and Western blot analyses, the Multi-PDZ domain Protein MUPP1, which comprises 13 protein interaction modules, was identified in rodent reproductive tissues. Further immunocytochemical experiments combined with immunogold electron microscopy revealed that MUPP1 is exclusively detectable within the acrosomal region of different mammalian spermatozoa and that the expression is most prominent at the outer acrosomal membrane. To assess the possible function of MUPP1 during the acrosome reaction, a functional secretion assay using permeabilized capacitated sperm loaded with different anti-MUPP1 antibodies was performed. The antibody treatment in these experiments significantly reduces the acrosome reaction rate compared to sperm incubated with IgG alone. These observations together with the finding that MUPP1 co-migrates in detergent-insoluble lipid rafts along with proteins involved in acrosomal exocytosis, like syntaxin-2, indicates that MUPP1 may assemble signalling molecules controlling acrosomal exocytosis in different mammalian species.

# The number of release-ready vesicles increases rapidly during homeostatic plasticity

Annika Weyhersmüller[1], Nicole Wagner[2], Jens Eilers[1], Stefan Hallermann[1]

[1]Carl-Ludwig-Institute of Physiology, University of Leipzig, Liebigstr. 27, 04103 Leipzig, Germany
[2]Institute of Physiology, University of Würzburg, Röntgenring 9, 97070 Würzburg, Germany

How can synapses change the probability of neurotransmitter release during synaptic plasticity? While synaptic release probability depends on the number of release-ready vesicles and the release probability of each vesicle, a dissection of both parameters during synaptic plasticity is difficult. Here we used the well established presynaptic homeostatic compensation upon interference with postsynaptic glutamate receptors at the *Drosophila* neuromuscular junction as a model for plastic alterations of synaptic release probability. As a genetic tool to induce synaptic homeostasis, we used animals lacking the glutamate receptor subunit IIA and expressing only the IIB-type receptor (GluRIIB; DiAntonio et al., 1999; J Neurosci 19:3023-3032). Combining short-term plasticity analysis, cumulative excitatory postsynaptic current analysis and quantal short-term plasticity modeling, we found an increase not only in the vesicular release probability, but also in the number of release-ready vesicles during homeostatic compensation. Consistently, in fluctuation analysis, the number of release-ready vesicles was almost doubled in homeostatically compensating animals compared to controls (690 ± 128 and 359 ± 58, n = 6 and 8 in GluRIIB and control, respectively; Mann-Whitney rank sum test: P = 0.03). Quantitative confocal image analysis (Schmid et al., 2008, Nat Neurosci 11:659-666) revealed an increase in the amount of the active zone protein Bruchpilot (BRP; 103 ± 4 and 88 ± 3 a.u., n = 8 each in GluRIIB animals and controls, P = 0.01) and stimulated emission depletion (STED) microscopy showed an enlargement of the presynaptic cytomatrix structure during homeostatic compensation (BRP ring diameter in STED images: 152 ± 5 and 136 ± 4 nm, n = 93 and 139 in GluRIIB and control, respectively, P = 0.01). Furthermore, we analysed homeostatic compensation on a shorter timescale by incubation with the glutamate receptor blocker Philanthotoxin for 10 minutes in semi-intact preparations (Frank et al., 2006; Neuron 52:663-677). Again, we found an increase in the number of release-ready vesicles as well as presynaptic structural alterations. Our results demonstrate that synaptic homeostasis regulates the number of release-ready vesicles on the time scale of minutes to weeks by structural protein redistributions.

# The role of LAP protein family members at the mammalian neuromuscular junction

Said Hashemolhosseini[1], Luca Simeone[1], Marion Straubinger[1], Muhammad Amir Khan[1], Tatiana Cheusova[1], Veronika Redai[1]

[1]Universität Erlangen-Nürnberg, Institut für Biochemie, Fahrstrasse 17, 91054 Erlangen, Germany

Erbin, a binding partner of ErbB2, was identified as the first member of the LAP family of proteins. Erbin was shown at postsynaptic membranes of the neuromuscular junction (NMJ) or in cultured C2C12 myotubes (1) to be concentrated, (2) to regulate the Ras–Raf–Mek pathway, and (3) to inhibit TGF-ß signaling. In the CNS, Erbin interacts with PSD-95. Furthermore, agrin–MuSK signaling initiates formation of AChR aggregates at the postsynaptic membrane. In search of proteins interacting with MuSK, we identified Erbin as aMuSK binding protein. We verified the interaction of MuSK with Erbin, or both concomitantly with ErbB2 by coimmunoprecipitation, and we mapped the interacting epitopes between Erbin and MuSK. We demonstrated elevated mRNA levels of Erbin at synaptic nuclei and colocalized Erbin and MuSK at postsynaptic membranes. We identified several Erbin isoforms at the NMJ, all of which contained the MuSK binding domain. By knocking down Erbin, we observed agrin-dependent AChR aggregates on murine primary skeletal myotubes and C2C12 cells, and in the absence of agrin, microclusters, both of significantly lower density. Complementary, AChR-e-reporter expression was reduced in myotubes overexpressing Erbin. We show that myotubes also express other LAP protein family members, namely Scribble and Lano, and that both affect physical dimensions of agrin-dependent AChR aggregates and density of microclusters formed in the absence of agrin. Moreover, MuSK–Erbin–ErbB2 signaling influences TGF-ß signaling. Our data define the requirement of Erbin on the cross talk between agrin and neuregulin signaling pathways at the NMJ.

# The Role of PSD-95 and Kinase Interactions in Synaptic Function

Derya Akad[1], Oliver M. Schlüter[1]

[1]European Neuroscience Institute, Molecular Neurobiology, Grisebach Strasse 5, 37077 Göttingen, Germany

Synaptic function in excitatory synapses is mediated by a highly compact network of synaptic proteins. One of these protein families located at the postsynapse is the Membrane Associated Guanylate Kinase (MAGUK) family. PSD-95, being a member of MAGUKs is known to play a key role in synaptic function by regulating the trafficking of AMPA receptors at glutamatergic synapses.
PSD-95 is proposed to function as a signaling scaffold, recruiting different kinases and phosphatases to the post-synaptic density. Furthermore, the function of PSD-95 itself is regulated by different kinsases, including cdk5. Cdk5 phosphorylates the N-terminus of PSD-95, regulating the subcellular distribution of PSD-95 and the interaction with the tyrosine kinase src. This interaction has been demonstranted to regulate the synaptic trafficking of NMDA receptors.
Using a molecular replacement approach, that is the silencing of endogenous PSD -95 with shRNAs and the expression of PSD-95 mutants from a bi-cistronic lentiviral vector, we analyzed the role of the cdk5 phosphorylation sites on the regulation of AMPA and NMDA receptor mediated synaptic transmission in CA1 pyramidal neurons of organotypic hippocampal slices.
We demonstrate that the phosphorylation mimicking mutant of PSD-95 could enhance AMPAR function resulting in an increase of the AMPAR EPSC amplitude. Using additional electrophysiological and biochemical assays, we will demonstrate data analyzing the role of the cdk5 phosphorylation in regulating PSD -95 function on both the AMPA and NMDA receptor component of synaptic transmission.

# Vertebrate-specific presynaptic protein Mover controls release probability at the calyx of Held

Christoph Körber[1], Darius Schwenger[1], Thomas Kremer[1], Thomas Dresbach[1], Thomas Kuner[1]

[1]University of Heidelberg, Institute for Anatomy and Cell Biology, Im Neuenheimer Feld 307, 69120 Heidelberg, Germany

Mover is a vertebrate-specific presynaptic protein previously discovered in a yeast-two-hybrid screen of bassoon-interacting proteins. Mover is expressed in subsets of excitatory and inhibitory synapses throughout the brain, including hippocampus, cerebellar cortex and brain stem. While a more global expression in all terminals would suggest an essential contribution to synaptic transmission, the pronounced subset-specificity of Mover expression points towards a synapse-specific modulatory function. Biochemical characterization revealed that Mover is attached to the surface of synaptic vesicles. However, the function of this protein remains unknown.
We have previously shown that Mover is expressed in the rat calyx of Held, a giant terminal in the auditory brain stem. To assess the function of Mover, we generated an in vivo knock-down of Mover using adeno-associated virus (AAV) mediated shRNA expression in globular bushy cells of the cochlear nucleus, the projection neurons forming the calyx of Held. Robust knock-down of Mover was first established in cultured hippocampal neurons by western blot analysis using an antibody directed against Mover. 3D Immunohistochemistry revealed a strong reduction of Mover expression in identified single calyces expressing mOrange in cis with the shRNA. Postsynaptic whole-cell recordings were established from cells receiving mOrange-expressing calyces and midline stimulation was used to evoke synaptic transmission. Calyceal Mover knock-down did not affect spontaneous synaptic transmission but increased the amplitude of evoked EPSCs. The size of the releasable pool was unaltered while short-term depression was accelerated and enhanced. Direct measurements of presynaptic calcium currents confirmed that these effects are not caused by altered calcium influx, but may be the result of differences in the calcium dependence of synaptic vesicle release. This was further substantiated by presynaptic capacitance measurements, showing that shorter depolarizing pulses were sufficient to evoke maximal release in Mover-depleted calyces compared to control calyces.
These findings are consistent with an increased release probability after Mover knock-down, suggesting that Mover could act as a negative regulator of release probability. The expression of Mover in certain subsets of synapses may thus constitute a novel mechanism to regulate release probability and short-term depression, and thereby tune the bandwith of synaptic transmission.

# VGLUT3-immunoreactive afferents of the lateral septum: anatomical evidence for a modulatory role of glutamate

Franziska Stöber[1], Eike Budinger[1], Riitta Miettinen[2], Karin Richter[3], Anett Riedel[4]

[1]Leibniz Institut for Neurobiology, Dept. Auditory Learning and Speech, Brenneckestraße 6, 39118 Magdeburg, Germany

[2]Institute of Clinical Medicine, Department of Neurology, University of Eastern Finland; CNServices Ltd, Microkatu 1, 70211 Kuopio, Finland

[3]Department for Biochemistry and Molecular Biology, Otto von Guericke University, Magdeburg, Institute of Biochemistry and Cell Biology, Leipziger Str. 44, Haus 36, D-39120 Magdeburg, Germany

[4]Department of Zoology/ Developmental Neurobiology, Otto von Guericke University, Magdeburg, Institute of Biology, Leipziger Str. 44, Haus 91, D-39120 Magdeburg, Germany

Through its extensive, predominantly reciprocal connections with various brain regions, extending from the telencephalon down to the spinal cord, the lateral septum (LS) is involved in higher cognitive functions like learning and memory, in neuroendocrine regulation, and in activities of the autonomic nervous system. Modulatory projections of the brain stem terminate in a layer-like fashion inside the LS forming basket-like axonal structures around their target neurons and playing a role in the adjustment of behavioural responses according to environmental demands. Utilizing multiple immuno-fluorescence labelling, we recently characterized a population of vesicular glutamate transporter 3 (VGLUT3) -immunoreactive ( -ir) afferents, which also terminate in a layer-like fashion throughout the entire rostro-caudal extend of the LS. At the light microscopic level, these VGLUT3-ir structures form prominent beaded structures, which ensheath the perisomatic area and proximal dendrites of their target neurons. However, the sub-cellular nature of the VGLUT3 -ir puncta and their source of origin remained unclear (Riedel et al., 2008. J. Chem. Neuroanat. 36).

Employing pre -embedding immunostaining for electron microscopy, we showed that the VGLUT3 -ir puncta outlining LS neurons represent pre-synaptic boutons. These are entirely filled with VGLUT3-ir vesicles and form primarily symmetrical synapses to their target neuron. However, we also observed some VGLUT3-ir boutons that formed asymmetrical synapses, which differed in their morphology from canonical asymmetric glutamatergic synapses typically formed by VGLUT1- and VGLUT2- expressing terminals.

Combining retrograde tracing with Choleratoxin B subunit and VGLUT3 -fluorescence labelling, we showed that neurons located in the median and dorsal raphe nuclei are the source of origin of the VGLUT3-ir LS afferents.

These data support the hypothesis that – in contrast to canonical glutamatergic neurons expressing VGLUT1 and VGLUT2 – VGLUT3-expressing neurons of the serotonergic raphe nuclei employ glutamate as a modulator or co-transmitter to LS.

# Poster Topic

## T8: Synaptic Plasticity, LTP, LTD

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*David Hinchliffe, Rupert Schmidt*

# Action of brain-derived neurotrophic factors at hippocampal mossy fiber-CA3 synapses

Sandra Maass[1], Petnoi Petsophonsakul[1], Volkmar Lessmann[1], Elke Edelmann[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipziger Straße 44, 39112 Magdeburg, Germany

Brain-derived neurotrophic factor (BDNF) is an important mediator for long-term changes in synaptic transmission. The action of BDNF in synaptic plasticity is best described for hippocampal long-term potentiation (LTP) in the CA1 region. The induction of this form of LTP relies on NMDA receptor (NMDAR) activation. However, the action of BDNF in NMDAR -independent types of LTP largely remains elusive. One form of NMDAR-independent hippocampal LTP is the CA3 mossy fiber LTP. Therefore, we focus here on the possible role of BDNF at mossy fiber - CA3 synapses in acute hippocampal brain slices.

We established field potential recordings in the CA3 region of acute slices from Wistar rats (P20-P25) and C57Bl/6J mice (6-9 weeks), respectively, by stimulating mossy fibers with an extracellular bipolar stimulation electrode in different regions of stratum lucidum of the CA3 region, or directly in the dentate gyrus. Field potentials were recorded from apical dendrites in CA3. Mossy fiber signals were distinguished from associative-commissural (A/C) signals by the pronounced paired pulse facilitation, as well as frequency- and train-facilitation. If mossy fiber signals were identified by these criteria, LTP was induced in presence of 50 µM DL-APV by a 1s 50 Hz stimulation protocol repeated three times every 20s. Purity of mossy fiber signals was routinely assessed by blocking of metabotropic glutamate signaling through application of 1µM DCG-IV. A/C fibers, which do not show any frequency facilitation, were stimulated in the absence of APV with the same stimulation protocol and served as a control.
After successfully establishing field potential recordings of mossy fiber LTP, we are currently analyzing the involvement of BDNF in these forms of LTP by using acute and chronic BDNF-deficiency models. Therefore we are analyzing mossy fiber LTP in heterozygous BDNF knock-out mice (6 -9 weeks of age), which chronically lack half of the BDNF content of wild type littermates. In order to analyze the involvement of acute BDNF-signaling in the induction/expression mechanism of mossy fiber LTP.experiments using pre-incubation with the tyrosine kinase inhibitor K252a, and with a scavenger (TrkB -Fc) of endogenously released BDNF were performed in C57Bl/6J mice,

Using this parallel analysis of NMDAR-independent mossy fiber LTP and NMDAR-dependent A/C-LTP, we should be able to directly compare the involvement of BDNF in both types of LTP in the same subregion of the hippocampal formation under identical recording conditions.

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# Activity dependent scaling of GABAergic excitation by dynamic $Cl^-$ changes in Cajal-Retzius cells

Sergej Kolbaev[1], Werner Kilb[1], Heiko J Luhmann[1]

[1]Universitaetsmedizin der Johannes Gutenberg-Universitaet, Institut fuer Physiologie und Pathophysiologie , Duesbergweg 6, D-55128 Mainz, Germany

The predominance of excitatory GABAergic responses during early phases of neuronal development implicates the question, how the excitability within immature neuronal networks is regulated in the absence of GABAergic inhibition. On the other hand, the limited capacity of NKCC1 mediated $Cl^-$ uptake systems may lead to a reasonable reduction the intracellular $Cl^-$ concentration ($[Cl^-]_i$) upon activation of $GABA_A$ receptors, thus decreasing the excitatory potential of GABAergic transmission. To unravel the activity- dependent $[Cl^-]_i$ dynamics and its functional implications we analyzed the GABA reversal potential ($E_{GABA}$) and the GABAergic effect on the rheobase (i.e. the threshold current required to induce an action potential) during repetitive activation of $GABA_A$ receptors and episodes of Carbachol -induced spontaneous GABAergic activity using gramicidin-perforated patch -clamp recordings from Cajal -Retzius cells (CRc) in tangential cortical slice preparations of immature mice (postnatal days 1-4).

Our experiments revealed that focal application of 10 µM muscimol provoked in all 29 CRc investigated a membrane depolarization, which in 24 cells induced a negative shift in the rheobase, indicating an excitatory effect of $GABA_A$ receptor activation. Repetitive focal muscimol applications (50 pulses at 0.5 Hz) induce a significant ($p<0.001$) decrease in $E_{GABA}$ from $-40 \pm 1$ mV to $-45 \pm 1.1$ mV (n=18), corresponding to a shift in $[Cl^-]_i$ from approximately 28 to 23 mM. This drop in $E_{GABA}$ led to a significant ($p<0.001$) reduction in the GABAergic rheobase shift from $-6.9 \pm 1.5$ pA to $-0.8 \pm 1.8$ pA, suggesting that the excitatory potential of $GABA_A$ receptor activation was nearly abolished under this condition. A similar reduction in $E_{GABA}$ was also induced by the activation of intrinsic GABAergic networks with Carbachol. Bath application of 30µM Carbachol enhanced the frequency of postsynaptic currents (PSCs) in CRc from less than 0.1 Hz to $4.4 \pm 0.7$ Hz (n=11), an effect that was completely suppressed by 3 µM gabazine. These Carbachol-induced GABAergic PSCs led to a significant reduction in $E_{GABA}$ from $-35 \pm 2$ mV to $-40 \pm 3$ mV (n=11), corresponding to a shift in $[Cl^-]_i$ from approximately 33 to 28 mM. In accordance with this reduction in $E_{GABA}$, the GABAergic rheobase shift was also significantly ($p=0.001$) reduced from $-7 \pm 2.6$ pA to $-4.4 \pm 2.5$ pA.

In summary, these observations demonstrate that the repetitive activation of $GABA_A$ receptors by extrinsic or intrinsic synaptic GABAergic inputs reduced $E_{GABA}$ and diminished the excitatory effect of $GABA_A$ receptor-mediated responses. This finding suggests that the limited capacity of $[Cl^-]_i$ homeostasis in immature neurons could be a substrate for synaptic scaling and homeostatic plasticity, a mechanism that may be used to avoid hyperexcitability in immature nervous systems.

# Activity-dependent regulation of GABAergic bouton plasticity

Anne Schuemann[1], Tobias Bonhoeffer[1], Corette J. Wierenga[1]

[1]Max Planck Institute for Neurobiology, Department for Cellular and Systems Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

In neuronal networks, several plasticity mechanisms act in concert to allow storage of new information, while at the same time maintaining a certain operating level of activity. During all these processes, a correct balance of excitation and inhibition is crucial. Although several forms of plasticity have been observed and described for glutamatergic synapses, the extent of GABAergic plasticity remains largely unexplored.
Our group recently demonstrated that GABAergic axons are capable of forming new synaptic contacts at a timescale of several hours. These contacts occur exclusively through the formation of new GABAergic boutons at pre-existing axon-dendrite crossings. Here, we examine how this form of structural GABAergic plasticity is affected by neuronal activity.
GAD65-GFP hippocampal slice cultures, in which mostly reelin-expressing, dendritically targeting interneurons are labeled, were used for this study. We imaged GFP-expressing GABAergic axons using two-photon microscopy for four to five hours at 30 min intervals.
GABAergic boutons consisted of two sub-populations. Although the majority of boutons was non-intermittently present throughout the imaging period (stable boutons), a significant fraction of boutons (30-40%) were present only part of the imaging period and often showed multiple appearances at the same position (non-stable boutons). Non-stable boutons had >2-fold smaller volumes and showed larger variation in their volumes (measured by the CV).
Activity blockade with TTX acutely stabilized GABAergic boutons, mostly by reducing the number and turnover of non-stable boutons. Bouton stabilization was maintained, although less pronounced, after 48 hours in TTX. Enhancing activity with 4-AP acutely reduced the number of stable GABAergic boutons and increased bouton turnover. Bicuculline also increased turnover, but only after 48 hours, suggesting that – at least initially – blockade of GABAA receptors could abolish the effect. These results indicate that activity affects plasticity of GABAergic boutons, and therefore potentially synapse formation. We are currently reducing the area of activity manipulation by applying local superfusion and other techniques in order to shed light on the spatial properties of GABAergic plasticity and its possible interactions with excitatory plasticity.

# Alpha-1 adrenergic receptors habilitate a stimulus-specific decrease in primary visual processing of adult mice

Mario Trevino Villegas[1], Sebastian Frey[1], Georg Köhr[1]

Max-Planck Institute, Molecular Neurobiology, Jahnstrasse 29, 69120 Heidelberg, Germany

Neuronal receptive fields are modified by experience through plastic mechanisms like long-term potentiation (LTP) and depression (LTD). It is clear, however, that experience-induced plasticity not only depends on the patterns of sensory input, but also on the level of neuromodulatory signals related to the behavioral state of the animal. Recent evidence reveals that neuromodulator receptors provide a robust control of the polarity of spike timing dependent plasticity (STDP) in the primary visual cortex (V1) in vitro (Seol GH et al., 2007). For example, a1-adrenoreceptors (a1-AR), which are coupled to phospholipase C, gate the expression of associative LTD and suppress LTP (Treviño M. and Kirkwood A., 2008-S-102674-SfN). Here, we explored whether a1-AR neuromodulation of plasticity is operational in V1 of adult mice in vivo. We found that systemic application of a1-AR agonists in conjunction with 1 hr of visual grating stimulation depresses active synapses from layer 2/3 pyramidal cells ex vivo. Notably, this manipulation also produced a rapid decrease of the visual acuity of the mice, a sensory discrimination threshold that depends on V1 processing. Plasticity in visual performance with this task is orientation-specific because it is not transferred if visual stimulation during neuromodulator action is orthogonal to testing orientation (an arrangement that segregates activated from nonactivated synapses), and is also absent when visual experience is provided under global blockage of NMDARs. Therefore, we propose that neuromodulators enable and control Hebbian plasticity of active synapses in V1 leading to a permanent change in the way specific visual information is transferred between and within superior cortical layers.

# Analysis of Dendritic Spine Plasticity with 2-Photon Glutamate Uncaging and 2-Photon Imaging

Daniel Meyer[1], Tobias Bonhoeffer[2], Volker Scheuss[3]

[123]Max Planck Institute of Neurobiology, Cellular and Systems Neurobiology, Am Klopferspitz 18, 82152 Munich, Germany

Synaptic plasticity, the activity-dependent, structural, and functional remodeling of neuronal connections, is thought to form the basis of learning and memory. One form of plasticity, synaptic strengthening, is associated with an increase in the volume of dendritic spines. It has been shown by electron microscopic studies that the major synaptic structures, on the postsynaptic side the spine head and postsynaptic density (PSD), and on the presynaptic side the bouton and active zone (AZ), correlate in size. Therefore, the increase in spine volume observed during synaptic strengthening would be expected to involve parallel increases in PSD, bouton and AZ size.

Here we examined the correlation between spine and PSD size during morphological spine plasticity. By 2-photon microscopy, we imaged pyramidal neurons in hippocampal slice cultures expressing tdTomato as cytosolic marker and eGFP tagged PSD-95, an abundant scaffolding protein of the PSD which we used as a reporter of PSD size. As expected, we found that PSD -95-eGFP levels in naïve spines correlate with their size. We then induced plasticity in individual spines by local 2-photon glutamate uncaging, and recorded the resulting spine growth and changes in PSD -95-eGFP level. We expected that after plasticity induction the increase in spine size would be accompanied by an increase of the PSD and thus PSD-95-eGFP level. However, as previously reported by Steiner et al. (Neuron, 2008) we found no PSD-95-eGFP increase in spines after plasticity induction when they were studied at room temperature. However, when performing the same experiments at 35 °C, we observed two populations of spines, one showing a significant increase in PSD-95-eGFP level after plasticity induction, and one which did not show this increase. We have now set out to explore the factors determining whether or not a significant PSD-95 increase accompanies persistent spine growth.

# Aromatase activity is essential for the induction of LTP in hippocampal slices

Ricardo Vierk[1], Günther Glassmeier[2], Lepu Zhou[1], Gabriele Rune[1]

1 Institut of Anatomy I: Cellular Neurobiology, University Medical Center Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg, Germany and 2 Institute of Physiology and Pathophysiology

Endogenous hippocampal estrogen synthesis maintains hippocampal spine synapses. We focused on the functional significance of spine loss after the inhibition of local estrogen synthesis by using long-term potentiation at CA3-CA1 synapses as cellular parameter of learning and memory. To this end, we reduced hippocampal estrogen synthesis in vitro and in vivo with letrozole, a reversible non-steroidal aromatase inhibitor. In hippocampal slice cultures of rats, letrozole treatment resulted in a significant decrease in 17ß-estradiol release into the medium, which was accompanied by a significant decrease in spine synapse number in the stratum radiatum of CA1. In these cultures, theta burst stimulation of CA3-CA1 Schaffer collaterals consistently failed to induce long-term potentiation after letrozole treatment. To prove the functional significance of aromatase activity in vivo, we used acute hippocampal slices of adult mice, which were treated with daily intraperitoneal injections of letrozole. After 6 hours of treatment LTP was already impaired by 30% and progressively increased, so that after 7 days of treatment LTP was no longer induced. Induction of LTP has been shown to be associated with phoshorylation of cofilin (p-cofilin), an actin-associated protein which stabilizes spines by its phosphorylation. We found a downregulation of p-cofilin after letrozole treatment. Taken together, our findings demonstrate an essential role of aromatase activity in long-term potentiation, which may account for memory deficits in letrozole-treated patients during breast cancer therapy.

# Astrocytes control spike-timing dependent long-term depression at cortical synapses

Thomas Nevian[1], Rogier Min[1]

[1]University of Berne, Department of Physiology, Bühlplatz 5, 3012 Bern, Switzerland

Spike-timing dependent plasticity (STDP) is an important mechanism for activity-dependent changes in synaptic strength. At neocortical synapses it has been implicated in developmental refinement of receptive fields. STDP might also be a mechanism for memory formation in neuronal networks. We investigated the molecular mechanisms of spike-timing dependent long-term depression (tLTD) at synapses between layer 4 spiny stellate and layer 2/3 pyramidal neurons in juvenile rat somatosensory barrel cortex, which are yet incompletely understood. This form of tLTD was dependent on retrograde signaling by endocannabinoids which were synthesized in the postsynaptic neuron resulting in a presynaptic modification of release probability. In addition, we found that astrocytes were required for the expression of tLTD. Blocking calcium signaling or exocytotic release of vesicles in astrocytes in close proximity to the postsynaptic layer 2/3 pyramidal neuron prevented the expression of tLTD. Supplementing d-serine, a coagonist for NMDA receptors did not recover tLTD. Our experiments suggest that retrograde signaling via astrocytes is a key step in the spike -timing dependent modification of synaptic efficacy.

# BDNF-knockdown in individual CA1 pyramidal neurons does not affect basal synaptic properties

Julia Daniel[1], Tanja Brigadski[1], Volkmar Lessmann[1]

[1]Institute of Physiology, Faculty of Medicine, Otto-von-Guericke University, Leipziger Str. 44, 39120 Magdeburg, Germany

The protein BDNF is a member of the mammalian neurotrophin family. Besides the important role of BDNF in neuronal survival and differentiation, BDNF is a critical regulator of acute and long-term changes in synaptic plasticity. At the synaptic level, BDNF has both, pre- and postsynaptic effects. The application of BDNF acutely stimulates neurotransmitter release and phosphorylation of ionotropic receptors, which can drive fast changes of glutamatergic and GABAergic synaptic transmission. Long-term application of BDNF to neuronal cultures regulates morphological synaptic changes, enhances glutamatergic as well as GABAergic transmission by pre- and postsynaptic mechanisms, and is also a mediator of synaptic homeostasis. Moreover, studies with BDNF knockout mice confirm the significance of BDNF in acute and long-term regulation of synaptic transmission. While many of the studies analysing the effect of BDNF on synaptic transmission have been performed by unrestricted administration or withdrawal of BDNF in complex neural networks, understanding more subtle mechanisms of synaptic changes by BDNF requires single cell overexpression or knockdown of the neurotrophin.
In order to analyse the consequences of a BDNF-deficit at a cellular level, we have developed an efficient method to knock down BDNF expression in single CA1 pyramidal cells. Hippocampal slices from newborn mice were prepared according to the Stoppini method. Using single cell electroporation, CA1 pyramidal cells were cotransfected at 10 DIV with a validated siRNA against BDNF and EGFP for later cell retrieval. Three days after transfection whole cell patch-clamp recordings of transfected cells were performed to analyse excitatory synaptic transmission. Electrophysiological properties like miniature synaptic currents, properties of action potentials, paired-pulse facilitation and synaptic fatigue revealed no significant difference between control and BDNF-deficient cells in a BDNF containing context. In addition, postsynaptic currents were recorded before and after high frequency stimulation of Schaffer collaterals and induction of LTP was analysed. Our results suggest similar basal electrophysiological and synaptic properties of BDNF-deficient cells grown in a BDNF-containing cellular context, suggesting that BDNF-withdrawal in a single cell can be compensated by the neighbouring BDNF-expressing cells.
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# Chemical-induced LTP elicits different effects on the morphology of hippocampal cultured neurons

Alberto Montalbano[1], Gabriele Baj[1], Giuseppe Tatò[1], Enrico Tongiorgi[1], Marina Sciancalepore[1]

[1]University of Trieste, Department of Life Sciences, Via A. Fleming, 22 , 34127 Trieste, Italy[1]

Long-term potentiation induced by chemicals (cLTP) is able to activate a large number of synapses and dendritic spines in cultured neurons. The purpose of this study was the characterization of the effects induced by administration of glycine or TEA in cultured hippocampal neurons both on amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs). Spontaneous mEPSCs were detected in whole-cell patch clamp recordings at 12-14 days in culture. The activation of NMDA receptors was achieved by brief (5 min) delivery of glycine (200 μM) whereas the blockade of $K^+$ channels was obtained by 5 min bath application of TEA (25 mM). These data were collected in the presence of TTX, strychnine and gabazine in the bath solution. Averaged mEPSCs were recorded and both amplitudes and frequencies were compared prior to (control) and 20 min after (washout) the application of the potentiating solution, showing a constant potentiation in both conditions. Furthermore we investigated the changes in number and length of specific dendritic spines types induced by these two drugs. At the end of the electrophysiology protocol cells were labeled with a fluorescent dye in order to collect measurements of density, length and head diameter of dendritic spines. Morphological analysis was conducted on the three main classes of dendritic spines: stubby, thin and mushroom. In conclusion, we found that both types of cLTP potentiate the miniature glutamatergic activity in cultured hippocampal cells. In addition a remodeling of different classes of dendritic spines was observed.

# Compartment-specific dopaminergic modulation of synaptic plasticity in hippocampal CA1 via NMDA receptors containing NR2B

Marina Herwerth[1], Vidar Jensen[2], Martin Novak[3], Witold Konopka[3], Oivind Hvalby[2], Georg Köhr[1]

[1]Max Planck Institute for Medical Research, Molecular Neurobiology, Jahnstaße 29, 69120 Heidelberg, Germany
[2]Institute of Basic Medical Sciences, University of Oslo, Norway
[3]Molecular Biology of the Cell I, German Cancer Research Center, Heidelberg, Germany

The dopaminergic system in nigrostriatal, prefrontal and hippocampal synapses is recognized to play an important role in synaptic plasticity, learning and pathogenesis of psychiatric disorders. Within the hippocampal CA1 area, in particular in the dorsal hippocampus, dopaminergic fibers have a higher density in stratum oriens (OR) than in stratum radiatum (RAD). We confirmed this in hippocampal sections of four week-old mice by intensively stained dopamine active transporter (DAT)-positive fibers. Previous field recordings in RAD demonstrated that dopamine (DA) agonists stabilize NMDAR-dependent LTP. Here, we examined the role of DA for NMDAR-dependent LTP in OR versus RAD in acute slices of four week-old mice. In the presence of the D1/5 agonist SKF38393 field LTP showed a tendency for a higher absolute value, particularly in RAD, suggesting a differential modulation. Field LTP was induced by high frequency stimulation, whereas additional experiments induced LTP in single CA1 neurons by postsynaptic depolarization and simultaneous low frequency synaptic stimulation. In the latter case, presence of DA agonists strongly reduced LTP in OR, but not in RAD. This compartment-specific effect was caused by a DA agonist-boosted inactivation of synaptic NMDAR-mediated currents (NMDA EPSCs) through a Ca2+-dependent and G-protein independent mechanism. Furthermore, to analyze NMDAR subtype-specific characteristics for this dopaminergic modulation, we employed two gene-targeted mouse lines. In NR2A-/- mice, NR2A is constitutively ablated and still the DA agonist-mediated compartment-specific effect on NMDA EPSCs and LTP persisted. By strong contrast, the DA agonist effects were absent in NR2BΔFb mice, which selectively lack NR2B in principal forebrain neurons. These experiments indicated that DA agonists regulated synaptic efficacy in OR via NMDARs containing NR2B subunits. Thus, DA hyperfunction in OR may cause NMDAR hypofunction restraining NMDAR-dependent LTP in OR and therefore could be important for hippocampal learning and memory as well as for the pathogenesis of schizophrenia.

# CPEB2 represses the constitutive translation of CPEB target mRNAs

Sada Lakshmi Turimella[1], Vamshidhar Vangoor[1], Lech Kaczmarczyk[1], Peter Bedner[1], Stefan Paßlick[1], Gerald Seifert[1], Ronald Jabs[1], Christian Steinhäuser[1], Martin Theis[1]

[1]Institute of Cellular Neurosciences, University of Bonn, Sigmund Freud Strasse 25, 53105 Bonn, Germany

Cytoplasmic Polyadenylation Element Binding proteins (CPEBs) in neurons are required for synaptic plasticity, learning and memory. They regulate the translation of key players involved in synaptic plasticity such as the a-subunit of calcium/calmodulin dependent protein kinase (CaMKIIa), tissue plasminogen activator (tPA) and the AMPA receptor subunit GluR2. Previous data on cultured neurons indicated that CPEB3 represses the basal translation of the CPEB target mRNA encoding GluR2. To find out if CPEB2 is also a basal repressor of translation, we generated mice with neuronal overexpression of CPEB2 (tet-off system) and measured hippocampal protein levels of two known CPEB targets. With immunoblotting and immunohistochemistry, we observed a downregulation of GluR2 and beta catenin. We have confirmed specificity of the effect by demonstrating that in these mice the protein levels of GluR1, another AMPA receptor subunit, remained unaltered. In mice overexpressing a CPEB2 mutant lacking the zinc finger in neurons, the protein levels of GluR2 and beta catenin were not changed indicating the importance of zinc finger-mediated RNA binding for translational repression. The observed effect on basal protein levels makes CPEB2 overexpressing mice a powerful tool to screen for new CPEB targets. Since GluR2 protein levels are downregulated in these mice, we are currently investigating the impact of CPEB2 on AMPA receptor function.

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# Distribution of ependymins and their binding partners in subcellular fractions of goldfish brain

Roman Göthe[1], Rupert Schmidt[1]

[1]Justus-Liebig-Universität Gießen, Biotechnology Center, Leihgesterner Weg 217, 35392 Gießen, Germany

Ependymins are secreted glycoproteins of the central nervous system (CNS) in goldfish (*Carassisus auratus*). They exist in a mono-N-glycosylated (= γ) and a bi-N-glycosylated (= ß) form. Ependymins are known to take part in memory consolidation [1] and neuronal regeneration. After active shock avoidance conditioning ependymin mRNA is rapidly induced in goldfish meningeal fibroblasts followed by enhanced ependymin synthesis and secretion. Memory consolidation was inhibited both, after blocking ependymin with anti-ependymin antisera and with antisense-oligodesoxynucleotides via intracranial injection [2].

Previous studies on goldfish brain exhibited a distinct distribution of ependymins and other proteins in various subcellular fractions of the CNS [3]. The major portion of ependymins was localized in the cytoplasm, whereas in the extracellular fluid, ependymins are the most prominent protein components (highest specific concentrations). Detailed studies of the pelleted fractions (P1 to P3) revealed a differentiated distribution of ependymins with the lowest amount in the mitochondrial fraction (P2) and the highest amount in the microsomal fraction (P3).

Because ependymins operate via the extracellular brain fluid in memory consolidation and regeneration processes, it is necessary to identify their interaction partners to understand ependymins' molecular function. Therefore, the different fractions were analyzed by 2D-gelelectrophoresis and Far-Western-Blotting with radioactive [$^{125}$I]-labeled ependymins. 17 different proteins between 20 and 55 kDa showed binding of ependymins. These proteins have a different distribution in various subcellular fractions. Some are present in almost all fractions, whereas others are present in only one fraction. Furthermore, [$^{125}$I]-ependymin binding to endogenic ependymin was shown in the extracellular fraction, in the vesicular fraction (P3) and in a fraction of glycoproteins separated by Concanavalin A affinity chromatography. These are the fractions where ependymin is the most prominent protein in goldfish brain. Apparently ependymin preferentially binds to other proteins present, as compared with homophilic binding to ependymin itself. Comparative studies exhibited that [$^{125}$I]-ependymin-ß has higher heterophilic binding preferences to other proteins than [$^{125}$I]-ependymin-γ, respectively.

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# Early stress experiences prevent emotional reinforcement of hippocampal long-term potentiation in adult male rats: active extinction of traumatic memories as a disease protective mechanism?

HAN WANG[1], Katrin Meyer[1,2], Volker Korz[2,3]

[1]Leibniz Institute for Neurobiology
[2]Otto von Guericke University Magdeburg, Institute for Biology
[3]Center for Behavioral Brain Sciences

Maternal separation (MS) paradigms are well established animal models to analyze stress related brain disorders. In order to study the effects of MS on hippocampal long-term potentiation (LTP), which is considered as a cellular model of memory formation, a repeated separation paradigm that male Wistar rats were deprived from their dams for everyday 6 hours from postnatal day (PND) 14 to PND 16 was used and the animals were tested for LTP in the dentate gyrus (DG) at adolescence (10 weeks old). Here, we show that the foregoing separation impairs the emotional LTP-reinforcement (transferring early-LTP into late-LTP) induced by exposing the animals to a 10-min. elevated-platform-stress immediately after the high frequency stimulation (HFS) triggering early-LTP in DG.
In order to reveal possible involvements of stress related steroid hormones on the observed LTP-modulation, we studied the expression patterns for a series of steroid hormone receptors, hormones and neurotrophic factors after stress in comparison to non-deprived rats. Adult stress strongly increased the level of corticosterone in 15 min. after HFS and hippocampal mRNA of the corticosterone binding mineralocorticoid receptor (MR), whose activation is proved to be necessary for emotional LTP -reinforcement, 1h after HFS in control rats, whereas deprived rats showed no significant increase of MR expression after adult stress. The similar difference appeared also in the expression of estrogen receptor (ER) mRNA, although there is no difference in 17ß-estradiol level in hippocampus between deprived and non-deprived animals. Furthermore, deprived rats show an increased hippocampal BDNF level 24h after HFS and stress as compared to controls. We hypothesize active extinction of traumatic memories in adult early deprived rats induced by steroid-BDNF interactions to protect from further traumatic overload. The involved mechanism might be due to epigenetic responses of steroid hormone receptor expression to stress during adulthood and differences in gene expression achieved by chromatin remodeling.

# Excessive 5-HT levels during development affect signal propagation and short term plasticity in the rat barrel cortex in vitro

Dirk Schubert[1], Martijn Selten[1], Moritz Negwer[1], Thomas Slippens[1], Judith Homberg[1], Rembrandt Bakker[1]

[1]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Department of Cognitive Neuroscience, Geert Grooteplein 21, 6525 EZ Nijmegen, Netherlands

Dysfunction of 5-HT transporters (5-HTT) leads to increased 5-HT levels during brain development. In rodents this causes distorted wiring in afferent pathways of sensory systems, which might have profound effects on structure and function in the primary somatosensory (barrel) cortex. We investigated how synaptic signal propagation and short-term plasticity are affected in the well-defined cortical microcircuits of the rat barrel cortex when 5-HTT expression has been genetically knocked out (5-HTT KO). We employed extracellular multi-electrode-array (MEA) recordings of local field potentials (LFPs), as well as whole cell patch clamp and layer specific electrical stimulation, on thalamocortical brain slice preparations of (i) juvenile wildtype and (ii) 5-HTT KO rats. The short term plasticity was tested by applying paired pulse stimulation (electrical stimuli at 50 ms interstimulus intervals). In 5-HTT KO rats under physiological conditions, paired pulse stimulation in the cortical layers II to VI led to increased amplitudes in the excitatory response of the initial LFP. However, we found layer specific changes in the short term plasticity: as compared to wildtype animals, in 5-HTT KO rats stimulation of the input layer IV resulted in significantly increased paired pulse facilitation (PPF) of excitatory responses, stimulation in the associative layers II/III resulted in decreased PPF. Stimulation of infragranular layers Va, Vb and VI resulted in no significant differences between LFP responses of 5-HTT KO and wildtype rats. Simultaneous recordings of stimulus evoked EPSPs in layers II/III, IV and Vb pyramidal cells showed, at least partially, similar changes in the paired pulse facilitation. This illustrates that in 5-HTT KO animals cortical short term plasticity might be changed on both neural population and single cell level. Furthermore, similar layer-specific alterations of LFP plasticity could be induced by exogenous applications of 5-HT in wildtype brain slices. In 5-HTT KO rats, however, this 5-HT application had no detectable effect on layer specific PPF. Our data indicate that increased 5-HT levels during brain development do not only affect the wiring in the afferent pathways of the rodent somatosensory system (e.g. thalamocortical afferences), but also the intracortical networks. This seems to comprise the pathway that transmits sensory information from the granular layer IV, which is a main target layer for thalamocortical afferences of the lemniscal pathway, to the associative layers II/III. Interestingly, changes of short term plasticity seem to be contrary for responses following stimulation of either layer IV or layers II/III, namely increased plasticity in the input layer and decreased plasticity in the associative layers. Given that the correct thalamocortical wiring is distorted in 5-HTT KO animals, these changes in cortical plasticity might be part of compensatory intracortical mechanisms that enable the animals to still make use of perceived tactile sensory information.

# Fucosylated proteins in the brain – where, what and why?

Nicole Höche[1], Karin Richter[2], Oliver Kobler[3], Wolfgang Tischmeyer[4], Karl-Heinz Smalla[4], Daniela C. Dieterich[1]

[1]Leibniz Institute for Neurobiology, RG Emmy Noether, Magdeburg, Germany
[2]Inst. of Biochemistry and Cell Biology, OvG University, Magdeburg, Germany
[3]Leibniz Institute for Neurobiology, Dept. Neurochemistry, Magdeburg, Germany
[4]Leibniz Institute for Neurobiology, Lab. Mol. Biol. Tec., Magdeburg, Germany

Protein glycosylation, especially fucosylation, is an important post-translational modification and provides a selective and temporal mean for controlling protein functions. The covalent attachment of carbohydrates like fucose provides the means to increase the functional diversity of proteins and to influence their biological activity. Interestingly, fucosylated carbohydrate structures in the brain have been implicated in molecular mechanisms that underlie neuronal development, learning and memory. Plasticity phenomena like hippocampal long-term potentiation (LTP) and memory formation, for instance, are accompanied by a transient increase in the incorporation of fucose into membrane glycoproteins. Most notably, inhibition of protein fucosylation does not interfere with LTP induction or memory acquisition, but prevents specifically the maintenance of LTP and long-term memory. The mechanisms underlying the particular importance of protein fucosylation for phenomena of long-term synaptic plasticity as well as the identity of synaptic fucosylated proteins, however, are largely unknown. To better understand the role of protein fucosylation in the context of neuronal plasticity, it is key to identify fucosylated synaptic proteins and the modes of their synthesis and modification, and to determine their subcellular localization. In the present study, we use the fucose-specific lectin from Aleuria aurantia (AAL) to investigate the distribution of fucose-containing carbohydrate moieties in the rat brain. We find strong AAL staining of membrane structures especially in synaptic neuropil regions. To identify fucosylated synaptic proteins, extracts from synaptic junctions were analysed either in an approach using AAL-affinity chromatography or in an unbiased targeted approach using immunoprecipitations and AAL blotting. For the targeted approach we focused on proteins previously implicated in neuroplasticity, i.e. neurotransmitter receptors, cell adhesion molecules, extracellular matrix proteins, voltage-gated potassium and calcium channels, growth factor receptors and ligand-gated ion channels. In addition, we performed an alternative strategy for tagging biomolecules using an azide-labelled fucose derivative to specifically label and identify de novo fucosylated proteins in primary cortical cultures after stimulation with NMDA. As a result of this combined approach we identified a diverse range of already known as well as so far unknown fucosylated proteins, which have been implicated in processes of neuroplasticity. Among the well-known candidates are neuroplastin and Thy 1.1, among the new fucosylated proteins are Caspr 2 and Kv 1.2.

# Interaction Partners of Neuronal Calcium Sensor-1 in Mouse Brain

Malte C. Stockebrand[1], Joanna Hermainski[2], Olaf Pongs[2]

[1]DFG Heisenberg Team Experimental Neuropediatrics, [2]Institute for Neural Signal Transduction, Center for Molecular Neurobiology Hamburg (ZMNH), Falkenried 94, 20251 Hamburg, Germany

The neuronal calcium sensor protein NCS-1 has been implicated in the modulation of synaptic efficacy and vesicular trafficking. Yeast NCS-1 orthologue Frq1 functions as regulatory subunit of phosphatidylinositol 4-kinase Pik1, playing an important role in Golgi function and vesicle trafficking. Given the high sequence conservation of NCS-1 proteins across phyla, we hypothesized that mammalian NCS-1 also functions in vesicular trafficking.

To address this hypothesis, we generated NCS-1 knockout mice as well as transgenic mice expressing GFP-tagged NCS-1 (NCS-1-EGFP) in forebrain. We prepared forebrain lysates from transgenic mice and isolated NCS-1-EGFP containing protein complexes by size-exclusion chromatography followed by immunoprecipitation with anti-GFP antibodies. Immunoprecipitated proteins were separated by SDS-PAGE. For control, we used brain lysate from transgenic mice expressing only EGFP under control of the same promoter. Specific protein bands, not apparent in controls were isolated and characterized by liquid chromatography coupled tandem mass spectrometry (LC-MS/MS).

The LC-MS/MS-analysis indicated several candidate interaction partners of NCS-1, but a mammalian Pik1 orthologue was not among them. Instead, we identified two novel candidate proteins, Bet3/TRAPPC3 and SNAP-47. Both proteins play a yet ill-defined role in vesicular trafficking in eukaryotic cells. Their interaction with NCS-1 could be confirmed by co-immunoprecipitation with anti-NCS-1 antibodies from brain lysate of wild type mice using NCS-1 knockout animals for control. Immunofluorescence analysis showed co-localization of NCS-1 with Bet3/TRAPPC3 and SNAP-47 in mouse neurons.

We propose that *in vivo* NCS-1 plays a role in $Ca^{2+}$-dependent regulation of intracellular vesicle trafficking.

# Learning-facilitated synaptic plasticity at CA3 mossy fiber and commissural-associational synapses reveal different roles in information processing

Hardy Hagena[1], Denise Manahan-Vaughan[1]

[1]Ruhr-University Bochum, Medical Faculty, Department of Neurophysiology, Universitaetsstr.150, 44780 Bochum, Germany

Persistent synaptic plasticity, in the form of long-term potentiation (LTP) and long-term depression (LTD), may comprise the cellular mechanism underlying long-term memory (Manahan-Vaughan and Braunewell, 1999; Braunewell and Manahan-Vaughan, 2001, Morris et al., 2003). It has been shown during the last decade, that LTP occurs at most hippocampal synapses as soon as novel spatial information processing is required and thus may comprise a fundamental encoding response. Object-place configurations, on the other hand, facilitate LTD. Whereas landmark features of an environment facilitate LTD in the dentate gyrus, small, discrete features of an environment facilitate LTD in area CA1. The CA3 region has remained unexplored on this level. Here, we investigate the role of two distinct synapses, the mossy fiber (mf)- and associational commissural (AC)- CA3 synapses, in synaptic plasticity in regard to different environmental features.
Male Wistar rats (7-8 weeks, Charles River, Germany) underwent chronic implantation of hippocampal electrodes under anesthesia, as described previously (Hagena and Manahan -Vaughan, 2010). Ten days later chronic monitoring of evoked potentials at mossy fiber- and AC - CA3 synapses was begun. Short-term depression (STD) was induced by low-frequency stimulation (LFS) at 1 Hz. Short-term potentiation (STP) was elicited using 100 Hz stimulation. To evaluate the effect of learning on synaptic plasticity, a holeboard was used. It contained 4 holes, each containing a small object in each hole, or alternatively, 3 large objects (landmark cues). The holeboard was introduced into the recording chamber during the plasticity-inducing stimulation only.
Exploration of the objects within the holeboard holes did not facilitate LTD, whereas exploration of the three large "landmark" cues led to a persistent facilitation of LTD (>24 h) at mf - CA3 synapses. At AC - CA3 synapses, novel exposure to the objects within the holes facilitated LTD. Exploration of the three novel large "landmark" cues during application of sLFS significantly impaired the induction of STD compared to controls. LTP was facilitated at both mf - CA3 and AC - CA3 synapses after exploration of novel space during application of STP.
Our data suggest a unique and distinct function for CA3 synapses in spatial information processing: whereas mf – CA3 synapses and AC – CA3 synapses respond identically with LTP upon exposure of the animal to novel space, they demonstrate very distinct LTD responses in association with different aspects of contextual spatial learning. This in turns supports the likelihood that the CA3 region serves as an integrator of different kinds of spatial information, that in turn contribute to the generation of spatial representations of the environment.

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# Long-term plasticity in BDNF knockout mice at distinct projections to the lateral amygdala in relation to age

Susanne Meis[1], Thomas Endres[1], Thomas Munsch[1], Volkmar Lessmann[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipzigerstr. 44, 39120 Magdeburg, Germany

The neurotrophin brain-derived neurotrophic factor (BDNF) has been studied intensely in the context of its trophic and growth-promoting effects during development. In addition, BDNF plays a key role as a modulator of synaptic strength. In the amygdala, recent data suggest a correlation between learning and LTP-like changes (Musumeci et al., 2009, J Neurosci. 29:10131-10143). Indeed, our own recent studies indicate that BDNF is crucial for amygdala synaptic plasticity as well as for acquisition and consolidation of amygdala-dependent fear learning. BDNF heterozygous knockout mice (BDNF+/- mice) which chronically lack BDNF show a learning deficit in response to a weak fear conditioning protocol when animals become older than 3 months of age (see poster by Endres et al.). Concurrently, parameters like general anxiety, and levels of activity remain unchanged between BDNF+/- mice and their wild type littermates.

To delineate the underlying cellular mechanism for the altered fear conditioning, we used field potential recordings in the lateral amygdala to study long-term potentiation at different ages. Different types of stimulation protocols were compared. These protocols comprised a high frequency stimulation pattern with three trains of 100 stimuli at 100 Hz separated by 30 s (HFS), or theta-burst stimulation (TBS), with two trains of 4 stimuli at 100 Hz, repeated 10 times at 5 Hz separated by 20 s, respectively.

In wild-type (WT) mice, LTP could be reliably induced at thalamo- and cortico-amygdala synapses. At the thalamic input, HFS-LTP was already abolished in BDNF+/- mice at P55 to P69, when the learning deficit is not yet developed (BDNF+/-: 93.8 ± 2.7 % of initial field potential values, n = 4; WT: 134.8 ± 7.0 %, n = 18). At the cortical input, LTP was preserved in knock-out mice when potentiated by HFS at 3 to 5 months, when the learning deficit is already established (BDNF+/-: 114.1 ± 7.3 % of initial field potential values, n = 14; WT: 121.9 ± 10.1 %, n = 12). In addition, LTP was unrelated to age when elicited by TBS in cortico-amygdala synapses (P56-P68: BDNF+/-: 126.9 ± 10.2 %, n = 8; WT: 114.2 ± 3.5 %, n = 9; P94-P140: BDNF+/-: 110.7 ± 10.3 %, n = 8; WT: 114.3 ± 4.7 %, n = 7).

Therefore, the temporal characteristics of these stimulation protocols may not reflect adequately conditions necessary for behavioral modifications. Alternative stimulation protocols in the two genotypes are currently under study.

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# Loss of profilin1 impairs synaptic plasticity of hippocampal CA1 pyramidal cells

Anika-Maria Zimmermann[1], Andreas Görlich[1], Doreen Schober[1], Ralph T. Böttcher[2], Mumna Al Banchaabouchi[3], Marco Sassoè-Pognetto[4], Eckhard Friauf[5], Walter Witke[3,6], Marco B. Rust[1,3]

[1]Neurobiology/Neurophysiology group, University of Kaiserslautern, Germany
[2]Max Planck Institute of Biochemistry, Martinsried, Germany
[3]Mouse Biology Unit, European Molecular Biology Laboratory, Monterotondo, Italy
[4]Department of Anatomy, Pharmacology and Forensic Medicine and National Institute of Neuroscience-Italy, University of Turin, Italy
[5]Animal Physiology Group, University of Kaiserslautern, Germany
[6]Institute of Genetics, University of Bonn, Germany

Profilins are actin -binding proteins that are localized at synaptic sites and have been implicated in synaptic plasticity and regulation of dendritic spine morphology. Recently, it has been shown that profilin2 is crucial for controlling presynaptic excitability and neurotransmitter release at glutamatergic synapses. Here, we tested the hypothesis that profilin1 is mainly involved in postsynaptic mechanisms, complementary to the presynaptic role of profilin2. To do so and to elucidate the role of profilin1 in the adult brain, we specifically deleted profilin1 in forebrain neurons by using conditional knockout mice on a CaMKII-cre expressing background. We show that synapse density and spine morphology in hippocampal pyramidal neurons are independent of profilin1. CA1 long-term potentiation could be induced, but was significantly decreased upon profilin1 deletion. Moreover, the ratio of AMPA receptor/NMDA receptor -mediated currents was increased and learning -induced spine formation was reduced. Despite these changes in synaptic plasticity, profilin1 mutant mice had no deficits in short-term working memory or spatial and aversive learning. Our in vivo findings demonstrate for the first time a crucial role of profilin1 in regulating structural and functional plasticity in dendritic spines. Based on the facts that synapse density and spine morphology as well as learning performance were unchanged, and long-term potentiation was inducible, our data further suggest overlapping functions of profilin1 and profilin2 for the general integrity of synaptic structure and physiology.

# Metaplasticity governs compartmentalization of synaptic tagging/capture through BDNF and PKM zeta

Sreedharan Sajikumar[1], Martin Korte[1]

[1]TU-Braunschweig, Div.Cellular Neurobiology, Spielmann Str.7, 38106 Braunschweig, Germany

Prior synaptic activity can integrate synaptic signals over a particular time and this form of metaplasticity can contribute to functional plasticity such as synaptic tagging/capture. Recent evidence suggests that synaptic tagging/capture processes are restricted to specific compartments or in clusters. As a compartment or cluster we consider simultaneously activated synapses that might act as a functional unit within a dendritic branch. In this study we have explored how long - term memory engrams are specifically clustered in the apical dendrites of rat hippocampal CA1 neurons and which molecules mediate processes of metaplasticity. Specifically we have investigated the role of two major proteins such as brain-derived neurotrophic factor (BDNF) and atypical protein kinase C isotype PKM zeta in maintaining clustered plasticity. For this purpose we recorded long-lasting LTP (L-LTP), LTD (L-LTD) and its associative interactions up to 6 hours in apical dendritic compartments. We also analyzed how it differentially act in a metaplastic condition elicited by the activation of metabotropic glutamate receptors (mGluRs). What we could show here for the first time is how metaplasticity can modify the threshold for the compartmentalization or clustering process of long -term memory engrams and how such threshold is maintained by BDNF and PKM zeta.

# Metaplasticity of early-LTP by ryanodine receptor activation and its effect on synaptic tagging and capture

Qin Li[1], Zhi Cheng Xiao[2], Wickliffe C Abraham[3], Martin Korte[1], Sreedharan Sajikumar[1]

[1]TU Braunschweig, Cellular Neurobiology, Spielmnnstrasse 7, 38106 Braunschweig, Germany

[2]Monash Immunology and Stem cell laboratories, Monash University, Clayton, Vic 3800, Australia

[3]Department of Psychology and the Brain Health and Repair Research Centre, University of Otago, Dunedin 9054, New Zealand

Activity-dependent changes in synaptic strength such as long-term potentiation (LTP) and long-term depression (LTD) are regarded to be cellular mechanisms underlying learning and memory. Regulation of such synaptic plasticity by prior neural activity has been termed metaplasticity. In our previous study (Sajikumar et al., 2009), we showed that priming of short-term potentiation (STP) by ryanodine receptor agonist, ryanodine (10 μM) or caffeine (10 mM) can prolong the persistence of STP up to 40 min. This primed STP can take part in processes like synaptic tagging and capture. Our present data shows that priming of early-LTP with ryanodine receptor activation can enhance potentiation and increase its persistence up to 4.30 h. In addition primed early-LTP takes part in synaptic tagging and capture processes even after 4 h, showing the priming processes enhance the endurance of the synaptic tag. These findings indicate that activating the ryanodine receptor increases the Ca2+ concentration through store operated calcium (SOC) channels that activates different kinases including CaMKII, MAPK and PKC leading to local protein synthesis. The locally synthesized proteins may prevent the synaptic tags from degradation for an extended period of time.

# Mice deficient in the $Cl^-/HCO3^-$exchanger AE3 display increased high-frequency oscillations and long-term potentiation, and a shift in GABA reversal-potential

Gaga Kochlamazashvili[1,2], Laura Cancedda[1], Christian A Hübner[3], Alexander Dityatev[1,2]

[1]Fondazione Istituto Italiano di Tecnologia , Neuroscxience and Brain Technologies, Via Morego 30, 16163 Genova, Italy;[2]Zentrum für Molekulare Neurobiologie Hamburg (ZMNH), Universitätsklinikum Hamburg-Eppendorf, Martinistrasse 52, D-20246 Hamburg, Germany;[3]Institut,für Humangenetik, Universitätsklinikum der Friedrich Schiller Universität Jena, Kollegiengasse 10, D-07743 Jena, Germany

The sodium-independent electroneutral anion-exchangers (AEs) exchange intracellular bicarbonate for extracellular $Cl^-$. Mice deficient in AE3 (AE3-/-) show a reduced seizure threshold after exposure to bicuculline, pentylenetetrazole or pilocarpine and increased seizure-induced mortality as compared to wild-type littermates (Hentschke et al., Mol Cell Biol 2006). Here, we studied generation of synchronized high-frequency oscillations of neuronal activity in the hippocampus of AE3 -/- mice under physiological conditions. Our recordings in the CA1 region of urethane -anaesthetized mice revealed an increase in power of gamma oscillations induced by short episodes (5 -20 pulses) of 100 Hz stimulation. Similarly, oscillations induced by such stimulation were more prominent in hippocampal slices from AE3-/- mice. These oscillations could be blocked by substitution of bicarbonate- to HEPES -based buffer. In vitro recordings of long -term potentiation in AE3 -/- mice revealed normal levels of potentiation elicited by 100 Hz stimulation (100 pulses) at 50% of supramaximal intensity, a protocol which did not induce oscillations. However, when 100 Hz stimulation (20 pulses) was applied at the supramaximal intensity, at which gamma oscillations are more prominent in AE3 -/- mice, higher levels of LTP were observed in mutants. These data suggest that synaptic stimulation followed by stronger oscillations can lead to stronger synaptic modifications. Analysis of synaptic transmission and paired -pulse facilitation of synaptic responses in vivo did not reveal any abnormalities in AE3-/- mice. However, paired-pulse inhibition of population spikes was stronger in the mutant, suggesting a stronger feedback GABAergic inhibition. Indeed, analysis of I–V relationships for membrane current responses elicited by puffing GABA at the soma of CA1 neurons in hippocampal slices revealed that GABA-reversal-potential was shifted by 6 mV to more negative values in AE3-/- mice, as compared to wild-type littermates. In summary, these data suggest a decrease in intracellular $Cl^-$ and an increase in intracellular bicarbonate concentration in AE3-/- mice, and demonstrate the importance of AE3 in generation of neuronal oscillations and synaptic plasticity. Supported by DFG (DI 702/6-1 to A.D and HU 800/3-1 to C.A.H.)

# Microheterogeneity, polysialylation and expression of the nervous system derived protein ependymin

Donato Penninella[1], Rupert Schmidt[1]

[1]Biotechnology Centre, Justus-Liebig-University Giessen, Leihgesterner Weg 217, 35392 Giessen, Germany

Ependymin is a specific CNS glycoprotein functionally involved in memory consolidation and neural regeneration processes in goldfish. After active shock-avoidance conditioning ependymin mRNA in meningeal fibroblasts is rapidly induced followed by enhanced synthesis and secretion of the protein into the extracellular and cerebrospinal fluids[1]. Intracranial injections of anti-ependymin antisera or antisense oligodeoxynucleotides both interfere specifically with memory consolidation, indicating that only de novo synthesized ependymin molecules are involved. Ependymin is capable of directing the growth of central axons in vitro and participates in neuronal regeneration in situ, presumably by its HNK-1 epitope. HNK-1 is a prominent glycosyl epitope on many cell-adhesion molecules (CAMs) and extracellular matrix molecules (ECM), including N-CAM and L1, which have all been implicated in synaptic plasticity.

2D-PAGE analyses of goldfish brain homogenate and extracellular fluid now revealed that ependymins are microheterogeneic proteins appearing in different isoforms. The major ependymins arise in two double bands with apparent molecular weights (MW) of 28.5 and 30.5 kD ("γ"; mono-N-glycosylated), and 35.5 and 37 kD ("ß"; bi-N-glycosylated), respectively. The γ-ependymins comprise isoforms with at least 9 different isoelectric points (pI) between pH 4.78 and 5.55, whereas ß-ependymins comprise isoforms with at least 8 different pI between pH 4.65 and 5.22. To investigate these microheterogeneic isoforms, single spots from 2D-gels (ependymins with accurately defined pI) were eluted and re-separated. The re-separation resulted again in the appearance of several isoforms with different pI. Furthermore, it was shown that de-glycosylated ependymins (N glycosidase F treatment in addition to 2 mercaptoethanol) comprise different microheterogeneic isoforms, demonstrating these are neither caused by N-linked glycans nor by differences in the amino acid sequences. We assume that the microheterogeneitic isoforms may be caused by stable conformational isomers.

Treatment with neuraminidase additionally revealed that ependymins also exist in different polysialylated forms. Polysialylation of N-CAM, another cell adhesion molecule involved in synaptic plasticity, was shown to reduce this protein's binding affinity to other cell adhesion molecules or N-CAM itself[2].

To further elucidate the functionality of ependymin it is important to study the expression mechanism of this protein. There is evidence that the expression is regulated by existing ependymin concentrations (negative feedback) and possibly also by stress hormones. Therefore, goldfish meningeal fibroblasts were cultivated in an attempt to modulate ependymin expression and posttranslational modifications by addition of several agents to the cell culture medium. First results show that ependymin is highly expressed in meningeal fibroblasts and is also secreted into the cell culture medium.

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# Modulation of LTP by cholinergic/glutamatergic receptors is essential to induce BDNF-dependent long-lasting memory

Sheeja Navakkode[1], Martin Korte[1]

[1]TU, Braunschweig, Cellular Neurobiology, Spielmannstrasse-7, 38106 Braaunschweig, Germany

The long-lasting forms of synaptic plasticity require the activation of neuromodulatory systems. Among all the neurotransmitter system, cholinergic system is of predominant interest after the finding that cognitive decline in aging and dementia is related to a decrease in cholinergic function. Cholinergic neurotransmission is known to affect activity-dependent plasticity in various brain areas, including the hippocampus. In order to analyze the cellular nature of this memory-promotion by acetylcholine, we could show here that long-lasting-LTP (L-LTP) could be induced by the co-application of a PDE4-inhibitor, rolipram and a cholinergic receptor agonist, carbachol at a concentration that by itself has no effect on basal synaptic transmission. We could in addition show that this chemical L-LTP is similar to electrical L-LTP in that it is dependent on protein synthesis, cAMP and NMDA-receptor activation. Occlusion experiments demonstrated that saturation of three TET (tetanus) late-LTP occluded carbachol-rolipram-LTP, showing that they share similar properties. Carbachol also transformed an early form of LTP (E-LTP) into L-LTP. In addition, we studied whether cooperation between glutamatergic and cholinergic receptors is essential to induce processes of functional plasticity. We could show that co-activation of acetylcholine/PDE4 inhibition should coincide with the release of glutamate to induce a long-lasting plasticity, showing a functional convergence of the two neuromodulatory systems. We have also evidence that both chemical L-LTP and carbachol reinforced early-LTP induced synaptic tagging/capture and that this is mediated by the neurotrophin, BDNF.

# Modulation of synaptic plasticity by intracellular pH in Purkinje neurons of the mouse cerebellar cortex

Alexander Neumeyer[1], Joachim W. Deitmer[1]

[1]General Zoology, FB Biologie, TU Kaiserslautern, P.O.Box 3049, 67653 Kaiserslautern, Germany

Proton buffering in the central nervous system is largely dependent on the $CO_2$/hydrogencarbonate buffer system. In aqueous solution, dissolved $CO_2$ and $H_2O$ react to $H_2CO_3$ which dissociates to $H^+$ and $HCO_3^-$. This slow reaction is accelerated by carbonic anhydrases (CA) which play an important role for proton homeostasis. The most prominent CA isoforms expressed in the brain are the intracellular CAII and extracellular CAIV and CAXIV. The regulation of pH is essential for inter- and intracellular communication. It was shown that intracellular pH ($pH_i$) plays an important role in a variety of cellular processes like modulation of receptors and ion channels, while many studies document that $pH_i$ undergoes large shifts under physiological and pathophysiological conditions (e.g. anoxia results in intracellular acidosis). Most studies about the influence of pH on synaptic activity of neurons however, are related to *extracellular* pH changes. The aim of the present study was to investigate the influence of *intracellular* pH changes on synaptic plasticity at a constant extracellular pH, studied at the glutamatergic parallel fibre (granule cell) to Purkinje cell (PC) synapse in the molecular layer of the cerebellar cortex. We used live-cell imaging employing the proton-sensitive fluorescent dye 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein, acetoxymethyl ester (BCECF, AM) and whole-cell patch clamp recordings from PCs during parallel fibre stimulation. Our experiments showed that intracellular alkalinization was accompanied by an increase in the excitatory postsynaptic current (EPSC) amplitude, while intracellular acidification resulted in a reduction of the EPSC amplitude in PCs. The short-term synaptic plasticity seemed to be mediated presynaptically, as indicated by opposite changes in the paired-pulse ratio, and postsynaptically, as indicated by postsynaptic responses evoked by focal puff application of a-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA) on PCs under the same conditions. Inhibition of CA activity by 6-Ethoxy-2-benzothiazolesulfonamide (EZA) modulated the course of intracellular pH change and the change in postsynaptic responses indicating the significance of intracellular pH changes and of CA activity for modulating synaptic plasticity.

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# Nogo-A restricts synaptic strengthening in the adult mouse hippocampus

Andrea Delekate[1], Marta Zagrebelsky[1], Martin E. Schwab[2], Martin Korte[2]

[1]TU Braunschweig, Zoological Institute, Cellular Neurobiology, Spielmannstraße 7, 38106 Braunschweig, Germany
[2]Brain Research Institute, University of Zurich and Dept. of Biology, ETH Zurich, Switzerland

Neuronal networks in the brain undergo constant remodeling processes during development and adult learning. These processes involve functional and structural alterations (plastic changes) at synapses and represent cellular mechanisms of learning and memory processes. An accepted model for activity dependent functional plasticity is the induction of Long-term potentiation (LTP) in the CA1 region of the hippocampus by high-frequency stimulation which leads to increased synaptic strength. While the molecular mechanisms positively regulating these functional changes are relatively well described, the molecules controlling the stabilization of synapses and restricting plasticity are still largely unexplored. In search for factors restricting plasticity processes, we investigated the role of the myelin-associated protein Nogo-A, whose function as negative regulator of structural changes in the CNS is well known. We analyzed synaptic transmission as well as long-term synaptic plasticity (LTP/LTD) in the presence of function blocking anti- Nogo-A or anti-Nogo receptor (NgR) antibodies and in the mature hippocampus of Nogo-A KO mice.

While baseline synaptic transmission, short term plasticity and long term depression were not affected by either approach, LTP was significantly increased following Nogo-A or NgR neutralization. Synaptic potentiation thus seems to be restricted by Nogo-A. Surprisingly, synaptic weakening was not affected by interfering with NogoA signaling. Further experiments with a $GABA_A$ receptor blocker implicate the gabaergic system as an additional possible target of Nogo-A function.

To complement the antibody and KO loss-of-function experiments, we applied the active Nogo-A specific Δ20 fragment to the hippocampal slices. This peptide is one of the two inhibitory domains of Nogo-A and is the epitope used to generate the Nogo-A blocking antibody. A five minutes pre-incubation of the tissue with Δ20 significantly decreased post-tetanic potentiation (PTP). This effect is in line with a synaptic weakening effect of Nogo-A.

The present results show a new role of Nogo-A expressed in the adult hippocampus in restricting physiological synaptic plasticity on a very fast time scale. Nogo-A could thus serve as an important negative regulator of functional and structural plasticity in mature neuronal networks. (Supported by the DFG, Az. ZA554/2-1).

# Pannexin1 modulates the excitatory postsynaptic potential response of hippocampal CA1 neurons in the mouse

Amr Abdulazim[1], Julian Hanske[1], Nora Prochnow[1], Georg Zoidl[1]

[1]Institute of Anatomy, Departement of Neuroanatomy and Molecular Brain Research, Universitätsstr. 150 , D-44780 Bochum, Germany

Panx1 channels have been revealed to be large-pore ion channels broadly distributed in the central nervous system (CNS).
Especially their expression at postsynaptic sites in the hippocampus in co-localization to the postsynaptic density protein 95 (PSD-95) indicates that Panx1 may contribute to hippocampal synaptic transmission. This is supported by the insight that Panx-1 channels seem to be opened by prior activation of N-methyl-D-aspartate receptors (NMDARs) and appear to conduct adenosine triphosphate (ATP) which is putatively involved in retrograde presynaptical inhibitory and interneuronal signaling.
In this study we used a transgenic Panx1 knock out mouse for field excitatory postsynaptic potential (fEPSP) recordings in hippocampal CA1 neurons of acutely dissected mouse hippocampal slices in vitro. Investigations were performed under different pharmacological conditions to characterize the hippocampal postsynaptic response properties of Panx1.
Long-term potentiation (LTP) measurements revealed an increase of the early LTP (0-5 min) in the Panx1 knock-out (KO) mice, whereas persistent late LTP did not show any difference between wild type (WT) and the KO.
Inhibition of Panx1 with mefloquine caused an increase of the amplitudes of fEPSP responses, indicating a putative inhibitory modulation of the postsynaptic hippocampal response by Panx1.
Results of LTP recordingds suggest a decisive Panx1 channel contribution to hippocampal plasticity.
These findings need to be verified in single cell recordings of CA1 pyramidal neurons and interneurons.
Given that Panx1 channels are opened by activation of NMDARs, this pathway and the inhibitory modulation property of Panx1 channels, in general, could display an inhibitory feedback loop that prevents the brain tissue from epileptic discharges or excitotoxicity. This needs to be evaluated in further studies.

# Plasticity-related gene 5 induces spine formation in immature primary neurons

Pierluca Coiro[1], Anja U. Bräuer[1]

[1]Chartiè, Institut für Zell- und Neurobiologie, Phillippstr. 12, 10115 Berlin, Germany

The shape of a neuron's dendritic arbour determines the set of axons with which it may form synaptic contacts, thus establishing connectivity within neural circuits. Dynamic cytoskeleton remodelling is an essential step during this process. Putative extracellular cues may act through membrane proteins that relay signals to a network of intracellular signalling pathways, which ultimately converge on the cytoskeleton. However, the molecular mechanisms involved in these steps are not well understood. Here, we show that a novel member of the vertebrate- and brain-specific PRG family, plasticity-related gene 5 (PRG-5), a multispanning membrane protein, localizes to and promotes the induction of spines in young primary neurons. The PRG family is a subgroup of the lipid phosphate phosphatase superfamily, which is able to dephosphorylate exogenous and intracellular lipid phosphates. Mutagenesis experiments in PRG-5 show residual amino acids that are important for the induction of spines. Our data show that PRG -5 may be involved in spine induction in primary neurons and thereby modulate extracellular molecular cues.

# Role of CB1 expression in hippocampal excitatory versus inhibitory neurons in regulating activity-dependent synaptic plasticity

Martin Polack[1], Lydia Steinke[1], Beat Lutz[2], Martin Korte[1]

[1]TU Braunschweig, Cellular Neurobiology, Braunschweig, Germany
[2]Institute of Physiological Chemistry & Pathobiochemistry, Mainz, Germany

Functional synaptic plasticity requires a cellular and molecular mechanism to highly potentiate synapses and to maintain these changes for a long time period. On the other hand, homeostatic mechanisms need to be in place in order to restrict the number of synapses which can be potentiated. Here endocannabinoids and their G-protein coupled receptor (CB1) might be important negative regulators of synaptic strength. The hippocampus contains one of the highest CB1 densities and administration of cannabinoids impairs memory similar to hippocampal removal. These observations suggest that the endocannabinoids might play selective actions on information processing within the hippocampus.
Using two different neuron type specific CB1 receptor KO mouse mutants we address the contribution to LTP (long-term potentiation) and to structural plasticity of CB1 expression respectively on inhibitory versus excitatory hippocampal neurons. In the hippocampus of mice with a restricted deletion of the CB1 receptor in GABAergic neurons, we observed a significant reduction in the dendritic complexity of pyramidal neurons as well as in dendritic spine density. Electrophysiological experiments in the CA3-CA1 region of the hippocampus revealed a highly significant decrease in LTP. Moreover, KO of the CB1 receptor specifically in glutamatergic neurons leads to an increase in their dendritic complexity. Preliminary electrophysiological data indeed indicate in these mice an increase in LTP supporting the structural analysis.
In order to investigate which of the possible endocannabinoids mediates functional synaptic plasticity, we analyzed FAAH KO mice. In FAAH KOs (block of anandamide degradation) one should expect to have more activation of CB1 receptor, but by only one of two major endocannabinoids (amandamide). And indeed FAAH KO mice showed a significantly decreased LTP. Taken together, we found evidence, that CB1 receptors play a role in regulating synaptic plasticity in inhibitory, as well as in excitatory neurons of the hippocampus. Furthermore we could show that the CB1 receptors are involved in controlling the structure of mature hippocampal pyramidal neurons and might be involved in the stabilization of neuronal networks.

# Serotonin - more than a neurotransmitter: transglutaminase-mediated serotonylation of glial cells and stem-cell-derived neurons

Patrick Schloss[1], René Hummerich[1], Susanne Kremer[1], Thorsten Lau[1]

[1]Central Institute of Mental Health, Biochemical Laboratory, J5, 68159 Mannheim, Germany

In the central nervous system serotonin plays important roles as a neurotransmitter as well as during neuronal development and in synaptogenesis. Outside the central nervous system, serotonin is covalently transamidated to procoagulant proteins involved in blood clotting. This process is mediated by transglutaminases and named "serotonylation". Serotonylated proteins then tightly bind to specific serotonin binding sites on fibrinogen and thrombospondin to form stable extracellular multivalent complexes needed for thrombus formation. Here, we have investigated whether transglutaminases can also covalently incorporate extracellular serotonin to neural proteins and whether this might affect extracellular protein expression. Our data reveal that recombinant transglutaminase specifically transamidates [³H] -serotonin to cell -surface proteins from C6 glioma cells and stem-cell derived neurons. Serotonylation of [³H] -serotonin is inhibited by the transglutaminase inhibitor cystamine and unlabelled serotonin. Transglutaminase-mediated transamidation of unlabelled serotonin to C6 cells induces an aggregation of extracellular protein matrices adjacent to and between single cells. Transglutaminase also transamidates the autofluorescent serotonin analogue 5, 7-dihydroxytryptamine and monodansylcadaverine (MDC) into living neural cells. Electrophoretic separation of MDC -labelled C6 cells identified several distinct fluorescent proteins one of which is fibronectin.

# Short-term synaptic plasticity of excitatory inputs to GABAergic interneurons of the mouse cingulate cortex.

Bernd Sutor[1], Florian Werthat[1]

[1]Institute of Physiology, Department of Physiological Genomics, LMU Munich, Schillerstrasse 46, 80336 Munich, Germany

We investigated the properties of stimulus-evoked excitatory synaptic responses of GABAergic interneurons located in the mouse posterior cingulate cortex. Cortical slices were prepared from transgenic mice in which a subpopulation of GABAergic interneurons had been labelled with the green fluorescent protein [1]. Whole-cell patch clamp recordings were taken from fluorescent neurons in layers 2 and 3 of the cingulate cortex, while synaptic responses were evoked by means of a monopolar fine-tipped stimulation electrode positioned at the border of layer 6 and the white matter.

Repetitive stimulation at a low frequency (0.05 Hz) and with low stimulus intensities induced EPSPs/EPSCs with maximum amplitudes ranging between 1.5 and 3 mV or 20 and 50 pA, respectively. The failure rate of these responses (number of failures per hundred stimuli) was found to be 0.6 - 0.7. The input-output-relationship of the EPSPs/EPSCs could be fitted with a sigmoid curve displaying a steep rise at low stimulus intensities. The maximum amplitudes were reached at these low intensities; a further increase in stimulus strength did not lead to an increase in the EPSP/EPSC amplitude or to a change in the failure rate. The stimulus-evoked synaptic responses were reversibly blocked by the AMPA receptor antagonists CNQX (10 µM) or NBQX (5 µM). The NMDA receptor antagonist D-AP5 (20 µM) had no effect on these synaptic potentials or currents.

Paired-pulse stimulation (inter-stimulus interval: 10 ms) led to a pronounced potentiation of the EPSP/EPSC with potentiation factors ranging between 3.5 and 5. This paired-pulse facilitation disappeared at inter-stimulus intervals larger than 150 ms. The potentiation factor increased with increasing stimulus number; when stimulated with 4 - 5 pulses (inter-stimulus interval: 10 ms), the EPSP amplitude invariably reached the threshold for spike initiation. The synaptically initiated action potentials occurred with a high probability and a small temporal jitter.

In addition to paired-pulse facilitation, frequency facilitation of the stimulus-evoked EPSPs/EPSCs was observed. At low stimulation frequencies (0.05 or 0.1 Hz), the responses occurred with a relatively high failure rate and small amplitudes. Enhancement of the stimulus frequency to 2, 3, or 4 Hz resulted in an up to 20-fold increase in the amplitude. The failure rate decreased to values around 0.05-0.1. The effects of high frequency stimulation reversed within 2 - 4 s after switching back to low frequency stimulation.

The magnitude of the frequency facilitation of stimulus-evoked EPSPs/EPSCs was reversibly reduced by application of the group III-mGluR agonist L-AP4 (10 µM). Activation of kainate receptors by addition of kainate (50 nM) to the bathing solution resulted in variable effects on both EPSCs and their frequency facilitation.

In conclusion, afferent stimulation of a subpopulation of GABAergic interneurons of the cingulate cortex evokes excitatory responses with unusual properties resembling in some aspects those of the mossy fiber synapses on hippocampal CA3 pyramidal cells [2].

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# Spike Timing-Dependent Plasticity in hippocampal CA1 region is dependent on brain-derived neurotrophic factor

Elke Edelmann[1], Volkmar Lessmann[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipziger Straße 44, 39120 Magdeburg, Germany

Long-lasting changes of synaptic transmission, like long-term potentiation (LTP) and long-term depression (LTD) are classically thought to be the cellular substrate for information storage in the brain. Conventionally, LTP is induced by high frequency stimulation or by theta-burst stimulation and is critically dependent on the presence of "brain-derived neurotrophic factor" (BDNF). To clarify a possible role of BDNF in LTP under more moderate stimulation conditions, we here focused on a low frequency induction protocol, which is called Spike Timing-Dependent Plasticity (STDP). This type of plasticity can be induced by precisely timed pairing of pre- and postsynaptic action potentials (APs).

In an attempt to establish STDP, we used different low frequency pairing protocols with different numbers of postsynaptic stimuli (one presynaptically induced excitatory postsynaptic potential (EPSP) and one, two or four postsynaptic APs) in acute hippocampal slices of P15-P20 Wistar rats and P25-P35 C57Bl/6J mice. Short positive pairings at +15 ms between pre- and postsynaptic activation lead to a significant timing-dependent LTP (t-LTP) compared to unpaired controls (e.g. pairing at +15ms in rats with 1 EPSP/ 1 AP: 1.71 ± 0.1 of initial values; control: 0.98 ± 0.1). Pairings with negative intervals lead to a timing-dependent LTD (e.g. t-LTD at -15ms in rats with 1 EPSP/ 1 AP: 0.85 ± 0.1 compared to control: 0.98 ± 0.1). The t-LTP induced by 1 EPSP/ 1 AP pairings at short positive pairings was blocked in the presence of 50 µM DL-APV (1.15 ± 0.15 compared to 1.78 ± 0.12), indicating a dependence of t-LTP on the activation of NMDA receptors.

Using either the Trk kinase inhibitor k252a or heterozygous BDNF mice, we investigate the involvement of BDNF/TrkB signaling in our STDP paradigms. Our results suggest a stimulus dependent involvement of BDNF in STDP induction: a low BDNF/TrkB sensitivity of the 1 EPSP/ 1 AP pairing (100 repeats) was observed (t-LTP in the presence of k252a at +10ms: 1.36 ± 0.2 vs. 1.74 ± 0.1 (control)). When using a stronger and more efficient paradigm for STDP induction (1 EPSP/ 2 AP pairings, 50 repeats), blocking BDNF/TrkB signaling seems to have a larger impact on the efficiency of STDP induction compared to control (k252a at 10ms: 1.38 ± 0.3 vs. 2.00 ± 0.4 (control)). A strong inhibitory effect of k252a on t-LTP is achieved when using a 1 EPSP/ 4 AP pairing paradigm with 25 repeats (k252a at 10ms: 0.98 ± 0.1 vs. 1.74 ± 0.2 (control)). Preliminary data from BDNF $^{+/-}$ mice show also a robust impairment of t-LTP with the 1 EPSP/ 4 AP paradigm in comparison to respective wild type littermates (at 10ms: WT 1.69 ± 0.2 vs BDNF $^{+/-}$ 0.58 ± 0.3).

We demonstrate an effective STDP protocol in acute hippocampal slices of rats and mice at low frequency stimulation with different numbers of postsynaptic stimulations. Based on our data, we suggest that BDNF is involved in hippocampal STDP. Acute inhibition of BDNF/TrkB signaling by application of k252a leads to a stimulus dependent reduction of t-LTP at short positive pairings. Likewise, chronic BDNF depletion leads to impaired t-LTP.

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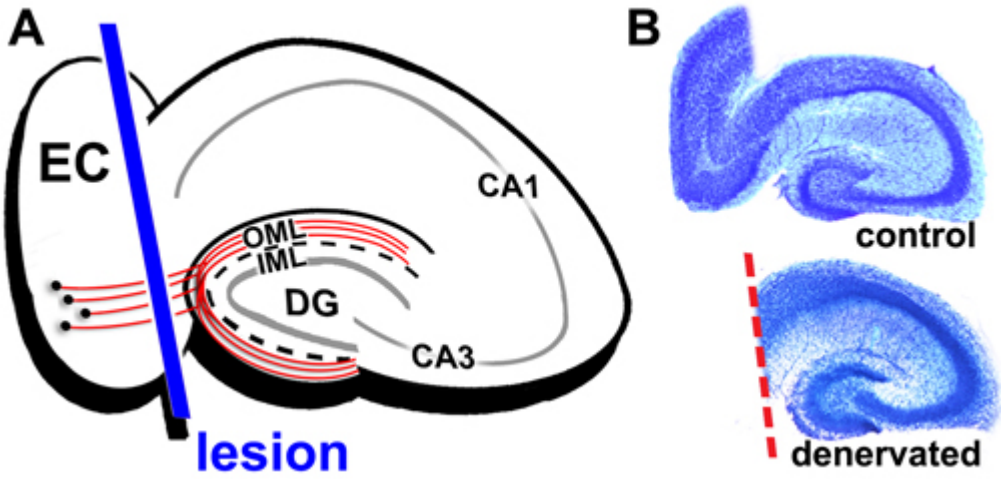
# Spine stability is a structural target of denervation-induced homeostatic synaptic scaling

Andreas Vlachos[1], Denise Becker[1], Carlos Bas Orth[1], Moritz Helias[2], Peter Jedlicka[1], Maike Neuwirth[1], Raphael Winkels[1], Markus Diesmann[2], Jochen Roeper[3], Gaby Schneider[4], Thomas Deller[1]

[1]Goethe-University Frankfurt, Institute of Clinical Neuroanatomy, Neuroscience Center, 60590 Frankfurt am Main, Germany
[2]RIKEN Brain Science Institute, Wako City, Saitama 351-0198, Japan
[3]Goethe-University Frankfurt, Institute of Neurophysiology, Neuroscience Center, 60590 Frankfurt am Main, Germany
[4]Goethe-University Frankfurt, Department of Computer Science and Mathematics, 60325 Frankfurt am Main, Germany

Denervation-induced spine reorganization was studied following entorhinal deafferentation of hippocampal granule cells in organotypic slice cultures of Thy1-GFP mice. The highly laminar organization of entorhinal afferents to the dentate gyrus made it possible to selectively denervate distal dendritic segments of granule cells without deafferenting their proximal dendritic segments. Thus, we could study how spines located on denervated and non-denervated dendritic segments of the same neuron react to the loss of innervation. Time-lapse imaging revealed alterations in spine loss and in the stability of newly formed spines but not in spine formation rate in the denervated layer. Patch-clamp analysis revealed homeostatic scaling of excitatory synapses within the same layer, demonstrating that denervated neurons locally adapt their synapses to maintain their afferent drive. The layer-specific functional and structural adaptations observed after denervation required the layer-specific upregulation of tumor necrosis factor alpha (TNFa). Since induction of homeostatic scaling in non-denervated control cultures also resulted in a destabilization of newly formed spines, we propose that TNFa-mediated destabilization of spines could be a general mechanism by which neuronal networks homeostatically adapt spine and thus excitatory synapse numbers to the level of network activity. Supported by DFG.



# Synaptic plasticity in the lateral Amygdala in GAD65-deficient mice

Maren Denise Lange[1], Kay Juengling[1], Hans-Christian Pape[1]

[1]Institut fuer Physiologie I, Westfaelische Wilhelms University, Muenster

GABA (Î³-aminobutyric acid) is the most abundant inhibitory neurotransmitter in the mammalian nervous system. It is synthesized by two isoforms of glutamic acid decarboxylase, GAD65 and GAD67 and is critically involved in the control of fear and anxiety by the amygdala. In fact, deficits in GABAergic metabolism are closely related to pathological conditions both in animal models and humans. Previous studies of our lab have shown that a genetically determined deficiency in GAD65 results in generalization of conditioned fear, impairment of fear extinction and increase in seizure susceptibility.

Therefore the present study was undertaken to characterize the functional role of GAD65 in synaptic transmission in the lateral amygdala (LA). Whole-cell patch-clamp recordings were performed in acute brain slices containing the LA of GAD65 deficient mice (GAD65 -/-) and age-matched wild-type littermates (GAD65 +/+). For evoked synaptic responses an extracellular bipolar stimulation electrode was placed in the LA, external and/or internal capsule. A theta-burst stimulation of thalamic and/or cortical afferents to the LA was used for LTP induction.

The GABAergic synaptic transmission on projection neurons (PN) in the lateral amygdala is significantly reduced in mice lacking GAD65, but the pharmacologically isolated glutamatergic transmission is unaltered. A theta-burst stimulation of thalamic afferents to the LA evoked an enhanced monosynaptic excitatory LTP in GAD65 deficient mice. This effect disappears in presence of a GABAB-receptor blocker. Additionally the stimulation of cortical afferents in the presence of gabazine evoked LTP in GAD65 deficient mice but not in GAD65 +/+.

The final finding of this project is the absence of disynaptic plasticity at thal/IN/PN synaptic connections and a reduced plasticity at LA/PN monosynaptic connections in GAD65 -/- mice.

In conclusion, these results indicate a critical role of GAD65 for GABAergic synaptic transmission and plasticity in the amygdala, which may correlate to the previously observed phenotype, i.e. generalized fear memory.

# The role of mammalian ependymin related protein (MERP) in a spatial learning task.

David Hinchliffe[1], Rupert Schmidt[1]

[1]Justus-Liebig Universität, Zentrale Biotechnische Betriebseinheit, Leihgesterner Weg 217, 35392 Gießen, Germany

The mammalian ependymin-related proteins (MERPs) form a wide spread family of proteins that can be found in vertebrates but as well in invertebrates. All in common is a relation to the piscine secretory glycoprotein ependymin. This is the pre-eminent protein in the cerebrospinal fluid of teleost fish, initially discovered in the ependymal zone of goldfish brain (Shashoua, 1977).

A hypothesis implicating ependymin in memory consolidation proposed two interlocked regulatory mechanisms in which stress hormones regulate the abundance of ependymin synthesis and metal cation concentrations influence ependymin polymerisation. The resulting ependymin-fibres provide an extracellular matrix to help establish or improve synaptic formations (Shashoua and Schmidt, 2004).

Typical of the ependymin structure is the L2/HNK-1 epitope, a feature of many neural cell adhesions molecules. Four cystein residues are highly conserved throughout the MERP family. Also potential N-linked glycosylation sites can be found. In teleosts bound oligosaccharides allow calcium to bind in turn (Schmidt and Makiola, 1991). In humans an ependymin-related protein was named 'upregulated in colorectal cancer gene-1' (UCC-1) after finding it in colorectal tumor cells (Nimmrich et al., 2001). Then a gene with 100% homology to the UCC-1 gene sequence was discovered in CD34+ hematopoietic progenitor cells. After completing the coding region by adding a 5' sequence, the whole protein was called MERP1 (Gregorio-King et al., 2002).

The two murine MERPs (mu-MERP) we are analysing here show an identity of 99% and 83% (mu-MERP1 and mu-MERP2), respectively, compared to the protein sequence of human MERP1 as derived from the theoretical translation of the open reading frame.

To analyse these proteins, we first localised the site of mRNA synthesis by *in-situ*-hybridisation. An immunohistochemical staining with a cross-reactive antibody against ependymin displayed where the proteins can be found in the brain of adult C57/Bl6J mice. Because of the structural relation to the teleosts' ependymin, a functional similarity is not unlikely. Therefore we performed a spatial learning task (Morris-Water-Maze) and compared the change of quantity of MERP-mRNA synthesis after various time points by RT-PCR.

# Poster Topic

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T9-9C Translational Regulation of Astrocytic Connexins and Glutamine Synthetase by CPEB3
*Vamshidhar Reddy Vangoor, Sada Lakshmi Turimella, Lech Kaczmarczyk, Jiong Zhang, Peter Bedner, Elena von Staden, Amin Derouiche, Ronald Jabs, Gerald Seifert, Christian Steinhäuser , Martin Theis*

# A non-enzymatic transport metabolon enhances lactate flux in astrocytes

Holger M. Becker[1], Malin H. Stridh[2], Marco D. Alt[2], Gunther Wennemuth[3], Joachim W. Deitmer[2]

[1]TU Kaiserslautern, Zoology/Membrane transport, P.O. Box 3049, 67653 Kaiserslautern, Germany
[2]TU Kaiserslautern, General Zoology, P.O. Box 3049, 67653 Kaiserslautern, Germany
[3]Saarland University, Department of Anatomy and Cell Biology, 66421 Homburg/Saar, Germany

High-energy metabolites, such as lactate, pyruvate, and ketone bodies, are transported into and out of cells via monocarboxylate transporters (MCT, SLC16), of which 14 isoforms have been described. In the brain, astrocytes express MCT1 and 4, which have been suggested to be responsible for the export of lactate to provide neurons with the energy substrate during increased activity. We have recently shown, that carbonic anhydrase isoform 2 (CAII) can increase lactate-induced acid/base flux via MCT1 and 4, heterologously expressed in *Xenopus* oocytes, in a non-catalytic manner (Becker et al. 2010 *J. Membr. Biol.* 234(2):125-35; Becker & Deitmer 2008 *J. Biol. Chem* 283(31):21655-67). In the present study, we tested whether carbonic anhydrase can facilitate lactate transport in astrocytes. Our experiments revealed, that knock-out of CAII induced a significant reduction in the rate of lactate-induced rate of acidification in mouse cerebellar astrocytes, as measured by *in situ* live-cell imaging with the pH-sensitive fluorescent dye BCECF in acute cerebellar slices of $CAII^{+/+}$ and $CAII^{-/-}$ mice. Blocking CA catalytic activity with 6-Ethoxy-2-benzothiazolsulfonamid (EZA, 10 µM) had no effect on $H^+$ flux, suggesting a non-catalytic facilitation of transport activity by CAII. The data could be confirmed by uptake experiments in mouse astrocytes culture: Knock-down of CAII and CAIV by siRNA led to a significant decrease in lactate flux, while inhibition of CA catalytic activity with EZA (30 µM) had no effect. To check for a direct interaction between MCT1 and CAII in astrocytes, we applied an *in situ* proximity ligation assay, which indicated close proximity (< 40 nm) of MCT1 and CAII as intrinsically expressed in cultured astrocytes. To identify the binding site for CAII, we carried out single site mutations of MCT1, heterologously expressed in *Xenopus* oocytes. The interaction between MCT1 and CAII seems to be facilitated by a cluster of three glutamate residues in the C-terminal of MCT1. While catalytic activity of CAII is apparently not necessary for enhancement of MCT1 transport activity, removal of the intramolecular $H^+$-shuttle, His64, in CAII abolished the interaction between the two proteins. Our results suggest that CAII, directly bound to MCT1, can facilitate lactate flux in astrocytes by acting as a "proton collecting antenna" for the transporter. By this mechanism CAII would facilitate proton movement at the pore of the transporter, suppressing the build-up of proton microdomains, and thereby increasing trans-membrane lactate flux in astrocytes.

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# Control of peripheral myelination by proteolytic processing and limited axonal transport of Neuregulin-1 type III

Viktorija Velanac[1], Tilmann Unterbarnscheidt[1], Maike N. Gummert[1], Tobias M. Fischer[1], Carla Taveggia[2], Michael Willem[3], Markus H. Schwab[1], Klaus-Armin Nave[1]

[1] Department of Neurogenetics, Max-Planck-Intitute of Experimetal Medicine, Germany

[2] San Rafaele Scientific Institute, Italy

[3] Adolf-Butenandt-Institute, Biochemistry, German Centre for Neurodegenerative Diseases (DZNE), Germany

The neuronal growth factor Neuregulin-1 (NRG1) type III serves as a master regulator of myelination in the peripheral nervous system (PNS). Proteolytic cleavage is thought to activate NRG1 type III, but the identity of the protease(s) as well as the number and order of cleavage events *in vivo* has been elusive. BACE1 (ß-site amyloid precursor protein-cleaving enzyme) cleaves NRG1 *in vitro* and myelination is strongly impaired in BACE1 null mutants, suggesting that BACE1-processing is required for the myelinating activity of NRG1 type III. However, a functional interaction between NRG1 type III and BACE1 *in vivo* has not been demonstrated.

In the present study, we analysed transgenic mouse lines that neuronally overexpress N-terminally epitope-tagged full-length NRG1 type III and a C-terminally shortend NRG1 type III-variant (HA-NRG1$^{GIEF}$), which mimics BACE1-processing in the juxtamembrane ‚stalk' region. HA-NRG1$^{GIEF}$-transgenic mice are hypermyelinated in the sciatic nerve, very similar to full-length NRG1 type III. Thus, BACE1-processing produces a myelination-inducing NRG1 type III-variant. Unexpectedly, overexpression of NRG1 type III also retains the potential to induce myelination in BACE1 null mutants, albeit at reduced capacity.

To study the subcellular localization and transport of NRG1 in spinal cord motor neurons, we took advantage of the expression of epitope-tagged NRG1-variants. Histological and biochemical analysis suggests that following NRG1 cleavage in the ‚stalk' region, N- and C-terminal portions of NRG1 are sorted to distinct subcellular domains and that vesicular transport of the N-terminal cleavage product into the axona might be rate-limiting. Finally, we observed an additional (smaller-sized) N-terminal product, compatible with the ‚shedding' of the EGF-like domain of NRG1 type III from the axonal surface. While these findings strongly suggest a functional interaction of BACE1 and NRG1 type III in peripheral myelination *in vivo*, they also point to more complex processing mechanisms involving other proteases.

# Cool Calcium Signals – or: Is Calcium-Induced Calcium Release Temperature-Sensitive?

Maren Stavermann[1], Bernd Nilius[2], Joachim W. Deitmer[1], Christian Lohr[3]

[1]General Zoology, University of Kaiserslautern, Germany
[2]Ion Channel Research, University of Leuven, Belgium
[3]Animal Physiology, University of Hamburg, Germany

Calcium-induced calcium release (CICR) plays a major role in intracellular calcium signaling in excitable cells such as neurons and muscle cells as well as in non-excitable cells such as glial cells. CICR is defined as a calcium release event from calcium stores via ryanodine receptors (RyR) or inositol 1,4,5-trisphosphate receptors (InsP3R) as a consequence of an initial, often moderate, cytosolic calcium elevation, caused, e.g., by calcium influx through voltage- or ligand- gated ion channels. Using olfactory ensheathing cells (OECs) in situ we focused on basic characteristics of CICR at different temperatures. Here we present our results from lifetime calcium imaging performed on intact isolated olfactory bulbs of postnatal mice (P0-P7). Decreasing the bath temperature from above 31°C to 22°C resulted in calcium transients and calcium oscillations. Cold-induced calcium signaling was abolished after calcium store depletion by cyclopiazonic acid, but was only weakly affected in calcium-free saline, indicating intracellular calcium release upon cooling. The cold-induced calcium responses were present in knock-out mice lacking cold-sensitive TRP-channels (TRPM8 or TRPA1) and were not affected by RyR antagonists in wild-type mice. The InsP3R blockers caffeine and 2-APB entirely suppressed cold-induced calcium transients, while photolysis of caged InsP3 mimicked cooling-evoked calcium signaling. A moderate intracellular calcium rise evoked by CPA or by photolysis of NP-EGTA (caged calcium) resulted in CICR at 22°C, but not at 31°C or in the presence of InsP3R blockers at 22°C. Furthermore, the calcium back-regulation following photolysis of caged calcium decelerated upon lowering the temperature. Hence, cooling could result in a moderate calcium rise sufficient to trigger CICR. Our findings demonstrate that calcium transients induced by lowering the temperature from 31°C to 22°C in OECs are caused by temperature-sensitive InsP3R-mediated CICR.
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# Differentiated dentate granule cells start to migrate under epileptic conditions

Gert Münzner[1], Stefanie Tinnes[1], Matthias Bechstein[1], Ute Häussler[1], Marie Follo[2], Carola A. Haas[1]

[1]Experimental Epilepsy Group, Neurocenter,Freiburg, Germany
[2]Department of Internal Medicine I,Freiburg, Germany

Characteristic features of temporal lobe epilepsy (TLE) are recurrent, focal seizures and Ammonshorn sclerosis including a widening of the granule cell layer, called granule cell dispersion (GCD). The electrophysiological and neuropathological characteristics of TLE, including GCD, can be mimicked by unilateral, intrahippocampal injection of the glutamate receptor agonist kainate (KA) in adult mice. Using this animal model we have previously shown that GCD develops within two weeks after KA injection due to displacement of differentiated granule cells (Heinrich et al., 2006, JNS 26(17). In order to unravel the mechanism of this intriguing finding, we used Thy-1-eGFP transgenic mice, in which eGFP is primarily expressed in a subset of differentiated dentate granule cells. With the aim to monitor GCD formation in real time, we established organotypic hippocampal slice cultures (OHC) from Thy-1-eGFP transgenic mice and developed a protocol to induce GCD in vitro. OHC were prepared from P8 mouse pups, kept in culture for seven days and were treated with 15 µM KA for 8 hours on three consecutive days. This treatment caused a significant displacement of eGFP-positive granule cells leading to a strong widening of the granule cell layer (123.6 ± 5.0 µm) when compared to untreated controls (78.8 ± 2.4 µm). As a next step, life cell imaging of control and KA-treated OHC was performed. To this end, eGFP-positive granule cells were observed by confocal time-lapse videomicroscopy in an aerated chamber for different periods of time (6h, 12h and 24h). During the whole observation period, images were acquired every 20 min to monitor the position of eGFP-positive granule cells. Preliminary results indicate that in KA-treated OHC differentiated granule cells move actively towards the hilar region. Further studies will clarify the exact mechanism of this migration process.

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# Elucidating the function of CPEB proteins in microglia

Lech Kaczmarczyk[1], Sada Turimella[1], Vamshidhar Vangoor[1], Pavel Dublin[1], Gerald Seifert[1], Harald Neumann[2], Christian Steinhäuser[1], Martin Theis[1]

[1]University of Bonn, Institute of Cellular Neurosciences, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

[2]Institute of Reconstructive Neurobiology, University of Bonn LIFE & BRAIN Center, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

Cytoplasmic Polyadenylation Element Binding proteins (CPEBs) translationally regulate protein synthesis in neurons crucial for LTP maintenance, memory and learning. Neuronal CPEBs also mediate hyperexcitability in a model of temporal lobe epilepsy (TLE). Unlike in neurons, CPEB function in microglial cells has hardly been investigated. Neuronal proteins regulated by CPEBs include a protease called tissue Plasminogen Activator (tPA), but the primary source of tPA in brain is microglia. In fact, microglial tPA mediates neuronal death in TLE models. Here we explored for the first time if microglial CPEBs are involved in tPA release and neurodegeneration during TLE. RT-PCR and immunoblotting demonstrateed abundant CPEB transcript and protein levels in BV-2 microglia and embryonic stem cell-derived microglia. Single cell RT-PCR following electrophysiological characterization identified CPEB1-4 mRNAs in hippocampal microglia. To study protein-RNA interactions, we performed RNA CoIP experiments and luciferase reporter assays. RNA CoIP consistently showed binding of tPA mRNA by CPEB3 which was in line with the luciferase reporter study, showing decreased reporter gene expression when CPEB binding sites were mutated. This suggests that CPEB binding to mRNA represses translation.

Microglial CPEB and tPA expression is also investigated in our TLE model. To this end, we use CX3CR1 EGFP/+ knock-in mice (expressing EGFP in microglia). Inducing status epilepticus in these mice altered CPEB 1-4 mRNA expression. Currently FACS purifications and Odyssey immunoblotting are performed to correlate transcript and protein expression levels, and compare with the cell culture results.

Our results suggest that CPEB-mediated translational regulation of tPA is not restricted to neurons and suggest an impact on neurodegeneration in TLE. However, due to the complexity of CPEB-mediated translational control, estimation of the quantitative contribution of CPEBs to translational control in microglia requires further investigation. These studies will shed new light on the function of microglial CPEBs and their potential role in neurodegeneration associated with TLE.

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# Extrasynaptic vesicular neurotransmitter release from olfactory receptor axons mediates neurovascular coupling via glial calcium signalling

Christian Lohr[1], Anne Thyssen[2], Daniela Hirnet[1], Hartwig Wolburg[3], Günther Schmalzing[4], Joachim W. Deitmer[2]

[1]Universität Hamburg, Abteilung für Tierphysiologie, Martin-Luther-King Platz 3, 20146 Hamburg, Germany
[2]Technische Universität Kaiserslautern, Abteilung für Allgemeine Zoologie, POB 3049, 67653 Kaiserslautern, Germany
[3] Universität Tübingen, Institut für Pathologie, Liebermeisterstr. 8, 72076 Tübingen, Germany
[4] RWTH Aachen, Institut für Pharmakologie und Toxikologie, Wendlingweg 2, 52074 Aachen, Germany

Neurotransmitter release is generally considered to occur at active zones of synapses, and only in few instances, ectopic release of neurotransmitters has been demonstrated. However, the mechanism of ectopic neurotransmitter release is poorly understood. We took advantage of the intimate morphological and functional proximity of olfactory receptor axons and specialized glial cells, olfactory ensheathing cells (OECs), in the nerve layer of olfactory bulbs, devoid of synapses, to study extrasynaptic neurotransmitter release. Axonal stimulation evoked purinergic and glutamatergic calcium responses in OECs, as measured by confocal calcium imaging using Fluo-4 AM, indicating ATP and glutamate release from olfactory receptor axons. In transgenic mice, in which receptor axons expressed the vesicle fusion marker protein synapto-pHluorin, stimulation evoked an increase in synapto-pHluorin fluorescence, indicative for vesicle fusion. In addition, synaptic proteins, such as synaptophysin and bassoon, as well as the vesicular glutamate transporter 2 were located in receptor axons. Transmitter release was dependent on calcium and could be inhibited by impairing vesicular neurotransmitter release with bafilomycin A1 and botulinum toxin A, respectively. To investigate the role of glial calcium signalling in neurovascular coupling, we co-visualized blood vessels by injection of sulforhodamine 101 and OECs by Fluo-4 AM in in-toto preparations of the olfactory bulb. Calcium transients in OECs evoked by ATP, axonal stimulation and laser photolysis of NP-EGTA ("caged calcium") resulted in constriction of adjacent blood vessels. Our results indicate that ATP and glutamate are ectopically released by vesicles along axons and mediate neurovascular coupling via glial calcium signalling.

# Functional and molecular analysis of $GABA_A$ receptors in hippocampal NG2 cells

Gerald Seifert[1], Michael Grauer[1], Christoph Schäfer[1], Stefan Paßlick[1], Ronald Jabs[1], Christian Steinhäuser[1]

[1]University of Bonn, Institute of Cellular Neurosciences, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

NG2 cells (also termed GluR cells) are equipped with functional AMPA and $GABA_A$ receptors and receive direct synaptic input from glutamatergic and GABAergic neurons. The functional impact of these neuron-glia synapses is still unclear. Here, we combined functional and molecular techniques to analyse properties of $GABA_A$ receptors in NG2 cells.

GABA activated slowly desensitizing receptor responses in NG2 cells (t about 2 s). The $GABA_A$ receptor agonist, muscimol, mimicked GABA -induced responses. Currents were sensitive to the $GABA_A$ receptor antagonist, bicuculline. To elucidate the $GABA_A$ receptor subunit composition, we tested the $Zn^{2+}$ sensitivity of receptor responses, as well as their modulation by benzodiazepines and barbiturates. Pentobarbital increased GABA-evoked responses about threefold. Preincubation of benzodiazepines, modulators of $GABA_A$ receptors, increased the receptor responses about twofold, while zolpidem, a modulator of a1 to a3 subunits, potentiated the GABA receptor responses at nM concentration. Micromolar concentrations of $Zn^{2+}$ blocked GABA responses effectively. Modulation of the responses by benzodiazepines and blocking by $Zn^{2+}$ strongly suggested expression of the γ2 receptor subunit. To further identify the receptor subunits, single cell transcript analysis was performed subsequent to functional characterization of NG2 cells. The subunits a 1, 2, 4, ß 2/3, and γ 2 were most abundantly expressed, matching properties resulting from pharmacological characterization.

To determine the effect of $GABA_A$ receptor activation on membrane potential, perforated patches were obtained from NG2 cells in situ. In the current-clamp mode, maximal activation of the GABA-mediated $Cl^-$ conductance depolarized the cells to –20 ± 6 mV (n = 6). Comparison of reversal potentials obtained with different $[Cl^-]_i$ (whole-cell mode) revealed a physiological $[Cl^-]_i$ of NG2 cells of about 60 mM. The GABA-induced depolarization might trigger $Ca^{2+}$ influx through voltage-activated $Ca^{2+}$ channels in the NG2 glial cells.

# Functions of ciliary neurotrophic factor (CNTF) in olfactory ensheathing cells (OEC): studies in neuron/OEC coculture systems

Heike Bömmel[1], Esther Asan[1]

[1]Universität Würzburg, Institut für Anatomie und Zellbiologie, Koellikerstr. 6, 97070 Würzburg, Germany

Olfactory sensory neurons (OSN) are continuously produced from neuronal precursors in the olfactory epithelium. Newly generated OSN extend their axons through the fila olfactoria into specific olfactory bulb glomeruli. Olfactory ensheathing cells (OEC), specialized glial cells of the fila, have been documented to promote axon growth in vivo and in vitro, and have therefore become candidate cells for transplantation-mediated support of axon regeneration after CNS lesions. CNTF, a neurotrophic factor which is implicated in neuroprotection and axonal regeneration particularly in the lesioned nervous system, is constitutively produced in high amounts in OEC in postnatal rodents and has been suggested to play a role in the life long neuroplasticity of the peripheral olfactory system.

We have established coculture systems using OEC and OSN from neonatal rats and mice to investigate neuron/OEC interactions, especially with respect to possible functions of CNTF. Quantitative analyses in these cocultures documented that OSN from both wildtype (wt) and CNTF deficient (ko) mice displayed significantly increased mean neurite length if cultured on CNTF-ko compared to wt OEC. Application of CNTF neutralizing antibodies led to enhanced mean neurite length in rat OSN/OEC cocultures and also in cocultures of rat cortical neurons with rat OEC, indicating that CNTF in the culture medium mediated the effect. In accordance with this suggestion, OSN displayed decreased mean neurite length in cocultures with CNTF-ko OEC upon addition of recombinant CNTF. Taken together, the findings suggested that CNTF produced by OEC mediated a reduction in mean neurite length of different neuron types in cocultures.

Further experiments were directed at defining the mechanism by which decreased mean neurite length could be mediated by CNTF production in OEC. BrdU labelling and TUNEL assays indicated that proliferation of neuronal precursors possibly present in the OSN isolates or survival rates of OSN were not enhanced in wtOSN when cultured on wt as compared to CNTF-ko OEC, i.e. under conditions in which reduced mean neurite length was observed in the wtOEC cocultures. In monocultures, addition of CNTF to the medium did not induce proliferation of rat OSN precursors and did not have an effect on OSN neurite length. On the other hand, rat OSN displayed longer neurites when cultured on laminin-111 versus laminin-211/221, showing that neurite outgrowth can be affected by specific components of the extracellular matrix (ECM). Preliminary experiments indicated that mean neurite length was significantly increased if wt OSN were cultured on ECM from CNTF-ko versus wt OEC.

The findings show that the coculture system is suited to assess CNTF functions for neuronal plasticity. Our preliminary data indicate that CNTF production in OEC mediates a reduction of mean neurite length of cocultured OSN, and also of other neuron types, possibly via an influence of the factor on production of specific molecules in OEC rather than by direct effects on neuronal proliferation, differential survival and/or neurite outgrowth. Further experiments will be carried out to test this hypothesis.

# Overexpression of the Fucosyltransferases 4 and 9 leads to activation of STAT 1 and STAT 2 in primary astrocytes

Beryl Schwarz-Herzke[1], Jürgen Konrad Mai[1]

[1]Heinrich-Heine Universitaet Duesseldorf, Institute for Anatomy, Universitaetsstrasse 1, 40225 Duesseldorf, Germany[1]

The CD15 antigen, or Lewis x (Lex), is a cell surface glycan consisting of a trisaccharide with the structure Gal-ß(1-4)-(Fuc-a(1-3))-GlcNac. It plays a role in CNS development and disease processes, but the regulation of the expression of this molecule is not well understood. The final biosynthetic step of the fucosylation during synthesizing CD15 is catalyzed by alpha-1.3 fucosyltransferases (fut). Two members of these futs are described in the murine brain: fut 4 and fut 9.
Primary astrocytes derived from neonatal mouse brains were transfected with three different expression vectors, containing a) the gene for the fut 4, b) the gene for the fut 9 and c) both genes. For microarray analyses total RNA was extracted and hybridized on mouse DNA chips (Affymetrix). Immunocytochemical stainings were performed using antibodies against STAT 1, STAT 2 and CD15. Total RNA was extracted and real time PCR was performed.
In transfected mouse astrocytes microarray analyses revealed expression of multiple genes involved in inflammation pathways. Among such genes a high transcription level was detected of STAT 1 and STAT 2 after overexpression of fut 4 and fut 9. Immunocytochemical stainings using antibodies against STAT 1 and STAT 2 have revealed that after transfection of the fut 4 and 9 as well STAT 1 as STAT 2 production were up regulated. These results indicate that 1. STAT 1 and STAT 2 are possible transcriptionfactors for fut 4 and/or fut 9 and 2. CD15 synthesis is connected with up regulation of inflammatory cell markers.
The transcription of STATs in response to specific cytokines and growth factors is well known in immunological pathways. Also phagocytotic processes activate the expression of inflammatory cell markers. This could be one possible interpretation for the high increase of CD15 in brains of high ages observed in immunohistochemistry.

# Glio-vascular interactions in the control of the blood-brain barrier.

Susan Noell[1], Andreas F. Mack[2], Karen Wolburg-Buchholz[3], Hartwig Wolburg[3], Petra Fallier-Becker[3]

[1]University of Tübingen, Dept. of Neurosurgery, Hoppe-Seyler Straße 3, 72076 Tübingen, Germany [2]University of Tübingen, Institute of Anatomy, [3] University of Tübingen, Institute of Pathology

The original finding by Paul Ehrlich (1885) that an infused dye did not stain the brain tissue (with the exception of the circumventricular organs, CVO), together with the complementary observation of his pupil Edwin Goldmann (1913) that the very same dye if applied into the cerebrospinal fluid (CSF) did stain the brain tissue (but not the CVO), has lead to the concept of biological barriers between blood and brain and blood and CSF. Today, we know that in most vertebrates the barrier is located within the endothelium (endothelial blood-brain barrier [BBB]) and in the epithelial choroid plexus cells and the tanycytes of the CVOs (glial blood -cerebrospinal fluid barrier [BCSFB]). The structure responsible for the restriction of the paracellular flux between endothelial and glial cells was identified as tight junctions (TJs). Today, we are faced with a multitude of TJ molecules, the best-known of them are occludin and the claudins, but also members of the immunoglobulin superfamily. The molecular composition and structure of BBB TJs is unique among all endothelial cell TJs in the body.

The quality of endothelial TJs depends on the brain microenvironment, including the surrounding basal lamina as well as the "second line of defense" which consists of pericytes, astrocytes, and microglia. The membrane domain of the astroglial endfeet is of particular importance for the brain physiology, in particular for the spatial buffering of the extracellular K+. The K+ taken up in synaptic regions is immediately redistributed and has to be extruded at the perivascular region or at the surface of the brain. The gate of extrusion of K+ at the astroglial endfoot membrane first of all is the K+ channel Kir4.1, which co -localizes together with the water channel protein aquaporin-4 (AQP4). AQP4 is the molecular equivalent of the orthogonal arrays of particles (OAP) which are well-known by electron microscopy since many years.

The basal lamina with its extracellular matrix (ECM) compounds such as laminins, fibronectin, collagens and the heparansulfate proteoglycan agrin has now be recognized to be important for the induction of polarity of astrocytes. Polarity of astrocytes in our context means heterogeneity of astroglial membrane domains. The OAP-related polarity stereotypically is reduced under pathological conditions like trauma, tumour, inflammation or stroke. Reduced polarity means insertion of potassium and water channels into wrong membrane domains leading to cytotoxic and vasogenic edemas. Vasogenic edema is a consequence of the disturbance of the BBB. However, we have to be aware of the complete inability to understand the step from the polarized astrocyte to the maintenance of the BBB (or from the reduction of polarity to the interruption of the BBB). Triggers the endothelial cell the astrocyte polarity or the astrocyte polarity the expression of the endothelial BBB parameters like TJs and BBB -specific transporters ? In any case, the composition of the basal lamina and its regulation by matrix metalloproteinases seem to be essential for the intact BBB. Understanding all the components of the BBB that may or may not operate together will be a great challenge in the future of BBB research.

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# Glucose transport and metabolism in acute brain slices: a multiphoton study

Patrick Jakoby[1], Elke Schmidt[1], Louis Felipe Barros[2], Joachim W. Deitmer[1]

[1]TU Kaiserslautern, General Zoology, P.O.box 3049, 67653 Kaiserslautern, Germany

[2]Centrao de Estudios Cientificos (CECS), Valdivia, Chile

Glucose is an essential energy source required to maintain brain metabolism. It is believed that the supply of energy is adjusted to energy demand in the brain and that different cell types may have their individual glucose handling. Addressing this issue, former studies were based on radioactively labeled glucose or PET scanning experiments. Since the spatial and temporal resolution of these techniques is relatively poor, we have used the fluorescent glucose analogues 2 NBDG and 6- NBDG to investigate the transport and metabolism of glucose in different cerebellar and hippocampal cell types in situ with multiphoton microscopy. Real time experiments by using 6- NBDG application revealed a biphasic uptake that is 2 to 5.5 times higher in glial cell rich layers compared to neuronal layers, i.e. granule cell layer or pyramidal cell layer. Comparing 2-NBDG and 2-NBDLG (L-glucose analogue), and using Cytochalasin B as glucose transport inhibitor, the second phase of NBDG uptake was identified as transport into cells. In contrast to this, the first phase seemed to be non-specific dye accumulation within the tissue. Bulk loading experiments at 21° C and at 35° C showed a temperature-dependent increase of 2-NBDG signals within all layers of the cerebellum, being highest in the molecular layer, due to the metabolism of 2-NBDG by hexokinase activity in Bergmann glial cells. Similar results were obtained in the hippocampus. Cell identification was confirmed by using monomeric red fluorescent protein 1 (GFAP-mRFP1) knock-in mice (obtained from F. Kirchhoff, Homburg/Saar) or by sulforhodamine 101 staining method. These results suggest that in acute cerebellar and hippocampal slices, glucose transport and glycolytic rate of astrocytes are considerably larger than those of neurons. Since the brain is mainly fueled by glucose, and as neurons consume significantly more energy than glial cells, these results are in line with the hypothesis suggesting substantial shuttling of energy-rich metabolites, as e.g. lactate, via monocarboxylate transporters from glial cells to neurons.
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# Human traumatic brain injury induced astrogliosis – involvement of P2Y1 receptors?

Kristina Bremicker[1], Marco Weber[2], Jan Dreßler[2], Heike Franke[1]

[1]Rudolf Boehm Institute of Pharmacology and Toxicology, University of Leipzig, Härtelstr. 16-18, 04107 Leipzig; [2]Institute of Legal Medicine, University of Leipzig, Johannisallee 28, 04103 Leipzig, Germany

Traumatic brain injury (TBI) is an important cause of death and disability in humans. TBI is highly correlated with the induction of astrogliosis, characterised by qualitative and quantitative changes of astrocytes, e.g. the expression of glial fibrillary acidic protein (GFAP), proliferation or alterations of the receptor expression. Knowledge of specific receptors and transduction mechanisms involved in astrogliosis are crucial for diagnosis and treatment. In previous in vivo studies in rats and mice after mechanical injury we have demonstrated the involvement of purinergic receptors, especially the P2Y1 receptor subtype, in the induction and maintenance of reactive gliosis and anti-apoptotic processes. The aim of the present study was to verify a possible influence of this receptor subtype in astrogliosis in human brain.

Based on human post mortem autopsy material of TBI patients, astroglial reaction around the traumatic area in the prefrontal cortex was investigated using histology, immunohistochemistry and Western blot techniques in comparison to a control group of matched age and gender.

The results indicate that TBI in human is associated with an elevated expression of the number of GFAP-positive cells, GFAP-immunoreactivity and protein content in relation to the post traumatic period as well as with a time-dependent up regulation of the P2Y1 receptor expression in the peritraumatic area. To confirm the localisation of the P2Y1 receptor subtype we performed multiple immunofluorescence studies in combination with laser scanning microscopy, indicating the astrocytic character of P2Y1-positive cells. The P2Y1 receptor expression was also found on a low number of MAP2-positve neurons. Furthermore, after TBI the expression of other marker for neuronal and glial cells as well as apoptosis was investigated.

In conclusion, present data show for the first time the involvement of the P2Y1 receptor subtype in injury-induced astroglial reaction in human, suggesting specific roles of purinergic receptors in glial cell pathophysiology in neurodegenerative diseases.

# Identification of glial functions modulating motor coordination in *Drosophila*

Silke Thomas[1], Imke Schmidt[1], Christian Klämbt[1]

[1]University of Münster, Institute for Neurobiology, Badestr. 9, 48149 Münster, Germany

The interaction of neurons and glial cells is crucial to establish a functional neuronal network. In the adult nervous system, glial cells remain important as they insulate axons to allow electric conductivity, support neurons with trophic factors and regulate synaptic homeostasis. However, only little is known about glial genes that modulate neuronal network function.

The principles of neuronal and glial functions appear conserved in all higher animals, but glial cell numbers vary extremely in different animals. Whereas glia comprises up to 90% of all cells in the human brain only 10% of all cells in the *Drosophila* nervous system are glial. The increasing number of glial cells throughout evolution underlines their importance for neuronal information processing. Anyway, the relatively few glial cells found in the *Drosophila* nervous system perform the same functional tasks as vertebrate glia. Furthermore, the *Drosophila* glia is morphologically rather similar to its vertebrate counterpart, suggesting that conserved mechanisms act in the nervous system of mammals and flies. The small number of *Drosophila* glial cells provides an enormous experimental advantage as all glial subtypes can be identified and individual cells can be targeted via specific Gal4 lines.

We performed a glia specific RNAi screen using the repoGal4 driver and UASdsRNA strains (Dietzl et al., 2007) to identify genes that are required in glial cells to allow neuronal network function. We tested about 5,000 RNAi lines (selected for membrane associated proteins, signal transduction and transcriptional regulators) for motor coordination defects in the adult fly upon pan-glial expression. Candidate genes were screened with specific glial subset driver lines to restrict gene function to one glial subtype. Further analysis of candidate genes inducing locomotion defects if exclusively suppressed in wrapping glial cells and analysis of larval locomotion will be presented.

# Impact of the NG2 proteoglycan on neuron-NG2 cell synaptic signaling

Stefan Passlick[1], Khalad Karram[2], Jacqueline Trotter[2], Gerald Seifert[1], Christian Steinhäuser[1], Ronald Jabs[1]

[1]Institute of Cellular Neurosciences, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany
[2]Molecular Cell Biology, University of Mainz, Bentzelweg 3, 55099 Mainz, Germany

Glial cells expressing the proteoglycan NG2 are widely distributed throughout the developing and adult gray and white matter of the CNS. Several properties distinguish them from astrocytes, mature oligodendrocytes and microglia. NG2 cells express different types of voltage-gated $K^+$, $Na^+$, and $Ca^{2+}$-channels. They also express a variety of ligand-gated receptors including group I metabotropic glutamate receptors and ionotropic AMPA- and $GABA_A$-receptors. Furthermore, NG2 cells are the only non-neuronal cells in the CNS that form synapses with neurons. In this respect, it is interesting that the NG2 protein (i) binds to the postsynaptic Glutamate Receptor Interaction Protein (GRIP) and (ii) contains two Laminin G/Neurexin/Sex Hormone Binding Globulin (LNS) domains in the extracellular region. GRIP is considered important for clustering of the GluR2 subunit of AMPA receptors. LNS domains are characteristic for postsynaptic neurexins that, by binding to presynaptic neuroligins, are important for synapse formation in neurons.

In this study we asked whether the NG2 protein is crucial for the formation of functional NG2 cell synapses by influencing clustering of postsynaptic receptors or neuroligin interactions. To address this issue, we investigated synaptic transmission between glutamatergic neurons and NG2 cells in NG2-EYFP-knockin (+/- and -/-) and wildtype mice (P8-15). We recorded whole-cell membrane currents from hippocampal NG2 cells during electrical stimulation of Schaffer collaterals and analysed the evoked excitatory postsynaptic currents (eEPSCs). Comparison of the kinetics and paired-pulse ratios of NG2 cell eEPSCs revealed no significant differences among the tested genotypes.

We conclude that the lack of the NG2 protein does not cause a general failure of synaptic signaling between glutamatergic neurons and NG2 cells in the hippocampus. It remains to be tested whether miniature EPSCs, which are not synchronised by presynaptic action potentials, are affected in NG2-defecient mice.

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# Inflammation in astrocytes induces abnormal $Ca^{2+}$ signaling caused by increased expression of VIA $Ca^{2+}$-independent phospholipase $A_2$ (VIA $iPLA_2$)

Georg Reiser[1], Mikhail Strokin[1]

[1]Otto-von-Guericke Universität Magdeburg, Institut für Neurobiochemie, Leipziger Str. 44, 39120 Magdeburg, Germany

Many $Ca^{2+}$-regulated intracellular processes are involved in the development of neuroinflammation. However, the changes of $Ca^{2+}$ signaling in brain under inflammatory conditions were hardly studied. Proper ATP-induced $Ca^{2+}$ signaling is a central event for the communication of the cells among astrocytic network, which functions in the tight concert with the synaptic network. We investigated the changes in $Ca^{2+}$ signaling in primary astrocytes after proinflammatory stimulation with lipopolysaccharide (LPS) and the role VIA phospholipase $A_2$ (VIA $iPLA_2$) in the observed changes. We measured $Ca^{2+}$ concentration in single cells using digital fluorescence microscopy and Fura-2 as fluorescence calcium-sensitive dye and alternative excitation wavelength 340/380 nm. We reveal that $Ca^{2+}$ responses to purinergic ATP stimulation are significantly increased in amplitude and duration after onset of inflammatory conditions (LPS 100 ng/ml for 6-24 h). We detected that increased amplitudes of $Ca^{2+}$ responses to ATP in LPS-treated astrocytes can be explained by substantial increase of calcium load in stores in endoplasmic reticulum. The mechanism implies enhanced calcium store refilling due to the amplification of capacitative calcium entry. Also for the increased duration of calcium responses in LPS-treated cells amplified capacitative calcium entry is the reason. Next, we established that the molecular mechanism for the LPS-induced amplification of calcium responses in astrocytes is the increased expression and activity of (detected by Western blot). Indeed, either gene silencing with specific siRNA or pharmacological inhibition of VIA $iPLA_2$ with S-bromoenol lactone reduced the load of the Ca2+ stores and amplitudes of calcium responses in LPS-treated astrocytes to values, which were comparable to those in untreated cells. Finally, our results show that calcium signalling in astrocytes is strongly disturbed under inflammatory conditions. Further, we demonstrated here that VIA $iPLA_2$ plays a key role in development of pathological calcium signaling. We propose that the regulation of VIA $iPLA_2$ activity is an instrument for neuroprotection during inflammation in brain after stroke (Strokin et al. 2006), brain bacteria invasion or encephalitis.

Reference: Strokin M., Chechneva O., Reymann K. G. and Reiser G. (2006) Neuroprotection of rat hippocampal slices exposed to oxygen-glucose deprivation by enrichment with docosahexaenoic acid and by inhibition of hydrolysis of docosahexaenoic acid-containing phospholipids by calcium independent phospholipase $A_2$. Neuroscience 140, 547-553.

# Modulation of $K^+$ buffering by aquaporin4 channels

Susan Strohschein[1], Kerstin Hüttmann[1], Siegrun Gabriel[2], Devin K. Binder[3], Uwe Heinmann[2], Christian Steinhäuser[1]

[1]Institute of Cellular Neurosciences, University of Bonn, Germany
[2]Institute of Neurophysiology, Charité Universitätsmedizin Berlin, Germany
[3]Center for Glial-Neuronal Interactions,University of California, Riverside, USA

Aquaporin4 (AQP4) is the main water channel in the brain and is primarily localized in astrocytes where the channels are thought to contribute to water and $K^+$ homeostasis during neuronal activity. The close apposition of AQP4 and inward rectifier $K^+$ channels (Kir4.1) gave rise to the hypothesis of direct functional interactions between both transmembrane channels. To get further insight into this issue, we investigated the impact of AQP4 on stimulus -induced alterations of the extracellular $K^+$ concentration ($[K^+]_o$) in the CA1 region of the hippocampus. $[K^+]_o$ was measured with $K^+$-selective microelectrodes, and field potentials were recorded simultaneously. In addition, gap junction coupling of astrocytes was investigated with tracer-filling of individual cells during patch -clamp recording. Comparative analyses were performed with wild -type and AQP4 knock out(ko) mice.

Retrograde fiber stimulation in the stratum oriens provoked smaller increases and slower recovery of $[K^+]_o$ in the stratum pyramidale of AQP4ko mice. Presumably, neuronal activity in the absence of AQP4 entails reduced glial swelling and a larger extracellular space as compared to controls. In a next step, we investigated the laminar profile of $[K^+]_o$ by moving the recording electrode from the stratum pyramidale towards the hippocampal fissure. At distances beyond 300 ìm from the stratum pyramidale, the stimulation-induced, normalized $[K^+]_o$ in AQP4ko mice significantly exceeded the corresponding values of controls. Astrocytes in AQP4ko mice displayed enhanced tracer-coupling which might underlie the improved spatial redistribution of $[K^+]_o$ in the hippocampus. These findings suggest that Kir4.1 and AQP4 channels are operating independently, and do not support the concept of direct functional interac-tions.

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# Morphological and functional analysis of astrocytes in the thalamus

Stephanie Griemsmann[1], Simon Höft[1], Gerald Seifert[1], Elena von Staden[1], Peter Bedner[1], Ronald Jabs[1], Martin Theis[1], David Cope[2], Vincenzo Crunelli[2], Christian Steinhäuser[1]

[1]Rheinische Friedrich-Wilhelms Universität Bonn, Institut für zelluläre Neurowissenschaften, Sigmund-Freud-Str. 25, 53105 Bonn, Germany

[2]University of Cardiff, School of Biosciences, Cardiff, United Kingdom

Aims: The thalamus serves as an important relay for information reaching the cortex. While astrocytes are well investigated in several brain regions such as cortex and hippocampus, little is known about properties and functions of glial cells in the thalamic nuclei. Recent studies have shown that astrocytes in the thalamus can modulate neuronal signalling upon intracellular $Ca^{2+}$ increases travelling through the astrocytic network. In the present study we investigated basic morphological and functional properties of astrocytes in the VPL and VPM nuclei of the thalamus in order to get a better understanding of neuron-glia interaction in this region of the brain.
Methods: We combined electrophysiological and immunohistochemical approaches with semi quantitative RT-PCR and Western blot analysis to investigate transmitter receptors, connexin expression, gap junction coupling and antigen profiles. Experiments were performed in wild type and transgenic mice with astrocyte-specific or glia-exclusive fluorescence labeling (hGFAP -EGFP, Cx43 -CFP ki, NG2 -EYFP ki) as well as in knockout mice (Cx30LacZ). We also investigated the red fluorescent dye SR101 for its properties as an astrocytic marker in the thalamus. Astrocytes were investigated in brain slices of mice aging between postnatal days 8-120.
Results: Antibody staining demonstrated that subpopulations of thalamic astrocytes express only weakly GFAP while staining strongly for glutamine synthetase. In freshly isolated, whole cell patch-clamped astrocytes, kainate induced small responses in a subset of the cells while much larger currents were evoked by GABAA receptor activation. Investigations of cell-cell coupling by biocytin filling of astrocytes of hGFAP-EGFP mice revealed profound gap junction coupling in young and adult mice (no of coupled cells: 105 +/- 31 cells), which decreased during aging. Unexpectedly, coupling was not decreased in heterozygous Cx43-CFP ki mice. In these mice SR101 labeling of astrocytes and subsequent Two-Photon microscopy identified a significant (41%) subset of SR101+ cells lacking CFP fluorescence. Subsequent semi-quantitative RT-PCR revealed a stronger expression of Cx30 in thalamic nuclei while Cx43 mRNA levels were higher in the hippocampus.Together, these data indicate a minor role for Cx43 in gap junction coupling of astrocytes in the thalamus. Consistent with these findings cell coupling analysis in Cx30 ko animals revealed a strong decrease in astrocytic cell coupling compared to wild type littermates.
Conclusions: These results indicate that thalamic astrocytes differ in various aspects from their counterpart in other brain regions and support the emerging concept of astrocyte heterogeneity.
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# Neuronal control of CNS myelination in conditional *Pten* mutant mice

Georg Wieser[1], Alexander Pieper[1], Bettina Weege[1], Klaus-Armin Nave[1], Sandra Goebbels[1]

[1]Department of Neurogenetics, Max Planck Institute of Experimental Medicine, Goettingen, Germany

In the vertebrate nervous system, oligodendrocytes assemble myelin, thereby decreasing the electrical capacity of axonal membranes and enabling rapid saltatory impulse propagation. The failure to achieve and maintain normal myelin causes severe neurological diseases including leukodystrophies and multiple sclerosis (MS). Thus, one of the major challenges in cellular neurobiology is the identification of neuronal and axonal signals that trigger oligodendrocyte precursor cell (OPC) proliferation and differentiation and myelination by mature oligodendrocytes. We have previously shown that the axonal growth factor Neuregulin-1 (NRG1) regulates myelination by Schwann cells. However, the corresponding neuronal signal(s) for oligodendrocytes in the CNS are distinct, and presently unknown.

To understand the respective signals in the developing and adult brain, we generated a mutant mouse line, which lacks the expression of the lipid phosphatase PTEN in granule cells of the cerebellum. In these mice, granule cells are hypertrophic and the normally small and unmyelinated parallel fiber axons increase in diameter. More importantly, this appears sufficient to trigger (i) the proliferation of OPC in the molecular layer, (ii) their maturation to oligodendrocytes, and (iii) the *de novo* myelination of parallel fibers starting at around postnatal day 40 (P40). By laser capture microdissection of mutant and control granule cells in combination with differential gene expression profiling the *Pten* mutant mouse model is used to identify neuronal candidate genes, whose expression regulates development and maturation of oligodendroglial cells.

# Nitric oxide induces HIF-1alpha protein stabilisation in primary astrocytes via PI3K/AKT/mTOR and MAPK pathways

Britta Brix[1], Fabrice Marcillac[2], Luc Pellerin[2], Olaf Jöhren[1]

[1]University of Lübeck, Institute of Pharmacology and Toxicology, Ratzeburger Allee 160, 23562 Lübeck, Germany
[2]University of Lausanne, Institute for Physiology, Switzerland

Hypoxia-inducible Factor 1 (HIF-1), a key mediator of cellular adaptation to hypoxia, is a heterodimeric complex comprising the subunits HIF-1alpha and HIF-1beta. HIF-1 controls transcription of genes amongst others those involved in glucose and iron metabolism, energy homeostasis or vascularisation. At physiological oxygen concentrations HIF-1alpha is hydroxylated followed by polyubiquitination and finally proteasomal degradation. Various chemical compounds for instance carbon monoxide or deferoxamine have been described to stabilise HIF-1alpha under normoxic conditions. In this study we investigated the impact of nitric oxide (NO) on glycolysis in murine primary astrocytes with respect to HIF-1alpha activity. Quantitative real-time PCR revealed that HIF-1alpha mRNA levels were markedly increased after treatment with the NO donor DETA-NONOate in astrocytes cultured under normoxic conditions. Concordantly, HIF-1 target genes like phosphoglycerate kinase, pyruvate dehydrogenase kinase, hexokinase 2 as well as glucose transporter-1 and monocarboxylate transporter 4 were found to be increased significantly at levels of mRNA. The NO-induced increase in HIF-1alpha and HIF-1alpha target gene expression was abolished by HIF-1alpha siRNA knockdown. HIF-1alpha protein stabilisation in response to NO was confirmed by immunocytochemistry and Western blot analysis.
To investigate the signaling pathways involved in HIF-1alpha protein stabilisation, astrocytes where incubated with the PI3K inhibitor LY29004, the mTOR inhibitor rapamycin or the Map kinase kinase inhibitor PD98059 prior to NO treatment. Immunoblotting analyses revealed that all applied inhibitors were capable of reducing NO induced HIF-1alpha protein stabilisation. In addition, NO induced HIF 1-alpha target gene expression was also diminished when astrocytes were incubated with these inhibitors. Further analysis by Western blotting showed that treatment of astrocytes with NO resulted in increased levels of phosphorylated AKT. In contrast, phosphorylation of the translation factors elF-4E and p70S6 kinase was not influenced by treatment with NO. Thus, our results indicate that PI3K/AKT/mTOR as well as MAPK signaling play a crucial role for NO induced HIF-1alpha stabilisation in astrocytes. Moreover, stabilisation of this protein seems to be independent of enhanced translation.
We conclude that HIF-1alpha plays a pivotal role in the regulation of glycolysis in NO-stimulated astrocytes and that PI3K/AKT/mTOR and MAPK pathways are involved in HIF-1alpha protein stabilisation.

# pH regulation of identified neurons and glial cells in acute mouse cerebellar slices

Marco D. Alt[1], Joachim W. Deitmer[1]

[1]General Zoology, FB Biologie, TU Kaiserslautern, P.O. Box 3049, D-67653 Kaiserslautern, Germany

Regulation of intra- and extracellular proton concentration is crucial for adequate protein function, fluidity of the membrane, cellular activity and a variety of metabolic processes. The amount of free protons is regulated by proton transport and chemical buffering. The most prominent buffer system in living organisms is provided by $CO_2$ and hydrogencarbonate. The reaction from $CO_2$ and water to $HCO_3^-$ and $H^+$ is catalyzed by carbonic anhydrases (CA), which greatly increase the reaction speed of the $CO_2/HCO_3^-$-dependent buffer system. Within the nervous system, the most prominent CA isoforms are the intracellular CAII and the extracellular isoforms CAIV and CAXIV. All three CA isoforms have been shown to interact with various acid base-coupled transporters like the electrogenic sodium bicarbonate cotransporter (NBCe1), the sodium proton exchanger NHE, and the chloride hydrogencarbonate exchanger (AE). These transporters are highly expressed in the nervous system, in glial cells and/or neurons, where they play a crucial role in pH homeostasis by either importing or exporting acid-base equivalents. This study addresses the physiological characterization of these pH-regulating mechanisms in the cerebellar cortex. Therefore we used *in situ* live-cell imaging with the proton-sensitive fluorescent dye BCECF. Experiments were performed on (1) GRFT mice, which express the red fluorescent protein mRFP under control of the glia-specific GFAP promoter to simplify cell type identification, and on (2) B6.D2-Car2$^o$ mice, which lack CAII expression due to a nonsense mutation in the Car2 locus. We studied the significance of $CO_2/HCO_3^-$ and of CAs for cytosolic buffer in Bergmann glia, astrocytes of the granule cell layer and granule cells. We were able to determine the contribution of NBCe1 and NHE to the cellular $H^+$ regulation in resting conditions, during acidification and alkalinisation in neurons and glia cells. The removal of $CO_2/HCO_3^-$ from the buffer induced a fast intracellular alkalinization, which was 30% slower in CAII KO mice as compared to wildtype mice. In the presence of the CA blocker 6-Ethoxy-2-benzothiazolsulfonamid (EZA, 10 µM), the rate of acidification in both, KO and WT, was reduced to the same low level (50% of the WT control).

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# Pharmacological Properties of Two Pore Domain $K^+$ Channel-mediated Currents in Hippocampal Astrocytes

Johannes Weller[1], Gerald Seifert[1], Christian Steinhäuser[1]

[1]University of Bonn, Institute of Cellular Neurosciences, Sigmund Freud Str. 25, 53105 Bonn, Germany

In the hippocampus, astrocytes predominantly express time- and voltage-independent currents, but the underlying ion channels are not well defined and subject to controversial discussion. This is partly attributable to the extensive intercellular coupling of astrocytes through gap junctions, impeding quantitative analysis of membrane properties in situ.

Previous studies suggested that two- pore domain $K^+$ (K2P) channels play an important role in setting astrocyte resting conductance. In the present study we examined K2P channel expression and addressed its functional regulation in mouse astrocytes. We combined electrophysiological analysis with single cell RT -PCR and immunohistochemistry of astrocyte-targeted transgenic mice. To avoid coupling artifacts and unmask putative K2P currents, electrophysiological experiments were performed in freshly isolated astrocytes (p8-12) in the presence of $K_v$ and Kir channel blockers. Application of TEA, 4-AP and $Ba^{2+}$ greatly reduced membrane currents in freshly isolated astrocytes. The blocker-resistant currents were amplified and further analysed in high $K^+$ (20 mM) bath solutions. The poly-unsaturated fatty acids, arachidonic acid and linolenic acid, increased putative K2P currents. Current responses were also sensitive to quinine, a K2P channel antagonist, and reversed close to $E_K$. Extracellular acidification led to a current increase in all cells tested. Single cell RT-PCR confirmed expression of K2P-channel transcripts TREK-1 and TWIK-1 while there was no evidence of expression of TRAAK, TASK-1 and TASK-3 subunits. Immunohistochemical analyses are under way to confirm these findings on the protein level and reveal the subcellular distribution of K2P channels.

Together, these findings indicate functional expression of TREK-1 channels in astrocytes of the hippocampus which possibly contribute to the significant resting conductance of these cells, neurovascular coupling and spacial $K^+$ buffering. Moreover, we demonstrate that these channels are regulated by various physiological and pathological stimuli, suggesting a function as signal integrators.

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# Profilin1 activity in cerebellar granule neurons is crucially important for glia cell binding and radial migration

Jan Kullmann[1], Alexander Neumeyer[2], Reinhard Fässler[3], Joachim W Deitmer[2], Eckhard Friauf[4], Walter Witke[5,6], Marco B Rust[1,6]

[1]TU Kaiserslautern, Neurobiology/Neurophysiology Group
[2]TU Kaiserslautern, General Zoology Group
[3]Max Planck Institute of Biochemistry, Department of Molecular Medicine
[4]TU Kaiserslautern, Animal Physiology Group
[5]University of Bonn, Institute of Genetics
[6]EMBL Mouse Biology Unit, Rom/Monterotondo

Migration of neurons along processes of radial glia cells is essential for the lamination of cortical structures during brain development of higher vertebrates. Cerebellar granule neurons (CGN) exploit Bergmann glia fibers for their migration from the external to the internal granule cell layer, and adhesion of CGN to Bergmann glia plays a pivotal role in controlling this migration. However, only little is known about the molecules and mechanisms that control the interaction between CGN and Bergmann glia. Here we demonstrate that the activity of the actin monomer-binding protein profilin1 in CGN is critical for the glia cell binding of CGN and their migration along fibers of Bergmann glia. We show that genetic deletion of profilin1 in the mouse brain causes a cerebellar dysplasia that is characterized by a reduced size of the cerebellum and aberrant layering of the cerebellar cortex, impaired radial migration of CGN, and the occurrence of differentiated ectopic CGN. While our data highlight the relevance of profilin1 for CGN/Bergmann glia interaction, they also demonstrate that profilin1 is not involved in the proliferation of CGN progenitors, the tangential migration of CGN or the development of Bergmann glia. Thus, our data revealed that during cerebellar development profilin1 is specifically relevant for glia cell adhesion and radial migration of CGN.

# Real time changes of morphology and intracellular diffusivity of hippocampal astrocytes

Christian Henneberger[1], Kaiyu Zheng[1], Dmitri A. Rusakov[1]

[1]University College London, Department of Clinical and Experimental Epilepsy, Queen Square House, Queen Square, WC1N 3BG London, United Kingdom

Glia cells such as astrocytes do not only provide structural and metabolic support to neurons but also affect synaptic transmission by shaping neurotransmitter concentration transients near synapses through neurotransmitter uptake and by releasing a number of active signalling molecules targeted at synapses. Thin astrocyte processes tightly enwrapping synapses are potentially particularly efficient because of their spatial proximity. However, the intracellular space of these small astrocyte processes is highly tortuous and intra-luminal diffusion is limited, which could restrict, for example, the invasion of fine processes by release triggering second messengers like calcium. This balance is likely to be adjusted dynamically because astrocyte coverage of synapse is not static and depends on development and the functional state of the synapse. Investigating the interplay between astrocyte morphology and synapse function therefore requires methods that monitor both, morphology and diffusivity, in real time and at high resolution.

We used two-photon (2P) fluorescence microscopy integrated with electrophysiology to study astrocytes in the stratum radiatum in an acute slice preparation of the rat hippocampal CA1 area. The morphological tracer Alexa 594 was delivered into astrocytes using the patch-clamp technique and excited at 800 nm. Passive astrocytes were characterized by their low input resistance (< 20 MO), low resting potential (< -85 mV), the absence of major voltage dependent conductances, their typical morphology, and extensive gap junction dye coupling.

Since fine astrocyte processes can be as small as 30-50 nm, conventional 2P imaging with a diffraction limit of ~200-300 nm is as such of insufficient spatial resolution. We therefore established a combination of image analysis parameters that can differentiate between changes in the number and size of individual astrocyte processes in a small given volume based on the analysis of astrocyte volume fraction, image entropy and segmentation. Acute changes of astrocyte process size were induced using hyper- and hypoosmolar solutions and used to verify this analytical approach. Additionally, intracellular diffusivity was assessed either by fluorescence life time imaging (2P-FLIM) or fluorescence recovery after photobleaching (2P-FRAP).

These techniques were then combined to investigate structural changes of astrocytes after induction of long-term potentiation (LTP) at Schaffer collateral synapses. Both, morphology and intracellular diffusivity were affected by LTP induction and required activation of N-methyl D-aspartate receptors. The observed changes are compatible with a retraction of astrocyte processes from synapses after LTP.

# Secretion of aldolase-C is accompanied of morphological changes in astrocytes following repetitive fluoxetine treatment.

Rodrigo Herrera-Molina[1], Mauricio Sandoval[2], Alejandro Luarte[3], Karl-Heinz Smalla[4], Eckart D Gundelfinger[5], Urzula Wyneken[6]

[145] Leibniz Institute for Neurobiology, Magdeburg, Germany.[1236] Laboratorio de Neurociencias, Universidad de los Andes, Santiago, Chile.

One of the most used drugs to treat depression is fluoxetine. Even though fluoxetine increases serotonin neurotransmission within few hours, its clinical effects have a time lag of weeks. This constitutes the "antidepressant drug paradox", which suggests that additional mechanisms develop following repetitive drug administration. To get insight into such mechanisms, rats were daily treated with therapeutic doses of fluoxetine (0.7 mg/kg) or with NaCl (0.9%) during 14 days. Next, subcellular fraction obtained from telencephalon were submitted to 2D electrophoresis and mass spectrometry. We found highly significant changes in 14 proteins with primary functions in metabolism, signalling, protein folding or other. Importantly, a 7-fold increase of the astrocytic glycolytic enzyme aldolase-C occurred in vesicular fractions of fluoxetine-treated rats. By Western blot, a 2.5-fold increase of aldolase-C in the cerebrospinal fluid was detected. By immunohistochemical analysis, a 4-fold increase in extracellular staining of aldolase-C invading the hippocampal pyramidal cell layer whereas that a decrease by 67% in colocalization of aldolase-C and astrocytic GFAP in stratum radiatum was observed. Intriguingly, astrocytes which decreased aldolase-c content presented more extended and abundant ramifications in fluoxetine-treated rats. Our results indicate that astrocytic aldolase-C might have an unanticipated extracellular role in the plastic changes induced by fluoxetine. Currently, we perform morphometric analysis of the astrocyte-neuron structural interface by three -dimensional modelling of z -stack projections obtained using high resolution confocal microscopy. We hypothesize that astrocytic morphological rearrangement affects astrocyte-neuron interaction in consequence of fluoxetine treatment.

Figure 1. Changes of aldolase-C correlate with morphological changes in the hippocampal CA1 region following fluoxetine treatment. Rats were daily treated with fluoxetine (Flx) or NaCl during 14 days, sacrificed under ketamine:xylazine (50:5 mg/kg) anaesthesia and perfused intracardially with 4% paraformaldehyde. GFAP (red) and aldolase -C (ALDO, green) stained sections were photographed with a 63X/1.4NA plane objective in an inverted microscope (AxioImagerA2). Deconvolution (Deconv) of images with AutoQuantX2 software was performed. A, Both, diffuse and vesicular-like ALDO-associated fluorescence are largely decreased by Flx in GFAP-astrocytes whose morphology is more complex. BI, GFAP and DAPI stained sections were obtained and photographed as in A. Bar=200 µm. a -d are magnifications of GFAP -astrocytes in stratum radiatum (s.r.) or stratum oriens (s.r.) of control (red frame) and Flx-treated animals (green frame) showed in BII. Bar=65 µm; b and d Bar=30 µm.

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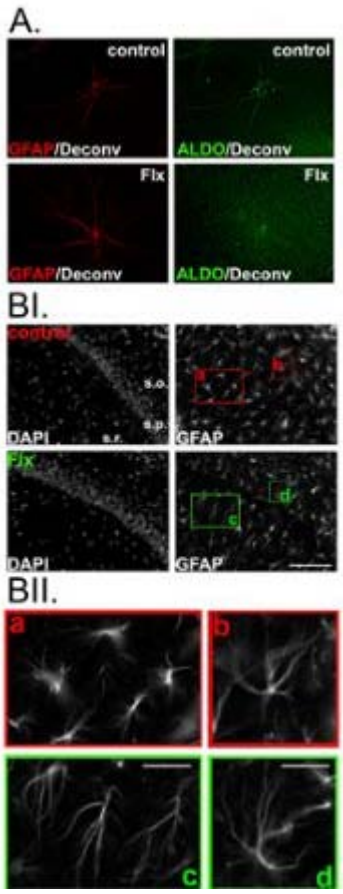
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# Study on the astroglial expression of glutamate receptors in the ventral respiratory group

Christian Schnell[1], Mahmoud Negm[1], Jens Fresemann[1,2], Swen Hülsmann[1,2]

[1]Universitätsmedizin Göttingen, Abteilung Neuro- und Sinnesphysiologie, Humboldtallee 23, 37073 Göttingen, Germany
[2]DFG Research Center Molecular Physiology of the Brain (CMPB)
[3]Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB)

Breathing requires a complex pattern of neuronal activity. An important part of the ventral respiratory network (VRG), located in the medulla, is the pre-Bötzinger complex (preBötC). In previous studies it has been shown, that glutamate modulates respiratory activity by activating glutamate receptors expressed by neurons in the respiratory network. While the role of astrocytes in uptake and conversion of glutamate to glutamine in the Krebs cycle is well
investigated, studies on astroglial expression of glutamate receptors in the VRG are rare. Because it is known from other brain regions, that astrocytes expressing glutamate receptors modulate synaptic stabilization and strength, in our study we looked for the functional expression of glutamate receptors in astrocytes in the VRG.
We used acute brainstem slice preparations of mice with eGFP or mRFP-labeled astrocytes or NMRI mice. In the latter, astrocytes were identified using the low $K^+$ method. Slices were loaded with Oregon Green to perform calcium imaging.
In a first approach we performed 2-photon calcium imaging to investigate the effect of group I mGluR and AMPA receptor agonist quisqualate on EGFP-labeled astrocytes in the VRG. Quisqualate triggered a calcium signal in virtually every astrocyte in the VRG. To discriminate between AMPA and group I mGluR mediated effects, we applied quisqualate also in the presence of DNQX. Blockade of non-NMDA receptors with DNQX significantly reduced the amplitude of the calcium signal, indicating that not only AMPA receptors but also metabotropic glutamate receptors are activated by quisqualate. Using widefield calcium imaging we could show that DHPG as an agonist of mGluR group I triggers calcium signals in a high number of low $K^+$-identified astrocytes. These calcium signals were not blocked by TTX, but significantly reduced after application of CPA to deplete intracellular calcium stores.
We got also evidence from immunohistochemical stainings for mGluR1 and mGluR5 for the presence of these receptors in the VRG. Although the soma of eGFP labeled astrocytes was only infrequently stained completely, we saw an overlap of astrocytic processes labeled by eGFP and stainings against group I mGluRs.
While we have demonstrated the functional expression of group I metabotropic glutamate receptors as well as non-NMDA receptors on astrocytes in the ventral respiratory group, the physiological role of these receptors remains to be clarified in future studies.

# Therapeutic potential of non-neuronal cells in Amyotrophic Lateral Sclerosis (ALS )

Hui Sun[1], Nadine Thau[1], Reinhard Dengler[1], Susanne Petri[1]

[1]Medical School Hannover, Department of Neurology, Carl-Neuberg Str. 1, 30615 Hannover, Germany

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by the death of motor neurons in the motor cortex, brain stem and spinal cord. The G93A transgenic mouse model expresses a mutant form of human superoxide dismutase (SOD1) and develops motor neuron disease similar to ALS.
It has previously been shown that surrounding non-neuronal cells (glia) have an influence on the survival of motor neurons. The dysregulation of intracellular Ca2+ homeostasis (exictotoxicity) is thought to contribute to the death of motor neurons.
We are therefore studying the interaction of neuronal and non-neuronal cells in vitro using a Calcium imaging system.
For this purpose, a co-culture system of motor neurons and astrocytes, derived either from mutant transgenic G93A-ALS mice or from wild-type animals, is established.
Transgenic and wild type motor neurons and astrocytes, respectively, are studied regarding survival, resistance to excitotoxic or oxidative stimuli and the occurrence of calcium transients.
This co-culture system presents a valuable in vitro screening tool for novel neuroprotective compounds in ALS.

# Translational Regulation of Astrocytic Connexins and Glutamine Synthetase by CPEB3

Vamshidhar Reddy Vangoor[1], Sada Lakshmi Turimella[1], Lech Kaczmarczyk[1], Jiong Zhang[1], Peter Bedner[1], Elena von Staden[1], Amin Derouiche[1], Ronald Jabs[1], Gerald Seifert[1], Christian Steinhäuser [1], Martin Theis[1]

[1]Institute of Cellular Neurosciences, University of Bonn, Sigmund Freud Strasse 25, 53105 Bonn, Germany

Cytoplasmic polyadenylation element binding proteins (CPEBs) are a family of translational regulators expressed in brain. They bind to mRNAs which contain the cytoplasmic polyadenylation element (CPE) and control protein synthesis. CPEBs in neurons play a role in synaptic plasticity, learning and memory. CPEB -1 was recently described in cultured astrocytes, and we investigated whether CPEBs 2-4 are also relevant in astroglia. All CPEB transcripts and proteins were detected in rat primary astrocyte cultures. In addition, CPEB-2, -3 and -4 were found in distinct glial subpopulations (astrocytes and NG2 cells) of the mouse hippocampus by single cell RT-PCR, non-radioactive in situ hybridisation and FACS analysis. Expression was less abundant in astrocytes compared to NG2 cells. We found that overexpression of CPEB3-EGFP in astrocytes during development caused enlarged ventricles, and that acute overexpression in adult mice induced reactive astrogliosis in cortex, hippocampus and thalamus. In these brain areas, CPEB-3 overexpression also led to downregulation of Connexin43 (Cx43), an astrocytic gap junction protein involved in extracellular ion homeostasis and glucose supply of neurons. Consistently, in the hippocampus of CPEB -3 overexpressing mice, intercellular gap junction coupling was strongly impaired. We showed previously that connexin function in radial glia-like cells is required for adult neurogenesis in the subgranular zone (SGZ), a neurogenic niche in the hippocampus. In the SGZ of CPEB-3 overexpressing mice we observed a significant reduction in the number of proliferating, Ki-67 positive cells. CPEB-3 overexpression also led to a downregulation of glutamine synthetase (GS), a key player in extracellular glutamate clearance. Since both interastrocytic coupling and astrocytic GS activity are downregulated in epilepsy patients presenting with hippocampal sclerosis, we hypothesise that upregulation of CPEBs in astrocytes contributes to the pathogenesis of epilepsy.

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# Poster Topic

# T10: Aging and Developmental Disorders

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T10-2A Classical microglial activation in a DNA repair defective accelerated model of aging
*Divya Raj, Nieske Brouwer, Marta Olah, Monique C de Waard, Ingrid van de Pluijm, Michel Meijer, Knut Biber, Erik Boddeke*

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*Nadine Strien, Daniel Wiswede, Marcus Heldmann, Thomas F. Münte*

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T10-3C The role of Disc1 during interneuron migration
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T10-4C Uncovering molecular mechanisms of early-onset age-related memory deficits in CB1 receptor knockout (Cnr1-/-) mice
*Anastasia Piyanova, Önder Albayram, Kerstin Michel, Rainer Buchalla, Andreas Zimmer, Andras Bilkei-Gorzo*

# Cadherin expression profiles in the allocortex/periallocortex of wild-type and Reeler mutant mice

Gudrun Stoya[1], Nicole Hertel[1], Christoph Redies[1]

[1]University of Jena School of Medicine, Institute of Anatomy I, Teichgraben 7, 07743 Jena, Germany

Cadherins are multifunctional adhesive molecules; their expression suggests a role as recognition cues in the layer formation of the developing cerebral cortex. An appropriate model for studying corticogenesis is the Reeler mutant mouse. While cortical regionalization is not affected, radial neuronal migration is disturbed. This may lead to an inversion of cortical layers, but recent results rather suggest a widespread and patchy expression of layer-specific markers in the adult Reeler neocortex (Dekimoto et al., 2010; Hertel et al., 2010).
By in situ hybridization, we examined the expression pattern of thirteen different cadherins during development of the hippocampus (dentate gyrus, CA regions, subiculum) and parahippocampal regions (presubiculum, parasubiculum, entorhinal cortex) in adult wild-type and Reeler mice.
All the cadherins investigated are expressed differentially and in a locally restricted fashion in wild-type mice. Every region shows combinatorial expression of several cadherins. The expression profiles are altered only in the radial dimension in the Reeler mutant. Layer formation differs in the allocortex and is largely absent in the periallocortex. Regional cadherin expression (dentate gyrus, CA regions, presubiculum etc.) is preserved in the hippocampal formation of the Reeler mutant.
In conclusion, we demonstrate a radial dispersion of cadherin-expressing cells in phylogenetical old cortical regions of the Reeler mutant, possibly caused by defects of radial glial cells and in neuronal migration.

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# Classical microglial activation in a DNA repair defective accelerated model of aging

Divya Raj[1], Nieske Brouwer[1], Marta Olah[1], Monique C de Waard[2], Ingrid van de Pluijm[2], Michel Meijer[1], Knut Biber[3], Erik Boddeke[1]

[1]University Medical Center Groningen, Department of neuroscience, A. Deusinglaan 1 9713AV Groningen,The Netherlands[2]Erasmus University Medical Center,Department of Genetics,PO Box 2040, 3000 CA Rotterdam, The Netherlands[3]University of Freiburg, Department of molecular psychiatry and psychotherapy, Molecular psychiatry, Hauptstrasse 5, 79104 Freiburg, Germany

Brain aging is characterized by cognitive decline in the majority of the population and is a prime predisposing factor for several neurodegenerative diseases. Neurodegeneration and glial activation have also been proposed to be cardinal features of aging. Although there have been several studies on aging neurons, effects of aging on glial functionality has nor been studied in detail. From previous studies, there have been two prominent schools of thought concerning the contribution of microglia to brain aging and neurodegenerative disorders. Chronic microglia induced neuroinflammation has been regarded by many as potentially detrimental and neurotoxic, thus contributing to aging and neurodegenerative disorders. The other idea comes from postmortem observations that a subpopulation of the activated microglia in aged and diseased brain also exhibits signs of cell death and cytoplasmic degeneration termed “microglial dystrophy”. This hypothesis proposes that microglia lose their supportive functions towards neurons resulting in age associated neurodegeneration.

We therefore attempted to address the aging microglia phenotype and functional characteristics in this mechanistic mouse model of aging. In the present study we have used DNA damage deficient mice model with accelerated aging characteristics to understand microglial aging response. ERCC1 in complex with XPF acts as a nuclease in the Nucleotide excision repair (NER) pathway. The mice used in the present study lack one allele of the excision repair cross -complementing group 1 (ERCC1) gene, whereas the protein derived from the other allele shows reduced activity, due to a seven amino-acid carboxy-terminal truncation. The accumulation of endogenous crosslinks as a result of ERCC1 functional inefficiency leads to deficient NER, cross-link DNA repair, cell cycle arrest, replicative senescence and genomic instability at the cellular level thereby causing accelerated ageing in mice.

Microglia isolated from these mice show robust activation as characterized by quantification of microglia morphology and extensive phenotyping of microglial activation markers by flow cytometry. Microglia isolated from this model also show increased phagocytic activity correlating to their activation status. There also is an exaggerated production of Reactive Oxygen Species(ROS) on injection of animals with Lipopolysaccaride (LPS). Furthermore, aging microglia are also characterized by accumulation of lipofuscin and ferritin suggesting active phagocytosis in vivo. Microglial activation associated with brain stem and spinal cord is drastic compared to other brain regions which might be associated with the motor neuron degeneration reported in this model. In this model of acclerated senescence, we find a robust activation of microglia in phenotypic and functional terms which may play a role in age related neurodegenerative pathology.

# Kainate Treatment Impairs the Proteolytic Processing of Reelin Necessary for Granule Cell Positioning

Stefanie Tinnes[1], Carola A. Haas[1]

[1]University of Freiburg, Experimental Epilepsy Group, Breisacher Straße 64, 79106 Freiburg, Germany

The extracellular matrix protein Reelin is an important regulator for the formation of cortical layers during development and maintains this lamination in the adult hippocampus. In temporal lobe epilepsy (TLE) patients and in a TLE mouse model, Reelin levels are decreased which causes a migration defect of adult granule cells (Haas et al., 2002, Heinrich et al. 2006). However, not only absolute Reelin levels, but also proper proteolytic processing, giving rise to several Reelin isoforms, is important for its biological function. Since matrix metalloproteases (MMP) are involved in the cleavage of Reelin, we investigated whether MMP-dependent processing of Reelin is impaired under epileptic conditions and contributes to the malpositioning of dentate granule cells in TLE.
As a prerequisite we showed that Reelin processing is decreased under epileptic conditions. Treatment of rat organotypic hippocampal slice cultures with kainate (KA) resulted in an increase of high molecular weight Reelin isoforms in tissue and a significant decrease of the secreted 180 kDa Reelin fragment. These changes of Reelin isoform composition could be mimicked by blockade of MMPs, confirming that KA indeed alters the proteolytic processing of Reelin. Not only epileptic conditions but also impaired proteolytic cleavage of Reelin by inhibition of MMPs caused a significant widening of the granule cell layer and an extracellular accumulation of unprocessed Reelin. Moreover, in KA -treated hippocampal slice cultures in situ zymography demonstrated a decrease of MMP-2 and MMP -9 (gelatinases) activity in the molecular layer and the granule cell layer of the dentate gyrus where extracellular Reelin accumulates after KA. In contrast, in other regions of the hippocampus cellular gelatinase activity appeared to increase. Taken together our results suggest that under epileptic conditions, proteolytic processing of Reelin by MMPs is impaired. As a consequence, Reelin accumulates extracellularly as a biological inactive form and contributes to granule cell dispersion (Supported by the DFG, SFB TR3).

# Lithium interferes with hippocampal development but does not involve GSK3beta

Joel Jarowyj[1], Bianka Brunne[1], Eckart Förster[3], Michael Frotscher[1]

[1]University of Freiburg, Institute of Anatomy and Cell Biology, Department of Neuroanatomy, Albertstraße 17, 79104 Freiburg-im-Breisgau, Germany

[2]University of Freiburg, Faculty of Biology, Schaenzlestraße 1, 79104 Freiburg-im-Breisgau, Germany

[3]University of Hamburg, Department of Anatomy I: Cellular Neurobiology, Martinistraße 52, 20246 Hamburg

Most hippocampal dentate granule cells are born early postnatally. Their migration is controlled by Reelin, a protein secreted by Cajal-Retzius cells in the marginal zone of the dentate gyrus. An identified component of the Reelin signalling cascade is the enzyme glycogen synthase kinase 3 beta (GSK3beta) that acts on the cytoskeleton by modulating tau phosphorylation. Activity of GSK3beta is downregulated by Reelin or by application of pharmacological inhibitors, such as lithium chloride (LiCl). Lithium is also known as a drug for the treatment of neurological disorders thought to be causally related to developmental neuronal migration defects. In a first study, we were interested whether LiCl may interfere with developmental processes known to be regulated by Reelin signalling. For this purpose we used hippocampal slice cultures. Different concentrations of LiCl were added to the incubation medium and the morphology of the cultures was analyzed after 6 days in vitro by double immunostaining with G10-Reelin antibodies (Chemicon) known to stain Cajal-Retzius cells and Prox-1 (Swant) antibodies specific for granule cells. With increasing concentrations of LiCl, we observed a loss of the proper arrangement of the dentate granule cell layer, suggesting that lithium interfered with the positioning of dentate granule cells. Next, we investigated by Western-Blotting whether LiCl treatment of the cultures affected Reelin content in the incubation medium. We found that Reelin content decreased with increasing LiCl concentrations. We also studied the effects of lithium on the morphology of Reelin-secreting Cajal-Retzius cells by immunostaining them with an antibody against Calretinin (Swant). Application of LiCl in the range of therapeutic concentrations induced neurite retraction of Cajal-Retzius cells in a dose-dependent manner. In order to compare the specificity of the LiCl-induced Reelin decrease, these experiments were repeated by replacing LiCl with sodium chloride (NaCl) or SB415286 (Sigma-Aldrich), a specific inhibitor of GSK3beta, to see whether the observed effects were mediated via GSK3beta. No similar effects were observed neither with NaCl nor with SB415286, indicating that these effects were lithium-specific, but were not induced by GSK3beta inhibition.
In ongoing experiments, we now study whether or not similar migration defects as observed in vitro are also seen in vivo in adult animals. Mice aged one month were injected with 0.3 M and 0.6 M LiCl or NaCl for 6 and 15 days, respectively, by daily intraperitoneal injections. 24h after the last treatment, the animals were perfused, the brains paraffin-embedded and sectioned, and the sections processed for cresyl violet staining.
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# Morphological and Molecular Characterization of Focal Cortical Dysplasias

Catharina Donkels[1], Susanne Fauser[2], Josef Zentner[3], Carola A. Haas[1]

1Experimental Epilepsy Research Group, 2Epilepsy Center, 3Department of Neurosurgery, University Clinic Freiburg, Germany

Focal cortical dysplasias (FCD) are malformations of the human neocortex which are highly associated with intractable epilepsy, especially in young children. The neuropathological characteristics of FCD extend from mild laminar disorganization and hypertrophic neurons (FCD type I) to severely developed laminar disorders with the appearance of dysmorphic neurons and cytomegalic cells (FCD type II). It is assumed that FCD arise due to local disturbances during prenatal development. To date very little is known about structural and molecular composition of these cortical malformations and to which extent they differ from normally developed neocortex. To address this question we used a combined morphological and molecular approach to analyze human resected FCD (type I and II) specimens from different cortical areas, obtained from patients with pharmaco-resistant epilepsy. In each FCD sample the degree of dyslamination was visualized by immunolabeling for NeuN and neurofilament H (SMI32), a marker for pyramidal cell layers 3 and 5, and, in parallel, the expression of genes encoding markers of laminar fate (e.g. reelin, Er81, Rorß, TLE4) , interneurons and cell maturity (e.g. doublecortin) was analyzed by real time RT-PCR. We found that in all specimens lamination was basically preserved, but there were strong differences with respect to lamina width, in some cases layers were even blurred. In addition, RT-PCR analysis revealed that laminar fate and interneuron markers were basically expressed, but with high variability mirroring the morphological observations. Taken together, our data indicate that FCD show not a homogenous disease pattern but a much more complex than initially assumed.

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# Pain and the Developing Brain: Is early procedural pain associated with neonatal brain abnormalities in very preterm infants?

Susanne Brummelte[1], Ruth E. Grunau[1], Kenneth J. Poskitt[1,2], Vann Chau[1,2], Steven P. Miller[1,2]

[1]University of British Columbia, Developmental Neurosciences & Child Health, L408- 4480 Oak Street, V6H3V4 Vancouver, Canada
[2]University of British Columbia, Department of Neurology

Procedural pain in the neonatal intensive care unit (NICU) triggers a cascade of physiological, behavioral and hormonal disruptions which may contribute to altered neurodevelopment in infants born very preterm, who undergo prolonged hospitalization at a time of physiological immaturity and rapid brain development. The aim of this study is to examine relationships between cumulative procedural pain (number of skin-breaking procedures from birth to term) and the incidence of brain injuries (white matter injury, intraventricular hemorrhage (IVH), cerebellar hemorrhage). Neonates born very preterm (24-32 weeks gestational age) were scanned with magnetic resonance imaging (MRI) early in life (as soon as stable for transport) and at term-equivalent age. Brain injuries were graded using validated scoring systems previously found to be predictive of adverse neurodevelopmental outcomes. We hypothesized that greater neonatal pain exposure would be associated with a higher incidence of brain injury, in particular IVH. However, preliminary results suggest that the relationship between cumulative pain in the NICU (adjusted for early illness severity and overall intravenous morphine exposure) and severity of brain abnormalities seen on MRI scans at term equivalent age appears to be highly complex and affected by multiple interacting clinical factors.

# Performance monitoring in the lifespan: Increased post-error adjustment and changed error monitoring in elderly subjects.

Nadine Strien[1], Daniel Wiswede[2], Marcus Heldmann[3], Thomas F. Münte[3]

[1]German Center for Neurodegenerative Diseases (DZNE), Location Magdeburg, Germany
[2]Department of General Psychology II, FSU Jena, Germany
[3]Department of Neurology, University of Lübeck, Germany

Society is challenged by an ever increasing proportion of older-aged individuals. To understand cognitive functions during life-time, it is important to separate effects of normal aging from pathological age-associated diseases. Performance monitoring processes are well-examined within cognitive neuroscience and therefore feasible to provide insight into the aging brain. Errors in reaction time tasks are associated with a sharp frontocentral negativation in Event-related brain potentials, which has been labelled Error-Related Negativity (ERN) (Gehring, Goss, Coles, Meyer & Donchin, 1993, Falkenstein, Hohnsbein, Hoormann & Blanke, 1991). It has been shown that the ERN amplitude is reduced in older subjects (Falkenstein, Hoormann & Hohnsbein, 2001, Band & Kok, 2000). There is agreement that the ERN is generated in the anterior cingulate cortex (ACC, see Ridderinkhof, 2004, for overview). According to the conflict monitoring theory, the dorsal ACC signals the occurrence of conflicts in information processing and triggers compensatory adjustments in cognitive control (Botvinick, Scheffler & Cohen, 2004).
The goal of present research was to explore age related effects on the performance in a choice reaction task. Specifically, we examined neurophysiological (EEG) and behavioural correlates of stimulus accuracy, stimulus sequence and post-error adaptation in a group of young and elderly subjects while performing a flanker task.
Results: Behaviour data: Elderly subjects made fewer errors, especially for incongruent flanker-stimuli, which is in line with previous research (Wild-Wall et al., 2008). Conflict adaptation (Botvinick, 2001, Mayr, Awh and Laurey, 2003, Nieuwenhuis et al., 2006) did not differ between groups, which contradicts the view of general impairments of cognitive control processes in the elderly. Both groups responded more slowly in correct trials directly after an error; an effect known as post-error slowing (Rabbitt, 1981, 2002). This post-error adjustment was more pronounced in elderly subjects.
ERPs: Errors committed in incongruent flanker trials were associated with an increased ERN in elderly subjects; whereas there were no differences between congruent and incongruent errors in young subjects.
Conclusion: Older people seem to show a larger cognitive control after incongruent erroneous responses. In line with Wild-Wall et al. (2008) we suggest that the processing of the targets, but not the processing of the flanker stimuli, appears to be enhanced in older subjects.

# Screen for Ageing Genes in Drosophila

Joachim G Schulz[1], Leen van Huffel[1], Antonio Laranjeira[1], Carlos G Dotti[1]

[1]University Leuven, Dpt. Human Genetics, Herestraat 49 bus 602, 3000 Leuven, Belgium

Age is the most important risk factor for neurodegenerative diseases such as Alzheimer's or Parkinson's disease, but little is known about the underlying factors. Based on the hypothesis that single genes might influence the rate of CNS ageing, comparable to what has been shown for lifespan, we performed a genetic screen to identify novel genes that, when knocked down by neuronal RNAi, will reduce the age-dependent decline in a behavioral assay (negative geotaxis). We tested 300 genes known to be up-regulated in old versus young fly heads and identified several candidates that will be further analyzed.

# Serotonin Receptor 5B Gene (Htr5b): A new Target Gene of MeCP2?

Steffen Vogelgesang[1], Till Manzke[1,2], Marcus Niebert[1,2], Gabriele Flügge[2,3], Diethelm W. Richter[1,2]

[1]Dept. Neuro- and Sensory Physiology, University of Göttingen, Göttingen, Germany

[2]DFG Research Center Molecular Physiology of the Brain, Göttingen, Germany

[3]Clinical Neurobiology Laboratory, German Primate Center, Göttingen, Germany

Severe symptoms of Rett syndrome (RTT) are interruptions of normal breathing by periods of breath holdings and apneas. Using transgenic and knockout animal models of RTT we studied gene expression at different postnatal development stages in the medullary pre-Bötzinger complex (pre-BötC) that is an essential center controlling respiratory rhythm generation. We identified the orphan serotonin receptor 5B gene (Htr5b) of which expression is differentially regulated in the pre-BötC of Mecp2 deficiency (Mecp2-/y). RNA quantification in wild-type mice revealed that Htr5b is upregulated after birth but is turned off between postnatal days 21 and 40. On the contrary, Htr5b-expression level in Mecp2-/y mice remains elevated in late development stages when the respiratory phenotype is fully developed. Since systemically applied recombinant MeCP2-TAT fusion protein to Mecp2-/y mice normalized Htr5b overexpression, our data indicate that Mecp2 is directly involved in downregulation Htr5b. We found that 5-HTR5B are coupled to inhibitory Gi-proteins and that the C-terminal part primarily localizes to the cytoplasm. In humans where the first exon of 5-HTR5B gene is interrupted, while the C-terminal second exon, corresponding to the protein found in mice, is readily expressed. During postnatal stages, 5-HTR5B might be an important serotonergic element involved in the development of the respiratory network rhythm, and deficit of Htr5b repression in Mecp2-/y mice might contribute to dysfunctions in the control of breathing. We also propose that the expression of the truncated protein in humans may translate to a possible pharmacological target to treat respiratory disturbances in RTT.

# The role of Disc1 during interneuron migration

André Steinecke[1], Christin Gampe[1], Jürgen Bolz[1]

[1]Friedrich Schiller Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstr. 1, 07743 Jena, Germany

The correct function of the mammallian neocortex is dependent on an exact balance between excitation and inhibition. This precise set is achieved by excitatory pyramidal neurons, constituting 80% of the cortical neurons, and inhibitory GABAergic interneurons which account for 20% of the nerve cells. A disruption of this balance may be associated with neuro-psychatric dissorders such as schizophrenia, major depression and epilepsy. GABAergic interneurons, other than excitatory neurons, derive from the ventricular zone of the medial ganglionic eminence (MGE) and migrate tangentially over a long distance to their destination in the cortex. This migratory behaviour follows a distinct mode, which has been studied extensivly over the last decade. Thereby these neurons built a highly dynamic leading process, scanning for signal molecules and defining the right orientation of the cells. Afterwards the nucleus is moved to the chosen direction by pulling and pushing this large cargo along the cytoskeleton of the neuron. This saltatory movement is strictly regulated by many molecules to ensure the right fashion and the direction of migration. One protein that has been studied widely in the development of pyramidal neurons is Disrupted-in-Schizophrenia 1 (Disc1). This gene was found in a Scottish family, where many members had a translocation of this gene, causing a higher predisposition for depression spectrum disorders. A knock down of this protein leads to a decreased radial migration of pyramidal neurons by disruption of the correct positioning of the centrosome (Young-Pearse et al. 2010). Our RT-PCR data confirm an expression of this protein in MGE-derived cells on embryonic stage E 14, which is the peak of interneuronal cell birth in mice. We could also detect Disc1 in these cells using immunocytochemical methods, revealing Disc1 is located in the filopodia of the leading process and in the rear of the nucleus. Therefor Disc1 could play an important role to maintain the correct movement of cortical interneurons. To investigate this hypothesis we use in-vitro and in-vivo approaches, such as migration assays, focal and in-utero electroporation, to study the migratory behaviour of cortical interneurons lacking Disc1 in detail.

# Uncovering molecular mechanisms of early-onset age-related memory deficits in CB1 receptor knockout (Cnr1-/-) mice

Anastasia Piyanova[1], Önder Albayram[1], Kerstin Michel[1], Rainer Buchalla[2], Andreas Zimmer[1], Andras Bilkei-Gorzo[1]

[1]University of Bonn, Institute of Molecular Psychiatry, Life&Brain Center, University of Bonn, Germany

[2]Institute of Physiological Chemistry und Pathobiochemistry, Johannes Gutenberg University, Mainz, Germany

Genetic deletion of cannabinoid 1 (CB1) receptor in mice has been shown to accelerate age-related memory decline and neuronal loss (Bilkei-Gorzo, 2005; Bilkei-Gorzo, 2010). The mechanism contributing to these processes has not been identified yet. It is now well established that endocannabinoids exert an antioxidative and neuroprotective effect in several pathological conditions (e.g., stroke, neurodegeneration), which is partly mediated by the cannabinoid receptor 1 (CB1). In our study we thus hypothesized that CB1 knockout animals could suffer from increased oxidative and cellular stress in the brain that would then contribute to age-related memory deficits. We first investigated the presence of oxidized macromolecules (lipids, proteins, DNA) and the aging marker lipofuscin in the brains of CB1 receptor knockout mice (Cnr1-/-) and their age-matched wildtype (WT) littermates (C57BL/6J). We found a similar age-related increase in the amount of oxidized lipids, proteins and DNA in both Cnr1-/- and WT mice. However, aged CB1 knockout mice exhibited increased lipofuscin accumulation in neurons. This could be indicative of increased cellular stress and impaired neuronal metabolism. To test if pro-apoptotic mechanisms are affected in mice lacking CB1 receptors, we next compared the age-related changes in caspase 3 activation between WT and Cnr1-/- mice. The expression of activated caspase 3 was significantly exacerbated in the hippocampus of 12- month-old Cnr1- /- mice, indicating apoptotic processes. In order to determine, which neuronal population in the hippocampus is more affected by the deletion of CB1 receptors, we checked levels of activated caspase 3 in 12-month-old GABA- and glutamate-specific CB1 receptor conditional knockout mice. We found increased caspase 3 activation in the brains of both mutant mouse lines as compared to WT mice. However, the effect seen in the GABA -specific knockout mouse brains was more prominent than in the glutamate-specific conditional knockout mice. Therefore, we state that GABA -ergic neurons are probably more affected by the deletion of CB1 receptors. Finally, we assessed endocannabinoid levels (2-AG and anandamide) in CB1 knockout mice. The 2- AG levels in the hippocampus were decreased in aged WT animals but increased in null mutants. Probably, this increase represents a compensatory mechanism aimed at preventing neurodegeneration occurring in these mice. Taken together, we show that CB1 knockout mice suffer from increased cellular stress in the hippocampus that results in accelerated neuronal aging and activation of pro -apoptotic mechanisms independent from oxidative stress.

# Poster Topic

# T11: Alzheimer's, Parkinson's and other Neurodegenerative Diseases

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*Marlena Schnieder, Christoph Dohm, Anja Baumann, Jan Liman, Mathias Bähr, Fred Wouters, Pawel Kermer*

T11-2A A potential role of estrogen in schizophrenia via regulation of ErbB3 expression
*Nicola Brandt, Uwe Wehrenberg, Lars Fester, Gabriele M. Rune*

T11-3A Activity and expression of calpain in P23H-1 and S334ter-3 Mutant Rhodopsin Transgenic Rats
*Blanca Arango-Gonzalez, Stine Mencl, Francois Paquet-Durand , Jasvir Kaur*

T11-4A Altered motor behavior in a mouse model of Batten disease is independent from GABAergic spinal inhibition
*Benedikt Gruenewald, Christian Geis, Andreas Weishaupt, Thomas Wultsch, Antonia Post, Andreas Reif, Klaus Victor Toyka, Manfred Heckmann, Claudia Sommer*

T11-5A Alzheimer´s disease-like modified tau protein affects expression and phosphorylation of heat shock protein 90
*Dennis Prieß, Christina Galonska, Roland Brandt*

T11-6A An optimized method for quantifying dendritic spines in a murine model of Alzheimer´s disease.
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T11-7A Analysis of binding motifs between the Golgi-localized, gamma ear-containing, ARF-binding (GGA) protein family and BACE1 and the amyloid precursor protein (APP)
*Bjoern von Einem, Anke Hellrung, Daniel Schwanzar, Cornelia Steinmetz, Christian Proepper, Tobias M. Boeckers, Daniela Strat, Angelika Rueck, Christine A.F. von Arnim*

T11-8A Anticonvulsant efficacy of systemic versus focal application of vigabatrin - Investigations in an acute seizure model
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T11-10A Axons of supraspinal origin control motor neuron regeneration in the lesioned spinal cord of adult zebrafish
*Veronika Kuscha, Angela Scott, Michell Reimer, Tatyana B. Dias, Sarah Frazer, Anton Barreiro-Iglesias, Catherina G. Becker, Thomas Becker*

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cellular parameters of ALDP-deficient astrocytes
*Nicol Kruska, Sabine Rönicke, Aurora Pujol, Georg Reiser*

T11-12A Current-clamp experiments on primary hippocampal neurons shed light on potentially converse roles of L-type calcium channels in the pathogenesis of neurological diseases
*Helmut Kubista, Petra Geier, Michael Lagler, Stefan Boehm*

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*Kerstin Schwabe, Joachim K. Krauss, Mesbah Alam*

T11-14A Differential growth factor expression in astrocytes from mouse models for Parkinson's disease
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T11-15A Distribution of microglia in the aging murine nigrostriatal system
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T11-16A Does inhibition of IL-1ß prevent or modify epileptogenesis in two different rat models of chronic epilepsy?
*Nadine Polascheck, Marion Bankstahl, Teresa Ravizza, Annamaria Vezzani, Wolfgang Löscher*

T11-17A Downregulation of PMCA2 increases the vulnerability of dopaminergic neurons to mitochondrial complex I inhibition
*Alexander Brendel, Christian Behl, Parvana Hajieva*

T11-18A Effects of disease-relevant alpha-synuclein- and LRRK2- mutants on neurital dynamics in primary midbrain dopaminergic neurons
*Jan Christoph Koch, Florian Bitow, Jessica Haack, Elisabeth Barski, Sebastian Kügler, Mathias Bähr, Paul Lingor*

T11-19A FGF-2 modulates proliferation and natural cell death in the developing ventral mesencephalon in mice
*Olga Baron, Andreas Ratzka, Claudia Grothe*

T11-20A First steps to an early diagnosis of Alzheimers disease via the enteric nervous systems
*Sandra Semar, Maryse Letiembre, Alex Liu, Markus Klotz, K. Fassbender, Karl-Herbert Schäfer*

T11-21A Formic acid and sodium dodecyl sulfate (SDS)-sensitive high-molecular Aß-oligomers and protofibrils are the predominant Aß-species in the native soluble protein fraction of the AD brain. Low-molecular Aß-oligomers occur after denaturation.
*Ajeet Rijal Upadhaya, Irina Lungrin, Haruyasu Yamaguchi, Marcus FÃƒÂ¤ndrich, Dietmar Rudolf Thal*

T11-22A GABAergic septo-hippocampal neurons and GABAergic hippocampal interneurons are early targets for neurodegeneration in a mouse model of amyloidosis and tauopathy
*Desiree Loreth, Raphael Poirier, Fiona Grueninger, Bernd Bohrmann, Michael Frotscher, Friedrich Metzger, Oliver Kretz*

T11-23A GGA3 is a potential CSF marker of Alzheimer´s disease
*Cathrin Schnack , Björn von Einem , Anke Hellrung, Markus Otto, Hayrettin Tumani, Sarah Jesse, Johannes Brettschneider, Christine von Arnim*

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*Christa Maria Erika Trattnig, Silke Patz, Gerda Gruenbacher, Ulrike Fasching, Wissa Sonja, Christian Guelly, Birgit Ebner, Beate Rinner, Alexandra Novak, Gerd Leitinger, Gertrude Havlicek, Ute Schaefer*

T11-25A IDENTIFICATION OF LOW MOLECULAR WEIGHT PYROGLUTAMATE ABETA OLIGOMERS IN ALZHEIMER DISEASE: A NOVEL TOOL FOR THERAPY AND DIAGNOSIS
*Oliver Wirths, Christian Erck, Henrik Martens, Anja Harmeier, Constanze Geumann, Sadim Jawhar, Sathish Kumar, Gerd Multhaup, Jochen Walter, Martin Ingelsson, Malin Degermann-Gunnarsson, Hannu Kalimo, Inge Huitinga, Lars Lannfelt, Thomas A. Bayer*

T11-26A NEW MARKERS FOR EARLY DIAGNOSIS OF ALZHEIMER DISEASE: THE FIRST STEP TOWARDS EFFICIENT TREATMENTS.
*Stefania Averaimo, Laura Gasparini, Simona Gornati, Michele Mazzanti*

T11-1B Impaired cerebellar GABAergic feedforward inhibition in CLN3 knockout mice
*Christian Werner, Benedikt Grünewald, Klaus Viktor Toyka, Claudia Sommer, Christian Geis*

T11-2B Impairment of N-cadherin function accelerates the effects of amyloid beta peptide (Aß) on glutamatergic synapses.
*Aksana Andreyeva, Katharina Horstmann, Kurt Gottmann*

T11-3B Induced pluripotent stem cell derived neurons: a human model for Alzheimer's Disease?
*Katja Nieweg, Aksana Andreyeva, Kurt Gottmann*

T11-4B Retracted

T11-5B Intrastriatal Botulinum toxin A injection ameliorates some motor abilities of hemiparkinson rats and leads to morphological changes in the brain
*Alexander Hawlitschka, Veronica Antipova, Eilhard Mix, Oliver Schmitt, Martin Witt, Reiner Benecke, Andreas Wree*

T11-6B In-Vivo Mouse Brain Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) and Immunohistochemistry (IHC) Reveals Gender Specific Pathology induced by Cuprizone
*Neele Hübner, Laura-Adela Harsan, Alexandru Parlog, Nicoleta Baxan, Jürgen Hennig, Dominik von Elverfeldt*

T11-7B Localization and subcellular distribution of the amyloid precursor family in the adult mouse brain
*Kristine Gampe, Christine Altmann, Sebastian Czaplinski, Susanne Walter, Walter Volknandt*

T11-8B Longitudinal study of the effect of traumatic brain injury on lateral ventricle and hippocampal volumes using fully automated MRI volumetry
*Veronika Brezova, Alexander Olsen, Toril Skandsen, Anne Vik, Asta Håberg*

T11-9B Mismatch in network dynamics in a model of temporal lobe epilepsy
*Ulrich Paul Froriep, Arvind Kumar, Delphine Cosandier-Rimélé, Ute Häussler, Carola A. Haas, Ulrich Egert*

T11-10B Modifying alpha-synuclein dimerization in living cells
*Susana Alexandra Gonçalves, Tiago Fleming Outeiro*

T11-11B Nervous system and general toxic effects in rats after subacute intratracheal application of nanosized lead oxide
*Gábor Oszlánczi, Edina Horváth, Andrea Szabó, András Papp, Péter Pusztai, Mária Szabó, Gábor Kozma, András Sápi, Zoltán Kónya, Tünde Vezér*

T11-12B Neurological and molecular biological characterization of the mutant mouse line Tom40, the protein that comprises the general import pore of mitochondria
*Ramona Maria Zeh, Lore Becker, Andreas Bender, Thomas Floss, Anja Schrewe, Julia Calzada-Wack, Frauke Neff, Helmut Fuchs, Valérie Gailus-Durner, Raffi Bekeredjian, Thomas Meitinger, Wolfgang Wurst, Martin Hrabé de Angelis, Holger Prokisch, Thomas Klopstock*

T11-13B Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease.
*Markus Morawski, Gert Brückner, Carsten Jäger, Gudrun Seeger, Thomas Arendt*

T11-14B Neuroprotective effects of hematopoietic stem cells in the G93A animal model of ALS
*Sarah Knippenberg, Nadine Thau, Ralf Hass, Reinhard Dengler, Susanne Petri*

T11-15B Neurotoxicity of nanosized manganese by subchronic exposure
*Andrea Szabó, Szabolcs Takacs, Zsuzsanna Máté, Edina Horváth, Gábor Oszlánczi, András Papp*

T11-16B Nigral injection of AAV-mediated overexpression of alpha-synuclein: A Parkinson-like model in the marmoset monkey
*Yi-Fang Cui, Enrique Garea-Rodriguez, Sebastian Kuegler, Christina Schlumbohm, Eberhard Fuchs*

T11-17B Non-local impairment and therapeutic restoration of visual plasticity mechanisms after a localized cortical stroke
*Franziska Greifzu, Silvio Schmidt, Karl-Friedrich Schmidt, Otto W. Witte, Siegrid Löwel*

T11-18B Overexpression of glutaminyl, the enzyme responsible for pyroglutamate Abeta formation, cyclase induces behavioral deficits and glutaminyl cyclase knock-out rescues the behavioral phenotype in 5XFAD mice
*Sadim Jawhar, Oliver Wirths, Stephan Schilling, Sigrid Graubner, Hans-Ulrich Demuth, Thomas A. Bayer*

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T11-20B Proteome analysis of a detergent insoluble fraction from spinal cords of SOD1 transgenic mice by label-free LC-MSE mass-spectrometry
*Martina Liebl, A. Murat Kaya, Stefan Tenzer, Susanne Petri, Jörg Kuharev, Hansjörg Schild, Christian Behl, Albrecht M. Clement*

T11-21B Rat models of Huntington’s disease – what can we learn about the neurodegenerative process and its impact on the adult SEZ niche?
*Yvona Mazurova, Ivana Guncova, Ivan Latr, David Astapenko*

T11-22B Read-through of a nonsense mutation as a treatment option for Usher type 1C
*Tobias Goldmann, Nora Overlack, Fabian Möller, Igor Nudelman, Timor Baasov, Uwe Wolfrum, Kerstin Nagel-Wolfrum*

T11-23B Reduced olfactory bulb volumes in patients with Parkinson Disease
*Jochen Klucken, Johannes Kahlis, Nina Mallog, Beate Winner, Mark Greenlee, Gerhard Schuierer, Juergen Winkler*

T11-24B Regulation of the processing of Amyloid Precursor Protein (APP) by GGA transport proteins
*Anke Hellrung, Björn von Einem, Daniel Schwanzar, Christine von Arnim*

T11-25B RET mediates the neuroprotective and neuroregenerative effects of GDNF in the MPTP model of Parkinson's disease
*V.V Durga Praveen Meka, Karsten Sollich, Anja Drinkut , Jorg Schulz , Sebastian Kugler , Edgar R. Kramer*

T11-26B Corticosteroid modulation of status epilepticus: the role of GRs and MRs
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T11-1C Role of inhibition in unleashing and quenching oscillations in the basal ganglia
*Stefano Cardanobile, Arvind Kumar, Stefan Rotter, Ad Aertsen*

T11-2C Screening for cone neuroprotective substance using 661W cells
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T11-3C Seeding effect of pyroglutamate amyloid beta 3-42 promotes plaque deposition and behavioral deficits in a novel bigenic mouse model of Alzheimer's disease
*Jessica Wittnam, Oliver Wirths, Thomas Bayer*

T11-4C Structural and functional interactions between wildtype and amyotrophic lateral sclerosis-causing mutant SOD1: A study with obligate SOD1 dimers
*Anna Weichert, Anna Besemer, Nadja Hellmann, Elmar Jaenicke, Heidrun Witan, Heinz Decker, Christian Behl, Albrecht M. Clement*

T11-5C Substantia nigra pars reticulata neurons projecting to the dorsal raphe nucleus appear to receive afferents from the subthalamic nucleus
*Henrike Hartung, Sonny Tan, Peter J Magill, Yasin Temel, Tervor Sharp*

T11-6C Synaptic and neurophysiological deficits in a fly model of Parkinson's disease (PD) with reduced locomotion.
*Christopher Elliott, Amanda Vincent, Laura Briggs, Elizabeth Emery, Matt Oswald, C. Adam Middleton, Rose Tomlins, Sean Sweeney*

T11-7C Synaptic proteome alterations in patients with sporadic Creutzfeldt-Jakob disease
*Julie Carimalo, Martin Nowak, Joanna Gawinecka, Barbara Ciesiekczykl, Walter Schulz-Schaeffer, Abdul R. Asif, Franco Cardone, Maurizio Pocchiari, Inga Zerr*

T11-8C The alpha-synuclein A30P-mutation negatively affects regeneration of dopaminergic neurons
*Lars Toenges, Patrizia Roszak, Eva Szegoe, Jan C. Koch, Christoph P. Dohm, Pawel Kermer, Sebastian Kuegler, Mathias Baehr, Paul Lingor*

T11-9C The brain region-specific effect of MPP+ on the expression of cytochrome c oxidase subunit IV isoforms and viability of astrocytes
*Marion Barbara Victor, Syama Sundar Boyalla, André Roemgens, Cordian Beyer, Susanne Arnold*

T11-10C The co-layer method as an efficient way for neurotrophic factor release by transplanted genetically modified neuronal progenitor cells in a rat model of Parkinson's disease
*Ieva Kalve, Andreas Ratzka, Meltem Özer, Maike Wesemann, Julia Jungnickel, Christiane Köster-Patzlaff, André Nobre, Claudia Grothe*

T11-11C The gender- and brain region-specific role of cytochrome c oxidase in neurodegeneration
*Susanne Arnold, Marion Victor, Syama Sundar Boyalla, Shilpee Singh, Magdalena Misiak, Cordian Beyer*

T11-12C The impact of Cytoplasmic Polyadenylation Element Binding Protein (CPEB)-mediated translational regulation on development and progression of temporal lobe epilepsy: Evidence from mice expressing a dominant negative CPEB in forebrain neurons
*Peter Bedner, Pavel Dublin, Sada Turimella, Vamshidhar Vangoor, Kerstin Hüttmann, Eric Kandel, Christian Steinhäuser, Martin Theis*

T11-13C The influence of the NKCC1-inhibitor bumetanide on alterations in seizure susceptibility after status epilepticus in mice
*Manuel Töpfer, Kathrin Töllner, Claudia Brandt, Wolfgang Löscher*

T11-14C THE NUCLEOLUS AS A SOURCE OF OXIDATIVE DAMAGE AND NEURODEGENERATION.
*Rosanna Parlato, Claus Rieker, Grzegorz Kreiner, Holger Bierhoff, Maria Armentano, David Engblom, Andreas Schober, Luca Bonfanti, Ingrid Grummt, Günther Schütz*

T11-15C The perivascular clearance of neuronal apolipoprotein E is modulated by amyloid ß-protein in mouse models of Alzheimer's disease
*Dietmar Rudolf Thal, Tom van Dooren, Christian Haass, Fred van Leuven, Harshvardhan Rolyan*

T11-16C The role of astrocytes in the Parkinson´s Disease pathology
*Sonja Mendritzki, Hermann Lübbert*

T11-17C The role of BAG1 in tau pathology
*Sandra C. Signore, Mathias Bähr, Fred S. Wouters, Pawel Kermer*

T11-18C The Role of Striatum and Hippocampus in Sequential Learning: Interaction, Dissociation or Competition?
*Moritz Thede Eckart, Moriah Hülse-Matia , Rebecca McDonald, David Loer, Rainer Schwarting*

T11-19C The role of voltage gated sodium channels in the pathogenesis of glaucomatous optic neuropathy
*Birte Könnecke, Shai Sandalon, Hani Levkovitch-Verbin, Katharina Hein, Muriel Sättler, Mathias Bähr, Mikael Simons, Ron Ofri*

T11-20C The transport of neurotrophins in neurodegenerative diseases
*Bianca Seifert, Volkmar Leßmann, Tanja Brigadski*

T11-21C Transcriptional regulators in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) – Histopathological and biochemical studies in the G93A ALS mouse model and in ALS post mortem tissue
*Nadine Thau, Sarah Knippenberg, Reinhard Dengler, Susanne Petri*

T11-22C Treatment of 5XFAD transgenic mice with Ibuprofen
*Antje Hillmann, Oliver Wirths, Thomas A. Bayer*

T11-23C Visualizing dopamine transporter activity with [123I]FP-CIT SPECT in 6-OHDA lesioned marmoset monkeys : A non-human primate model of Parkinson's disease
*Enrique Garea-Rodríguez, Yi-Fang Cui, Christina Schlumbohm, Eberhard Fuchs*

T11-24C Zebrafish: A new model of Parkinson's disease
*Tomás Ribeiro da Silva Lopes da Fonseca, Ana Dulce Correia, Tiago Fleming Outeiro*

T11-25C A severe epileptic phenotype due to a moderate loss of M-current in the $KCNQ2^{Nmf134}$ mouse model
*Daniel Milkereit, Axel Neu, Fabio Morellini, Quyen Le, Dirk Isbrandt*

T11-26C The role of the C-C chemokine CCL17 in Alzheimer's disease
*Kim Neitzert, Onder Albayram, Sathish Kumar, Jochen Walter, Irmgard Förster, Andras Bilkei-Gorzo, Mira Kron, Wolfgang Maier, Andreas Zimmer, Judith Alferink*

# A novel in vitro model of a-synuclein aggregation and toxicity

Marlena Schnieder[1], Christoph Dohm[1], Anja Baumann[1], Jan Liman[1], Mathias Bähr[1], Fred Wouters[2], Pawel Kermer[1]

[1]Dept. of Neurology and Center for Molecular Physiology of the Brain, University of Göttingen Medical School
[2]Laboratory for Molecular and Cellular Systems, Department of Neuro- and Sensory Physiology, Georg-August University Göttingen

Recent findings concerning the molecular mechanisms of Parkinson's disease (PD) identified a number of causes for neuronal demise like protein misfolding and aggregation, mitochondrial dysfunction and the impairment of protein degradation pathways.
In this context, a -Synuclein misfolding has been proposed as key initiator event in the pathogenesis of PD.Misfolded a-synuclein can self associate, forming oligomers, fibrils and Lewy bodies, the pathological hallmark of PD. Oligomeric forms are considered to be the true toxic intermediates of aggregating a-synuclein.
Here, we present a novel in vitro model of a-synuclein duplications and triplications. Transient overexpression of these a-synuclein constructs in SH-SY5Y cells resulted in increased cell death and inhibition of the ubiquitin-proteasome pathway. Furthermore, we studied the formation of oligomers and aggregates in SH-SY5Y cells overexpressing these a-synuclein constructs.
Our findings are introducing a -synuclein duplications and triplications as a potent and simple model for investigating PD and potential neuroprotective strategies in vitro.

# A potential role of estrogen in schizophrenia via regulation of ErbB3 expression

Nicola Brandt[1], Uwe Wehrenberg[1], Lars Fester[1], Gabriele M. Rune[1]

[11]University Medical Center Hamburg-Eppendorf, Institute of Anatomy I: Cellular Neurobiology, Martinistr. 52, 20246 Hamburg, Germany

It has been proposed that Neuregulin (NRG1) and its cognate receptors ErbBs, as well as estrogen, play a role in schizophrenia. ErbB3 expression is reduced in brains of 50% of all chronically schizophrenic patients (Hakak et al., 2001, Tkachev et al., 2003; Aston et al., 2004) and impaired maturation of dendritic spines and behavioral deficits, that have been associated with schizophrenia-like symptoms were found in mice lacking NRG1/ErbB signalling in the CNS. Fading estrogen secretion at menopause in vulnerable women leads to relapse of schizophrenic symptoms or to new late-onset schizophrenia, which gave rise to the estradiol protection hypothesis for schizophrenia. Given this background, we studied estrogen responsiveness of ErbB3 expression in hippocampal cultures, ErbB3 was significantly upregulated in response to estradiol, as shown by image analysis of immunoblots, quantitative immunhistochemistry of ErbB3, and quantification of ErbB3 transcripts by TaqMan PCR. Inhibition of hippocampal estrogen synthesis in the cultures, resulted in a downregulation of ErbB3 expression and spine synapse loss. The In Situ Proximity Ligation Assay (Söderberg et al., 2006) revealed an increase in ErbB receptor dimerization, which is a prerequisite of NRG1 signalling. Our data suggest that the decline in brain-derived estrogen in women at menopause may contribute to the relapse of schizophrenia symptoms and they are in favour of the estrogen protection hypothesis in schizophrenia.

# Activity and expression of calpain in P23H-1 and S334ter-3 Mutant Rhodopsin Transgenic Rats

Blanca Arango-Gonzalez[1], Stine Mencl[1], Francois Paquet-Durand [1], Jasvir Kaur[1]

[1]Universitaetsklinikum Tuebingen - CENTRE FOR OPHTHALMOLOGY, INSTITUTE FOR OPHTHALMIC RESEARCH, Röntgenweg 11, 72076 Tübingen, Germany

Purpose: To examine the expression and activity of Calpain during photoreceptor cell death in transgenic rats with P23H and S334ter rhodopsin mutations.
Methods: Retinas of P23H -1, S334ter -3 and wild type Sprague Dawley (SD/CD) rats were examined by conventional histological techniques, immunohistochemistry and immunoblotting using specific antibodies for calpastatin and calpain isoforms 1, 2 and 3. Monitoring of calpain activity at the cellular level was investigated using enzymatic in situ assays (Paquet-Durand et al., 2006; 2007) on unfixed cryosections.
Results: Calpain activity was considerably increased in photoreceptor cells in the P23H-1 and S334ter-3 retinas and only very few cells were positive on CD rats. Ubiquitous calpains 1 and 2 are evenly distributed throughout the layers of retina and immunostaining did not show marked differences between P23H-1 or S334ter-3 mutants and their corresponding wild-type controls. Interestingly, up regulation in the calpain 3 labelling in the ONL was observed in both transgenic rats, being more intense in the S334ter. Immunostaining against calpastatin, the endogenous inhibitor of calpain, was performed. Calpastatin labelling was less intense in both rhodopsin transgenic rats. In both mutants, labelling of the inner segments was weaker and less condensed than in wild type. Immunoblot showed that calpastatin expression was decrease in both mutant models.
Conclusions: Our results indicate that increased expression and activation of calpain in rhodopsin transgenic rats play a critical role in photoreceptor degeneration. These results coincide with previous studies using animal models with non-rhodopsin mutations for retinitis pigmentosa, indicating that this pathway could be a general mechanism involved in photoreceptor cell death, which elevates the possibility of using calpain inhibitors as therapeutic agents to prevent or delay photoreceptor degeneration.

# Altered motor behavior in a mouse model of Batten disease is independent from GABAergic spinal inhibition

Benedikt Gruenewald[1], Christian Geis[1], Andreas Weishaupt[1], Thomas Wultsch[2], Antonia Post[2], Andreas Reif[2], Klaus Victor Toyka[1], Manfred Heckmann[3], Claudia Sommer[1]

[1]Dept. of Neurology,Unviversitaetsklinikum Wuerzburg
[2]Dept. of Psychiatry, Universitaetsklinikum Wuerzburg
[3]Inst. of Physiology, Universitaet Wuerzburg

Juvenile neuronal ceroid lipofuscinosis (JNCL, Batten disease) is an autosomal recessive disorder caused by mutations in the CLN3 gene. It is the most prevalent form of the neuronal ceroid lipofuscinosis (NCL), a group of rare neurodegenerative disorders affecting up to 1:12500 live births. The disorders are characterized by the accumulation of autofluorescent material within patients' cells which is not yet defined and patients develop a progressive and selective neuronal loss. Additionally, JNCL patients may have autoantibodies against the glutamate decarboxylase 65 (GAD 65). Symptoms in patients affected by the JNCL appear around age 4-10 starting with loss of vision, seizures, cognitive decline and personality changes. The function of CLN3 is not yet fully understood and the pathogenic mechanisms are still unclear. The cln3 knockout mouse (cln3-/-) is a genetically engineered model of the JNCL resembling the human disorder in some behavioral aspects and in its cellular pathology. Motor dysfunction, learning deficits, and visual deficits were described, and autoautoantibodies against GAD 65 were found. Behavioral changes in the animal model point toward a supraspinal pathology, whereas certain motor deficits may rather result from spinal dysfunction

Here, we analyzed the spinal cord function in the cln3-/- mice using behavioral methods and in-vivo electrophysiology. Motor performance was tested with RotaRod analysis, the rope climbing test and gait analysis. Spinal reflex pathways were investigated by investigating frequency-dependent depression of the Hoffmann reflex. Spinal inhibition was specifically tested in-vivo by recording GABAergic dorsal root potentials (DRP), resembling presynaptic inhibition due to primary afferent depolarization. Further, we addressed supraspinal function by testing anxiety related behavior in the open field (OF) and elevated plus maze (EPM), as well as fear conditioning.

In the behavioral analysis, cln3-/- mice showed coordination deficits in the climbing test, whereas forced walking behavior on the Rotarod and gait analyses were unchanged compared to their wild-type littermates. Analysis of the frequency-dependent depression of the Hoffmann reflex and spinal presynaptic inhibition revealed no significant differences between the experimental groups. In the EPM and after fear conditioning, cln3 -/- mice showed increased anxiety associated behavior.

We conclude from the in-vivo electrophysiological recordings that the behavioral motor deficits of cln3-/- mice are not likely to result from disturbed synaptic transmission at the spinal cord level. We suggest that coordination deficits may result from dysfunction of cerebellar pathways. Anxiety-related behavior in the cln3-/- mice model mimics human pathology in afflicted patients and may be related to deficits in amygdala-hippocampal network. It remains to be elucidated in further studies whether autoantibodies against GAD 65 are involved in the pathophysiology of JNCL.

# Alzheimer´s disease-like modified tau protein affects expression and phosphorylation of heat shock protein 90

Dennis Prieß[1], Christina Galonska[1], Roland Brandt[1]

[1]University of Osnabrück, Neurobiology, Barbarastraße 11, 49076 Osnabrück, Germany

Alzheimer's disease is associated with an increased phosphorylation (hyperphosphorylation) of the microtubule-associated protein tau. Hyperphosphorylation may convert tau to a toxic species, however the downstream cascades, which mediate neuronal cell death, remain elusive. To identify potential candidate proteins that are affected by tau modification, we employed cells that express pseudohyperphosphorylated (PHP) tau to simulate a permanent disease-like modification of tau protein. As a control, human wildtype (wt) tau expressing cells were used. We had previously shown that PHP tau establishes key structural and functional changes of hyperphosphorylated tau and induces cell death in contrast to wt tau (Fath et al., J. Neuroscience 22 (2002)). We performed a phosphoproteomic approach, where we used a combination of stable isotope labeling, phosphoprotein affinity chromatography and mass spectrometry to identify proteins that are differentially synthesized or phosphorylated in PHP tau versus wt tau expressing PC12 cells. We identified heat shock protein 90 (Hsp90) as a candidate protein. Quantitative Western blotting showed that the amount of protein was not affected. In contrast, Hsp90 exhibited a lower phosphorylation in PHP tau compared to wt tau expressing cells, as detected by 2D gel electrophoresis and mass spectrometry. We identified specific amino acid residues as potential candidate sites which are differentially phosphorylated. In PHP-tau expressing mice, the amount of Hsp90 protein was reduced in old age.

The data suggest that tau hyperphosphorylation induces a dephosphorylation of Hsp90 and may reduce Hsp90 expression after long term exposure. It will be interesting to determine whether changes in Hsp90 are causally linked to tau-induced cell death in neurons.

# An optimized method for quantifying dendritic spines in a murine model of Alzheimer´s disease.

Linda Reimers[1], Claudia Perez-Cruz[1], Mark Maasland[1, 2], Ulrich Ebert[1], Corinna Klein [1]

[1]Neuroscience Discovery Research, GPRD, Abbott, Ludwigshafen, Germany
[2]Image Processing, Fraunhofer Institute for Industrial Mathematics ITWM, Kaiserslautern, Germany

Dendritic spines appear as small protrusions on neuronal dendrites, typically consisting of a head and a neck. More than 90% of all excitatory synapses of the CNS are located on the dendritic spines or rather on their heads. According to their size, shape and presumably function, spines can be classified into different groups: mushroom, stubby, thin and filopodia spines. Mushroom and thin spines are considered to form mature synapses in the adult brain and are therefore responsible for neuronal signal transmission. Filopodia spines are thought to be precursors developing into mature spines upon strengthening of the synapse.
Pathological alterations resulting from different diseases such as Alzheimer´s disease (AD) can cause changes in spine density of specific brain regions. Here we compare two methods of spine quantification: the Rapid Golgi Cox staining (random impregnation of single neurons with chromates) and the biolistic delivery of a fluorescent dye (random labeling of single neurons by tungsten particles coated with a fluorescent carbocyanine dye and delivered by air pressure). We used both techniques to analyze the spine morphology and quantity of pyramidal neurons in the CA1 region of the hippocampus of amyloid precursor protein (APP) transgenic mice (Tg; Tg2576), a murine model of AD, and their wildtype littermates (Wt).
We found the total amount of spines in Wt mice to be approximately the same with both techniques (gene gun 1,451 ± 0,081 spines/µm; Golgi 1,652 ± 0,034 spines/µm). Comparing Wt with Tg animals we could not detect a deficit in total spine density in Tg mice with either method (Tg gene gun 1,289 ± 0,060 spines/µm; Tg Golgi 1,697 ± 0,055 spines/µm), however, the values are comparable to those previously reported for this AD model. Using the Golgi staining, we are only able to identify either mushroom or filopodia spines unambiguously, leading to an overestimation of these spine classes (mushroom: ~2.5 fold, filopodia: ~4-fold compared to the Golgi method), whereas the gene gun procedure enables a more precise classification. Assuming mushroom spines to be mainly responsible for synaptic transmission we expected a decrease in the tg animals. However, neither the quantification by Golgi impregnation nor by gene gun labeling showed a significant difference in this spine population. Currently we are developing an algorithm for a faster automated analysis of spine density and classification, based on the three-dimensional acquisition of the fluorescence-labeled spines under the confocal laser scanning microscope.

# Analysis of binding motifs between the Golgi-localized, gamma ear-containing, ARF-binding (GGA) protein family and BACE1 and the amyloid precursor protein (APP)

Bjoern von Einem[1], Anke Hellrung[1], Daniel Schwanzar[1], Cornelia Steinmetz[1], Christian Proepper[2], Tobias M. Boeckers[2], Daniela Strat[3], Angelika Rueck[3], Christine A.F. von Arnim[1]

[1] Dept. of Neurology, Ulm University, Helmholtzstrasse 8/1, 89081 Ulm, Germany

[2] Dept. of Anatomy and Cell Biology, Ulm University, Albert Einstein Allee 11, 89081 Ulm, Germany

[3] Institute for Laser Technologies in Medicine and Metrology, ILM, Helmholtzstrasse 12, 89081 Ulm, Germany

The proteolytic processing of APP by BACE1 is the initial step in the production of amyloid-beta (Aß), which accumulates in senile plaques in Alzheimer's disease (AD). Essential for this cleavage is the transport and sorting of both proteins through endosomal and Golgi compartments. A family of proteins called Golgi-localized ã-ear-containing ARF-binding (GGA) was found to have striking functions in cargo sorting in this pathway. Recently, it was shown that GGA1 and GGA3 can interact with BACE1, that they are expressed in neurons and GGA3 is reduced in AD brain tissues. Using Fluorescence Lifetime imaging Microscopy (FLIM), Immunoprecipitations and ELISA assays with GGA -VHS deletion and point mutations, we found that the GGA VHS-domain that was predicted to be the main interaction domain for GGA cargo binding has only limited influence upon the processing of APP and the interaction of BACE1 and GGAs. Therefore, we extended our approach to identify other domains responsible for GGA-BACE1 interaction using GGA deletion mutants with FLIM and Immunoprecipitation assays. Since it was additionally found that GGA1 also confines APP to the Golgi and perinuclear compartments and thereby impacts Aß generation, we tested the hypothesis that all three GGAs can modulate not only BACE1 independently of their VHS domain but also APP transport via direct or indirect binding.
In conclusion, our data suggest that — in addition to its role in BACE1 trafficking — GGAs affect APP processing by directly modulating transport and localisation of APP.

# Anticonvulsant efficacy of systemic versus focal application of vigabatrin - Investigations in an acute seizure model

Sonja Broeer[1], Bianca Backofen-Wehrhahn[1,2], Marion Bankstahl[1,2], Wolfgang Loescher[1,2], Manuela Gernert[1,2]

[1]University of Veterinary Medicine Hannover, Germany, Dept. of Pharmacology, Toxicology, and Pharmacy, Buenteweg 17, 30559 Hannover, Germany and

[2]Center for Systems Neuroscience Hannover, Germany

About 50- 90% of patients suffering from temporal lobe epilepsy are pharmacoresistant, meaning that severe seizures occur despite systemic treatment with antiepileptic drugs (AEDs). An increase of the dosage of systemically applied drugs is limited by the occurrence of unwanted side effects. Alternative therapeutic strategies to systemic treatment, which are studied by our group, include focal drug administration by local microinfusion of AEDs. Focal approaches to treat epilepsies require appropriate target structures within the brain. Especially the subthalamo-nigral network has repeatedly been shown in experimental epilepsy to be a highly interesting target for modulation of different seizure types emanating from the limbic system. We have chosen the subthalamic nucleus (STN) as a potential target structure for pharmacological inhibition of the subthalamo-nigral network.

Drugs that increase the function of the inhibitory neurotransmitter GABA have an anticonvulsant effect when applied to the epileptic focus or to seizure-associated regions. Vigabatrin (Gamma-Vinyl-GABA; GVG) irreversibly inhibits the degradation of GABA, resulting in an elevation of the GABA -concentration in the synaptic cleft. Using different epilepsy models, previous experiments proved anticonvulsant effects of GVG when applied systemically or when applied locally to the substantia nigra pars reticulata (SNr). We now examined (1) the time course and efficacy of the anticonvulsant effect of GVG after systemic application in an acute seizure model, which allows repeated determination of seizure threshold at different times after drug administration, and (2) whether anticonvulsant effects also occur after local application into the STN, which is poorly investigated in comparison to the SNr. (3) The experiments were conducted with a focus on possible time-dependent differences, comparing systemic versus local GVG injection.

By using the timed intravenous pentylenetetrazole (PTZ) infusion seizure threshold test, we evaluated the seizure susceptibility of adult female Wistar rats before and after GVG application. The PTZ threshold was determined at least 48 hours prior to GVG injection and again either 6, 24, 48 or 96 hours after GVG treatment. We could demonstrate a significant anticonvulsant effect after systemic treatment with GVG (600 mg/kg intraperitoneally), with a maximum efficacy 6 hours after application. For local microinjection of GVG, rats were anaesthetized with isoflurane (induction 3%, maintenance 1.5%), which did not influence seizure threshold 6 hours after anaesthesia. GVG (40 Î¼g/Î¼l) was injected bilaterally in a volume of 250 nl into the STN during surgery. PTZ seizure threshold was determined either 6, 24, 48 or 96 hours after surgery and compared to the control PTZ threshold determined before GVG injection. Microinjection of GVG into the STN most effectively increased the seizure threshold after 24 hours. It is likely that the delayed maximum effect after local microinjection is due to the fact that GVG has to diffuse throughout the target region before a significant modulation of the subthalamo-nigral network occurs. Further studies will be needed to decide whether the STN is a more efficient target than other parts of the epileptic network such as the SNr.

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# Autophagy modulation alters alpha-Synuclein aggregation and toxicity

Anne-Maria Poehler[1], Pamela J. McLean[2], Edward Rockenstein[3], Bradley T. Hyman[2], Eliezer Masliah[3], Juergen Winkler[1], Jochen Klucken[1]

[1]Division of Molecular Neurology, University Hospital Erlangen, Erlangen, Germany [2]MassGeneral Institute for Neurodegenerative Disease, Department of Neurology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, USA [3]Department of Neurosciences, University of California San Diego, La Jolla, CA, USA.

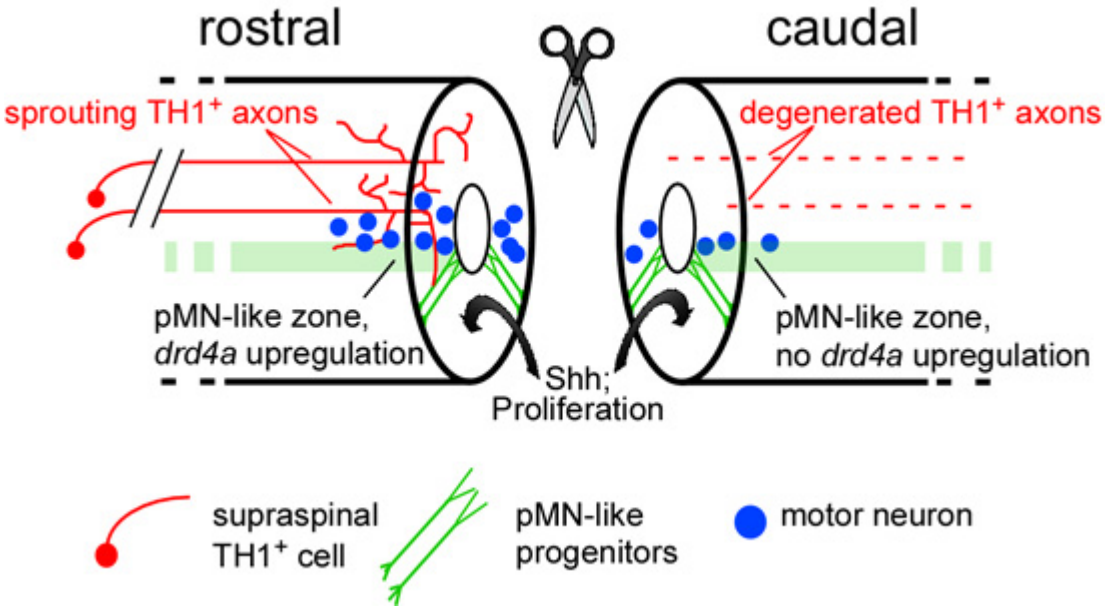
Accumulation of alpha-synuclein (aSyn) within Lewy bodies and neurites is one of the pathological hallmarks in Parkinson's disease and Dementia with Lewy bodies (DLB). Impaired degradation of alpha -synuclein is considered to result in its aggregation. In addition to the ubiquitin-proteasome degradation, the autophagy-lysosomal pathway (ALP) may be involved in intracellular degradation processes for alpha-synuclein. We explored ALP in human brain tissue from DLB patients, in a transgenic mouse model of synucleinopathy, and in a cell culture model for alpha -synuclein aggregation. We showed that ALP is dysregulated in human DLB brains. Using transiently transfected H4 neuroglioma cells with an aggregating form of alpha-synuclein (C-terminally tagged alpha -synuclein), we observed a substantial potentiation of alpha -synuclein toxicity after ALP inhibition using BafA1. In addition, we demonstrate an altered regulation of ALP related markers like LC3 and Lamp2a. Likewise, treating alpha-synuclein transgenic mice with intraventricular infusion of BafA1 resulted in a reduction of MAP2 immunoreactivity suggesting a loss in dendrites. More importantly, the increased toxicity was paralleled by a reduction in aggregate formation in alpha -synuclein transgenic mice and in transfected H4 cells. These findings allow two important conclusions: first, aggregate formation may reflect rather a detoxifying intracellular mechanism in synucleinopathy, and secondly that a BafA1 sensitive ALP process results rather in alpha-synuclein aggregate formation than only in degradation. Understanding alpha -synuclein aggregate formation involving the ALP and its potential role to induce alpha -synuclein mediated toxicity will provide the basis to define new protective targets for the treatment in synucleinopathies.

# Axons of supraspinal origin control motor neuron regeneration in the lesioned spinal cord of adult zebrafish

Veronika Kuscha[1], Angela Scott[1], Michell Reimer[1], Tatyana B. Dias[1], Sarah Frazer[1], Anton Barreiro-Iglesias[1], Catherina G. Becker[1], Thomas Becker[1]

[11]University of Edinburgh, Centre for Neuroregeneration, Little France Crescent, EH16 4SB Edinburgh, United Kingdom

After a spinal lesion, zebrafish regenerate lost motor neurons (Reimer et al., 2008, J Neurosci 28, 8510-6). We hypothesised that signals released by descending axons are involved in cellular regeneration around the lesion site. Dopaminergic axons of supraspinal origin sprout rostral, but are almost completely absent caudal to the lesion site. Expression of the dopamine receptor drd4a is only increased rostral to the lesion site.
Numbers of regenerating motor neurons are almost 2-fold higher rostral than caudal of the lesion site. Conversely, numbers of newly generated vsx1+ interneurons are significantly higher caudal than rostral to the lesion site. This is consistent with specific influences of descending axons on cellular regeneration in the lesioned rostral spinal cord.
To test whether dopamine is involved in motor neuron regeneration, we ablated tyrosine hydroxylase positive, mostly dopaminergic axons by injecting the toxin 6-hydroxydopamine. At 2 weeks post-lesion, we observed a significant motor neuron decrease in toxin injected fish only rostral to the lesion site. As a gain-of-function experiment, we injected the dopamine agonist NPA after spinal lesion, which increased motor neuron numbers only rostral to the lesion site. These results suggest that dopamine released by descending axons, is specifically involved in the generation of motor neurons in the lesioned spinal cord of adult zebrafish.



# Comparative analysis of the toxic effects of branched-chain fatty and very long chain fatty acids on cellular parameters of ALDP-deficient astrocytes

Nicol Kruska[1], Sabine Rönicke[2], Aurora Pujol[3], Georg Reiser[4]

[124]Otto-von-Guericke Universität Magdeburg, Institut für Neurobiochemie, Leipziger Str. 44, 39120 Magdeburg, Germany
[3]Institut d'Investigacio Biomedica de Bellvitge (IDIBELL, Barcelona, Spain

In diseases with peroxisomal impairment, like X-linked adrenoleukodystrophy (X-ALD), Refsum disease and Zellweger syndrome, the accumulation of very long chain fatty acids and/or the branched-chain fatty acids, phytanic acid and pristanic acid, is a characteristic hallmark. Elevated levels in tissue and plasma of patients suffering from these diseases lead to manifold neurological symptoms and neurodegeneration. Recent studies in neural cells of rat hippocampus revealed that VLCFA as well as phytanic acid and pristanic acid exert strong toxic effects on mitochondrial functions and Ca2+ homeostasis leading to cell death (1,2). Furthermore, we could demonstrate that after long-term application of VLCFA in rat hippocampal oligodendrocytes the expression level and the localization of the myelin basic protein (MBP), which is essential for forming the myelin sheath in cells, is changed dramatically. These findings, which are consistent with the pathology of X-ALD, encouraged us to investigate the effects of VLCFA on cellular parameters, like the generation of reactive oxygen species (ROS), in brain cells of an X-ALD mouse model. Oxidative stress is one of several characteristics in X-ALD, which is caused by the impaired function of the peroxisomal membrane transporter adrenoleukodystrophy protein (ALDP) due to mutations in its coding gene ABCD1. In the X-ALD mouse model, the ABCD1 gene is disrupted leading to an ALDP-deficiency. Characteristics of ADLP-deficient animals were already described (A. Pujol). For elucidating the effect of VLCFA in X-ALD, we used ALDP-deficient astrocytes and analyzed cell physiological processes (mitochondrial membrane potential, ROS formation and cell death) with specific fluorescent dyes. Here, we demonstrate that VLCFA can induce the generation of ROS and reduce the mitochondrial membrane potential in ALDP-deficient astrocytes. Additionally, we could detect a stronger toxic activity of VLCFA on ALDP-deficient cells compared to control cells by analyzing the cell death by the release of lactate dehydrogenase. However, the formation of ROS due to VLCFA application was significant but only weak, compared to that seen after treatment of ALDP-deficient cells with the branched-chain fatty acids phytanic acid and pristanic acid. Oxidative stress has been described as a major hallmark in X-ALD. In our studies, oxidative stress caused by VLCFA in astrocytes from ALDP-deficient animals seems to play a minor role in inducing detrimental changes in X-ALD, when compared to the toxic effects of branched-chain fatty acids.

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# Current-clamp experiments on primary hippocampal neurons shed light on potentially converse roles of L-type calcium channels in the pathogenesis of neurological diseases

Helmut Kubista[1], Petra Geier[1], Michael Lagler[1], Stefan Boehm[1]

[1]Medical University of Vienna, Center of Physiology and Pharmacology, Institute of Pharmacology, Waehringerstrasse 13a, A-1090 Vienna, Austria

Potentiation of L-type voltage-gated calcium channels (LTCCs) is known to arise in the course of neuronal aging, from oxidative stress signalling, and in the presence of oligomeric amyloid-ß-protein. Therefore, enhanced LTCC activity may contribute to pathomechanisms of neurological diseases. Considering this, inhibition of LTCCs would appear as a logical therapeutic strategy. However, LTCC antagonists have been widely studied e.g. for anti-epileptic activity, albeit with opposing findings. Attempts to identify beneficial effects of LTCC modulators in stroke therapy also provided inconclusive results. Since LTCCs show coupling to both excitatory and inhibitory Ca2+-dependent ion channels, their inhibition may have contrary outcomes. In this study we examined the effects of pharmacologically -induced functional LTCC up- regulation on spontaneously occurring and experimentally induced neuronal depolarisations. Current clamp recordings in perforated patch mode were performed on hippocampal neurons in primary culture. Pulsed current-injections of various durations (from 50 ms to 10 s) were applied in the presence of TTX for stepwise depolarisation and the availability of LTCCs was modulated by the dihydropyridines Bay K8644 and isradipine. Varying pulse length and current strength, we found that weak depolarising stimuli tend to be enhanced by LTCC activation which, when compared to traces recorded in isradipine, revealed voltage bumps (meaning that depolarisations rose above the response elicited in the presence of isradipine). In contrast, in the course of stronger depolarisations LTCCs counteract excitation, showing up as hyperpolarising sags (meaning that the responses - after an initial rise above the trace recorded in isradipine – already during the pulse hyperpolarise towards or even below the response elicited in the presence of the LTCC inhibitor so that the voltage response declines to or even traverses the isradipine trace in overlays). Experiments using apamin and exchange of external Na+ with choline or application of flufenamic acid indicate that bumps and sags involve Ca2+ --mediated activation of nonspecific cation (CAN) channels and KCa2.x ("SK") channels, respectively. In the absence of TTX, brief (100 ms range) unprovoked electrical events (e.g. excitatory postsynaptic potentials) were enhanced by LTCC activation, and paroxysmal depolarisation shift -like signals occurred. In contrast, under the same conditions depolarisation -patterns lasting for seconds were shortened. Our data indicate that excessive LTCC activity contributes to formation of brief depolarization shifts (which are putative key events in epileptogenesis) but may also be neuroprotective in the case of long-lasting depolarisations, such as those occurring under ischemic conditions. Hence, LTCC activity may cause opposing effects (e.g. of promoting or counteracting nature) on differently shaped pathological electrical events. This needs to be considered when trying to establish LTCC modulators in the treatment of various forms of abnormal neuronal electrical activities (supported by the FWF Austrian Science Fund, P19710).

# Deep brain stimulation of the pedunculopontine nucleus reverses hyperactivity of the subthalamic nucleus in the rat 6-hydroxydopamine Parkinson model

Kerstin Schwabe[1], Joachim K. Krauss[1], Mesbah Alam[1]

[1]Medical School Hannover, Department of Neurosurgery, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Deep brain stimulation (DBS) of the nucleus pedunculopontine tegmentum (PPN) is an apparent and promising surgical therapy for dopamine refractory motor dysfunction, gait disturbances and postural instability in late stages of Parkinson's disease (PD), but also for progressive supranuclear palsy (PSP) and multisystem atrophy. Lesions of the PPN have been shown to reduce the elevated discharge rate of the subthalamic nucleus (STN) in the 6-hydroxydopamine (6-OHDA) rat model of PD. In rats with unilateral 6-OHDA induced nigrostriatal lesions we examined the effect of 25 Hz low frequency stimulation of the PPN on single unit activity and oscillatory local field potentials (LFPs) of the STN, and on the electrocorticogram (ECoG) of the primary motor cortex region. Stimulation of the PPN suppressed the firing rate in the STN, without affecting the firing pattern. It also reduced the activity in the beta band (15-30 Hz) of the STN, which is elevated in 6-OHDA lesioned rats, without affecting the enhanced beta activity of the motor cortex.
We showed an important modulatory influence of PPN stimulation on altered neuronal STN activity in the PD 6-OHDA rat model. Our observations are in line with previous studies on the effect of PPN lesions in this PD rat model.

# Differential growth factor expression in astrocytes from mouse models for Parkinson's disease

Pascal Malkemper[1], Hermann Lübbert[1,2]

[1]Ruhr-University Bochum, Department of Animal Physiology , Universitätsstr. 150, 44780 Bochum, Germany
[2]Biofrontera Bioscience GmbH, Leverkusen, Germany

Introduction: Transgenic astrocytes of specific mouse models for Parkinson's disease (PD) fail to influence neuronal differentiation in indirect coculture systems (Schmidt et al. 2010). The molecular basis of this phenomenon is unknown and might comprise secretion of toxic molecules as well as a lack of neurotrophic support. Glial cell line-derived neurotrophic factor (GDNF) and brain-derived neurotrophic factor (BDNF) have been shown to be involved in PD pathogenesis and have even been used in clinical trials (Howells et al., 2000; Gill et al., 2003). Therefore, the current study investigated the expression levels of these neurotrophic factors in primary astrocytes of three different mouse models for PD using a competitive RT-PCR approach.

Results: Depending on the observed mouse model, astrocytes derived from cortex and mesencephalon showed differential mRNA contents of GNDF but not of BDNF compared to astrocytes derived from non-transgenic littermates. Highest differences were found in astrocytes derived from mice with deletions in exon 3 of the parkin gene. Co-culturing with cortical neurons prior to RNA isolation did not affect expression levels.

Conclusion: In conclusion, astrocytes from PD mouse models show differences in the expression of important neurotrophic factors. Whether this leads to the observed failure to induce neuronal differentiation needs to be elucidated but the data again underlines the importance of astrocytes and neurotrophic factors in the pathophysiology of Parkinson`s disease.

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# Distribution of microglia in the aging murine nigrostriatal system

Ahmed Sharaf[1], Kerstin Krieglstein[1,2], Björn Spittau[1]

[1]Albert-Ludwigs-Universität Freiburg, Institute of Anatomy & Cell Biology, Department of Molecular Embryology, Albertstraße 17, 79104 Freiburg, Germany

[2]Albert-Ludwigs-Universität Freiburg, Freiburg Institute for Advanced Studies (FRIAS), Albertstraße 19, 79104 Freiburg, Germany

Parkinson´s disease (PD) is characterized by a dramatic loss of midbrain dopaminergic (mDA) neurons in the substantia nigra (SN). Although the exact mechanisms for most forms of PD leading to disease onset and progression remain elusive, an inflammatory response mediated by microglial cells is a common constituent of all different forms of PD. Moreover, aging is one of the strongest risk factors for developing PD. A detailed analysis of the microglia distribution and density in the adult and aging nigrostriatal system is the prerequisite to understand the contribution of microglia to the disease onset and progression of PD. In this study we used male C57BL/6 mice at different postnatal stages ranging from P0 to 24 month in order to determine changes in microglia numbers and their morphology, respectively. Using Iba1 as an immunohistochemical marker, we detected the highest numbers of microglia in the nigrostriatal system at 3 month of age. From that time point the numbers of microglia declined till the age of 24 month. Interestingly, the morphology of microglia changed during normal aging. We observed reduced ramification and stronger staining intensity of microglia at 12 month, suggesting a shift to an activated phenotype. At 18 month and 24 month of age, microglia in the nigrostriatal system again exhibited a more ramified appearance, indicating a critical time period with activated microglia in the nigrostriatal system at 12 month of age. Taken together, our results show age-dependent changes of microglia numbers and their morphology in the aging murine nigrostriatal system.

# Does inhibition of IL-1ß prevent or modify epileptogenesis in two different rat models of chronic epilepsy?

Nadine Polascheck[1], Marion Bankstahl[1,2], Teresa Ravizza[3], Annamaria Vezzani[3], Wolfgang Löscher[1,2]

[1]University of Veterinary Medicine, Department of Pharmacology, Toxicology, and Pharmacy, Hannover, Germany

[2]Center for Systems Neuroscience, Hannover, Germany

[3]Mario Negri Institute for Pharmacological Research, Laboratory Experimental Neurology, Department of Neuroscience, Milano, Italy

Inflammatory cytokines are suggested to play a central role in the pathophysiology of epilepsies. A strong increase in IL-1ß production after status epilepticus (SE) has been described in electrical as well as in chemical models of epilepsy (Vezzani et al., 2002, Epilesia; Ravizza et al., 2008, Neurobiol. Dis.; Kuteykin-Teplyakov et al., 2009, Epilepsia). Several studies have shown that pharmacological inhibition of IL-1ß by antagonizing its receptor or by inhibiting its production has anticonvulsive effects and impairs kindling epileptogenesis (Vezzani et al., 2002, Epilepsia; Ravizza et al., 2006, Epilepsia; Ravizza et al., 2008, Neurobiol. Dis.; Marchi et al., 2009, Neurobiol. Dis.).

In the present study, production and action of IL-1ß were blocked by administering an IL-1 receptor antagonist (anakinra) and a caspase 1 inhibitor (VX-765) directly after termination of SE for the next seven days. Anakinra was continuously administered by subcutaneously implanted minipumps and VX-765 was daily injected intraperitoneally. SE was induced either by electrical stimulation of the ventral hippocampus or by lithium and pilocarpine administration. To investigate the effect of IL-1ß inhibition on the development of spontaneous seizures, rats were EEG- and video-monitored during the treatment period. Seven days after SE induction blood and CSF samples were taken before the animals were perfused. Brain slices were immunohistochemically stained to visualize IL-1ß-protein and to evaluate effects on neurodegeneration.

Measurements of IL-1 receptor antagonist by quantitative ELISA detected high concentrations of anakinra in plasma and CSF samples. In line with these results, IL-1ß stained cells were strikingly decreased in the VX-765-anakinra-treated animals seven days after SE. The histological analysis of neurodegeneration indicate a neuroprotective treatment effect in selected brain areas.

The pharmacological treatment used in this study was able to reduce IL-1ß in brain regions involved in seizure generation and propagation. Whether this positively effected the early phase of epileptogenesis will be seen after completion of the EEG- and video-analysis.

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# Downregulation of PMCA2 increases the vulnerability of dopaminergic neurons to mitochondrial complex I inhibition

Alexander Brendel[1], Christian Behl[1], Parvana Hajieva[1]

[1]University Medical Center of the Johannes Gutenberg-University, Institute for Pathobiochemistry, Duesbergweg 6, 55099 Mainz, Germany

Parkinson's disease is an age-associated disorder characterized by a selective degeneration of dopaminergic neurons. The molecular mechanisms underlying the specific vulnerability of this subset of neurons is not fully understood.
Employing SH-SY5Y cells and primary mesencephalic neurons, we here demonstrate a significant increase in cytosolic calcium after inhibition of mitochondrial complex I, which is a well-established in vitro model of Parkinson's disease. This increase in calcium is correlated with a downregulation of the neuron-specific plasma membrane $Ca^{2+}$-ATPase isoform 2 (PMCA2). Two other important mediators of calcium efflux, sarcoplasmic reticulum $Ca^{2+}$-ATPase (SERCA), and $Na^{+}$-$Ca^{2+}$-exchanger (NCX), remained unaltered, indicating a specific role of PMCA2 in maintaining calcium homeostasis in dopaminergic neurons. The observed PMCA2 downregulation was accompanied by reduced levels of CREB phosphorylation dependent on protein kinase A (PKA).
In order to investigate the potential influence of PMCA2 on neuronal vulnerability, experimental downregulation of PMCA2 by means of siRNA was performed. The results demonstrate a significant impairment of cell survival under conditions of PMCA2 suppression. Hence, increased cytosolic calcium levels as a consequence of insufficient calcium efflux may lead to an increased vulnerability of dopaminergic neurons.
Our findings point towards a dysregulation of calcium homeostasis in Parkinson's disease and suggest a specific molecular mechanism.

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# Effects of disease-relevant alpha-synuclein- and LRRK2- mutants on neurital dynamics in primary midbrain dopaminergic neurons

Jan Christoph Koch[1], Florian Bitow[1], Jessica Haack[1], Elisabeth Barski[1], Sebastian Kügler[1], Mathias Bähr[1], Paul Lingor[1]

[1]University Medicine Goettingen, Neurology, Robert-Koch-Str.40, 37075 Goettingen, Germany

One of the main hallmarks of Parkinson's disease (PD) is the progressive loss of dopaminergic neurons in the substantia nigra pars compacta. Clinical symptoms, however, do not appear until a large fraction of nigral neurons is degenerated. In normal ageing, the number of dopaminergic neurons also decreases over time, which however does not result in clinical symptoms. This is suggestive of compensatory mechanisms in normal ageing, which may be dysfunctional in PD. Axonal regeneration and sprouting may be part of such compensatory mechanisms. Since alpha-synuclein and LRRK2 represent the two most commonly PD-associated proteins, we set out to study their role in neurital dynamics, such as growth, regeneration and vesicular transport.

We generated primary rat embryonic midbrain cultures (E14) containing ~5-10% dopaminergic neurons. Cultures were then transfected using the nucleofection technique with plasmids expressing either human alpha-synuclein (WT, A30P, A53T), LRRK2 (WT, KD, G2019S, R1441C, R1441C-KD) or EGFP (control). Cotransfection with an EGFP-expressing plasmid allowed the identification of transfected neurons. Neurite morphology was evaluated after immunocytochemical staining and vesicular transport was monitored via life-imaging of synaptophysin in vitro.

Here, we present data on neurite outgrowth, branching behaviour, regeneration, growth cone morphology and vesicular transport in dopaminergic and non-dopaminergic neurons. Our data demonstrate differential effects of alpha-synuclein, LRRK2 and their disease-relevant mutants on neurital dynamics, suggesting new roles of these proteins in the pathogenesis of Parkinson's disease.

# FGF-2 modulates proliferation and natural cell death in the developing ventral mesencephalon in mice

Olga Baron[1], Andreas Ratzka[1], Claudia Grothe[1,2]

[1]Hannover Medical School, Neuroanatomy, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Center for Systemic Neurosciences (ZSN), Hannover

Progredient loss of mesencephalic dopaminergic neurons (mDA) in the substantia nigra pars compacta (SNpc) is the main cause for characteristic symptoms in Parkinson's disease. Insight in the regulation of the SNpc development may benefit to the understanding of disease pathophysiology, and improvement of therapeutic approaches. Our previous studies revealed in addition to a protection function of FGF-2 in mature mDA neurons, an involvement of FGF signalling in developing substantia nigra pars compacta (SNpc) (Grothe & Timmer 2007, Brain Res. Rev.). The increased numbers of tyrosine hydroxylase immunoresponsive (THir) neurons in SNpc of adult FGF-2 deficient mice, as well as decreased numbers in FGF-2 overexpressing mice suggest a regulatory role of FGF-2 for proper development of SNpc (Timmer et al., J. Neurosci., 2007). To elucidate the physiological function of FGF-2 in the nigrostriatal development, we analyzed embryonic (E14.5), newborn (P0), and juvenile (P28) FGF-2 deficient mice.

Our recent stereological analysis (CASTgrid, Olympus) on the content of THir cells in the SNpc of FGF-2 depleted mice could further delineate the potential onset of the phenotype between E14.5 and P0. Additionally, the examination of the SNpc and ventral tegmental area (VTA) in juvenile mice (P28) showed a specific increase in number of THir cells in SNpc. The unchanged number of THir cells in VTA is suggestive for an unaltered migration of mDA neurons of FGF-2 deficient mice. To unravel the underlying mechanism, immunohistochemical analysis of proliferation rates in E14.5 animals and of apoptosis rates in P0 animals was performed. The significant increase in Ki67ir cells in the subventricular zone of FGF-2 deficient mice points out a modulating influence of FGF-2 on proliferative progenitors in ventral mesencephalon. The number of cells positive for cleaved caspase-3 was unchanged in the P0 animals, whereas the number of cells positive for apoptotic bodies but negative for cleaved caspase-3 was significantly decreased in the mutants. This might indicate either a later onset of natural cell death or reduction of caspase independent cell death. The examination of the expression levels of mDA relevant genes by qRT-PCR in E14.5 and P0 did not reveal a possible target modulated by FGF-2.

However, both physiological alterations could determine the phenotype in the ventral mesencephalon of the adult FGF-2 depleted mice: longer or enhanced proliferation of progenitors may cause an increased generation of mDA neurons, while altered wiring control during the maturation may lead to reduced natural cell death. Moreover, the impact of signalling pathways activated downstream of FGF receptor will be investigated in the recently established in vitro model of nigrostriatal co-culture explants.

# First steps to an early diagnosis of Alzheimers disease via the enteric nervous systems

Sandra Semar[1], Maryse Letiembre[2], Alex Liu[2], Markus Klotz[1], K. Fassbender[2], Karl-Herbert Schäfer[1]

[1]

[1]University of Applied Sciences Kaiserslautern, Biotechnology, Amerikastrasse 1, 66482 zweibrücken, Germany
[2]University of Saarland, Medical Faculty, Department of Neurology, Homburg, Germany]

Neurodegenerative diseases lead to specific or general degeneration of neural structures within the central nervous system. In Parkinsons disease the dopaminergic population is reduced in the substance nigra, but also in the enteric nervous system.
So far, changes in the ENS of patients with Alzheimers disease (AD) have not been reported. If the ENS is also involved in AD, the gut could be used for an early diagnosis of the disease, while the clinical signs are not yet appropriate or fully developed. The perspective of an early treatment might lead to a less severe progression of the disease.
We therefore investigated the expression of amyloid precursor protein in the ENS of neurodegenerative patients, as well as in two different AD mouse models. Immunohistochemical stainings for human APP, as well as for neuronal and glial markers were performed. Smooth muscle tissue with included myenteric plexus from the mouse models were analyzed with RT-PCR. Proteomic analysis (2-D-DIGE) were performed on brain and gut tissue.
We could find APP upregulation in both AD mouse models, correlated with increasing levels of nestin and GFAP. In one of the mouse models, a significant reduction of the myenteric plexus could be detected, while the other showed only slight modifications.
The early diagnosis of neurodegenerative disease associated pathological proteins in the enteric nervous system can be a powerful tool. To find appropriate markers a much more detailed analysis of the correlated changes in CNS and ENS is necessary. Furthermore, the onset of the expression of APP and correlated proteins have to be investigated in detail to find suitable markers for a reliable diagnosis.

# Formic acid and sodium dodecyl sulfate (SDS)-sensitive high-molecular Aβ-oligomers and protofibrils are the predominant Aβ-species in the native soluble protein fraction of the AD brain. Low-molecular Aβ-oligomers occur after denaturation.

Ajeet Rijal Upadhaya[1], Irina Lungrin[2], Haruyasu Yamaguchi[3], Marcus FÃ¤ndrich[4], Dietmar Rudolf Thal[5]

[1 2 5]University of Ulm, Neuropathology, Helmholtzstrasse 8/1, 89081 Ulm, Germany

[3]Gunma University of Health Sciences, Maebashi, Gunma, Japan

[4]Max Planck Research Unit for Enzymology of Protein Folding and Martin-Luther University Halle-Wittenberg, Halle, Germany

Alzheimer's disease (AD) is characterized by the aggregation and deposition of the amyloid β-protein (Aβ). Soluble Aβ-aggregates have been considered to be most toxic. All kinds of soluble Aβ-aggregates (e.g. Aβ-dimers, Aβ-dodecamers, high molecular Aβ-oligomers, protofibrils, and fibrils) have been described in the human brain. Here, we analyzed the sucrose -soluble fraction of human brain lysates from AD and control cases to characterize the native forms of soluble Aβ-aggregates by blue native-polyacrylamid gel electrophoresis (BN-PAGE) as well as sodium dodecyl sulfate-PAGE (SDS-PAGE). BN-PAGE analysis revealed a high-molecular smear (> 1000 kDa) of Aβ-positive material in the AD brain whereas low-molecular and monomeric Aβ-species were not detected. SDS-PAGE analysis, on the other hand, allowed the detection of Aβ-monomer, dimer and trimer bands in AD cases but not in controls. To confirm dissociation of amyloid fibrils and high molecular oligomers into monomers and low-molecular oligomers in SDS-PAGE we used synthetic Aβ40 to grow Aβ-fibrils, protofibrils and high molecular oligomers that were detectable at the electron microscopic level and in BN-PAGE as a high molecular smear. A monomer band was also observed whereas low-molecular oligomers were less prominent. In contrast, SDS-PAGE revealed Aβ-dimers, trimers and dodecamers but no high-molecular Aβ. Immunoelectron microscopy of immunoprecipitated oligomers, and protofibrils/fibrils showed nearly no Aβ-positive material in controls but globular Aβ-oligomers and Aβ-protofibrils in AD cases. Here, we used the B10-antibody domain (B10AP) that detects protofibrillar and fibrillar Aβ and the A11-antibody that detects oligomeric Aβ-aggregates to analyze Aβ-aggregates. B10AP and A11 positive Aβ-aggregates in human AD brain sections were no longer detectable after formic acid pretreatment. However, some amyloid plaques were detected with the A11-antibody after formic acid pretreatment. These results indicate that high-molecular Aβ-species are most prevalent in the soluble fraction of the AD brain and form globular oligomers and protofibrils whereas low-molecular oligomers predominantly occur after protein denaturation. Thus, denaturation of high-molecular Aβ-aggregates by potentially contributes to the generation of soluble low-molecular Aβ-species especially near amyloid plaques. Amyloid plaques may, thereby, serve as a reservoir for the production of low-molecular Aβ oligomers.

# GABAergic septo-hippocampal neurons and GABAergic hippocampal interneurons are early targets for neurodegeneration in a mouse model of amyloidosis and tauopathy

Desiree Loreth[1], Raphael Poirier[2], Fiona Grueninger[2], Bernd Bohrmann[2], Michael Frotscher[1], Friedrich Metzger[2], Oliver Kretz[1]

[1]University Freiburg, Center for Neurosciences, Albertstr. 23, 79104 Freiburg, Germany
[2]Roche CNS Discovery Research, Basel, Switzerland

Alzheimer's disease is a neurodegenerative disorder characterized by brain accumulation of amyloid-ß peptide, which is believed to initiate a pathologic cascade resulting in progressive impairment of brain plasticity and cognitive functions as well as in neuronal cell death. Here, we analysed a triple -transgenic mouse model of amyloidosis and tauopathy (3tg mice) overexpressing human mutations of amyloid precursor protein, presenilin 2 and tau. We indentified a significant neuronal degeneration affecting GABAergic septo-hippocampal projection neurons. In the axons of these neurons localized in the fimbria fornix, accumulation of hyperphosphorylated tau is detectable at 7 months of age. At 12 months of age, a 30% loss of parvalbumin-positive cells in the medial septum is observed by stereology. At later time points this cell loss is even more pronounced and accompanied by a significant reduction in fimbria-fornix diameter, gliosis and disappearance of phospho-tau staining in this region. Moreover, within the hippocampus of 3tg mice GABAergic interneurons are found to be dramatically reduced in all hippocampal subfields, supported by evidence of reduced NPY and pro-enkephalin RNA expression, whereas Y2R and GABA -A Alzheimer's disease is a neurodegenerative disorder characterized by brain accumulation of amyloid-ß peptide, which is believed to initiate a pathologic cascade resulting in progressive impairment of brain plasticity and cognitive functions as well as in neuronal cell death. Here, we analysed a triple-transgenic mouse model of amyloidosis and tauopathy (3tg mice) overexpressing human mutations of amyloid precursor protein, presenilin 2 and tau. We indentified a significant neuronal degeneration affecting GABAergic septo-hippocampal projection neurons. In the axons of these neurons localized in the fimbria fornix, accumulation of hyperphosphorylated tau is detectable at 12 months of age. At 12 months of age, a 30% loss of parvalbumin-positive cells in the medial septum is observed by stereology. At later time points this cell loss is even more pronounced and accompanied by a significant reduction in fimbria-fornix diameter and a disappearance of phospho-tau staining in this region. Moreover, within the hippocampus of 3tg mice Reelin-positive GABAergic interneurons are found to be dramatically reduced in all hippocampal subfields, supported by evidence of reduced NPY, Y1 receptor, and pro -enkephalin RNA expression, whereas Y2R and GABA -Aalpha2 receptors were upregulated. This apparent disequilibrium of inhibitory circuits resulted in strongly enhanced long-term potentiation in the medial-perforant path input to the dentate gyrus at 13 months of age compared with wild-type controls. Our data indicate that inhibitory neurons are early targets of neurodegeneration in a mouse model of amyloidosis and tauopathy and point to a possible role of disinhibition in the pathophysiological cascade of Alzheimer's disease.

# GGA3 is a potential CSF marker of Alzheimer´s disease

Cathrin Schnack [1], Björn von Einem [1], Anke Hellrung[1], Markus Otto[1], Hayrettin Tumani[1], Sarah Jesse[1], Johannes Brettschneider[1], Christine von Arnim [1]

[1]Uniklinikum Ulm , Deparment of Neurology, Helmholtzstraße 8/1, 89091 Ulm , Germany

Alzheimer´s disease (AD) is a slowly progressive neurodegenerative disease that results in significant memory loss and behavioural and personality changes. The major neuropathological hallmarks of the disease are extracellular amyloid plaques that are composed of AB40 and A B42 and intracellular neurofibrillary tangles which are composed of hyperphosphorylated protein tau. The first pathophysiological symptoms probably appear many years prior to clinically detectable cognitive deficits. The diagnosis of Alzheimer´s disease is currently based primarily on clinical symptoms. Biomarkers in CSF like Amyloid beta and tau can help in early and differential diagnosis. More and better biomarkers in CSF to get an improved early and differential diagnosis for pathological aging processes and to give deeper insight into pathophysiological events and signal pathways are needed. Early diagnosis of AD would be a great value for distinct identification of initiate states of disease. The aim of our study was to identify new potent biomarkers in CSF of Alzheimer´s patients versus controls.

With immunanalytical assays we analyzed CSF from 26 AD patients, 10 PD patients, 10 ALS patients and 30 non-demented control subjects for specific proteins involved in intracellular sorting and transport processes associated with the pathophysiology of AD. In our investigation we detected significantly altered levels of Golgi-localizing, G-Adaptin Ear Homology Domain, ADP-ribosylation Factor-binding protein 3 (GGA3) in CSF of AD patients. Members of the GGA protein family are involved in APP processing and regulation of BACE. GGA proteins facilitate membrane trafficking events at the Golgi, perhaps by sorting or recruiting cargo into forming vesicles. Also association with neuropsychological profile and Abeta and tau levels in CSF was investigated. In contrast no difference of GGA proteins could be detected in CSF of PD and ALS patients versus control subjects.

We conclude that GGA3 seems to be a promising CSF biomarker for diagnosis of AD particularly in differential diagnostically cases and might indicate an important role of GGAs in pathophysiolocial processes of AD.

# Identification and characterisation of cerebral microparticles in cerebrospinal fluid following cerebral damage

Christa Maria Erika Trattnig[1], Silke Patz[1], Gerda Gruenbacher[1], Ulrike Fasching[1], Wissa Sonja[2], Christian Guelly[3], Birgit Ebner[3], Beate Rinner[4], Alexandra Novak[4], Gerd Leitinger[5], Gertrude Havlicek[5], Ute Schaefer[1]

[1]Medical University Graz, Research Unit Experimental Neurotraumatology, Auenbruggerplatz 33a, 8036 Graz, Austria
[2]Department of Neurosurgery, Graz, Austria [3]Core Facility Molecular Biology, Center for Medical Research, Medical University Graz, Austria
[4]Core Facility Flow Cytometry, Center for Medical Research, Medical University Graz, Austria
[5]Core Facility Ultrastructure, Center for Medical Research, Medical University Graz, Austria

Background
Microparticles (MPs) are spherical bodies emerging by a cell budding process with an average size of 100-1000nm. Their role in cell-cell-communication is well known, but evidence of the release of MPs into the cerebrospinal fluid CSF is sparse and little is known about their role in the central nervous system. These membrane covered particles may be released into the CSF, possibly as a response to pathophysiological changes, such as brain injury, in the CNS.
Material and Methods
We characterized stem cell derived microparticles in the cerebrospinal fluid by RT-PCR (Reverse Transcription Polymerase Chain Reaction), qRT-PCR (quantitative Real-Time PCR), flow cytometry, and microarray analysis. MPs were visualized in electron and fluorescence microscopy.
Results
The concentration of RNA in MPs derived from CSF varied significantly in different patient groups: Patients with subarachnoid bleeding (SAB) 0,39ng/µl ($p>0,076$), traumatic brain injury (TBI) patients 0,17ng/µl as compared to healthy controls 0,1ng/µl. 50% of the MP-RNA was demonstrated to be microRNA. 80 different miRNAs were identified in MPs derived from CSF of SAB or TBI injured patients. miRNA-9, miRNA-30b, miRNA134, 128a, 411, 301a, 124, 124*, 29b, 129-3p and let7 are indicated to have a cerebral function and were detected in MPs of SAB or TBI patients, but rarely in healthy controls. MPs derived from CSF were demonstrated to carry ß-Actin, MAP2, ARC and LimKI mRNA. Within neuronal cells these transcripts are transported in large granules. The concentration of ß-Actin, MAP2 and ARC was increased in MPs derived from the CSF of TBI/SAB patients as compared to healthy controls. Additionally RPBs (Ribonucleotid Binding Protein) like hnRNP, A2/B1, Argonaute-2, ZBP-1 and Staufen-2 were determined in the MPs of patients with SAB. These proteins are known to be components of RNA granules.
MPs with a size of 200-400nm were visualized by electron and fluorescence microscopy. Furthermore, our results suggest that the MPs derived from CSF of patients are cell specific. Up to 15% of MPs were shown to be stem cell derived CD133+ positive in the patient groups.
Conclusion
We were able for the first time to demonstrate that microparticles in the liquor of patients with cerebral injury may contain specific miRNA and mRNA molecules, possibly packed into the MPs as RNA granules. The composition of MPs derived from TBI patients indicates a role of these MPs in neuronal cell-cell communication in response to brain injury. CSF derived extracellular MPs might serve as blueprints of the cerebral donor cells and provide information about the prevailing cellular status of these cells.

# IDENTIFICATION OF LOW MOLECULAR WEIGHT PYROGLUTAMATE ABETA OLIGOMERS IN ALZHEIMER DISEASE: A NOVEL TOOL FOR THERAPY AND DIAGNOSIS

Oliver Wirths[1], Christian Erck[2], Henrik Martens[2], Anja Harmeier[3], Constanze Geumann[2], Sadim Jawhar[1], Sathish Kumar[4], Gerd Multhaup[3], Jochen Walter[4], Martin Ingelsson[5], Malin Degermann-Gunnarsson[5], Hannu Kalimo[6], Inge Huitinga[7], Lars Lannfelt[5], Thomas A. Bayer[1]

[1]University of Goettingen, Dept. of Psychiatry, Molecular Psychiatry, von-Siebold-Str. 5, 37075 Goettingen, Germany [2]Synaptic Systems, Göttingen, Germany [3]Free University of Berlin, Berlin, Germany [4]University of Bonn, Germany [5]Uppsala University, Sweden [6]University of Helsinki, Finland [7]Netherlands Brain Bank, Amsterdam, The Netherlands

N-terminally truncated Aß peptides starting with pyroglutamate (AßpE3) represent a major fraction of all Aß peptides in the brain of Alzheimer disease (AD) patients. AßpE3 has a higher aggregation propensity and stability and shows increased toxicity compared to full-length Aß. In the present work, we generated a novel monoclonal antibody (9D5) that selectively recognizes oligomeric assemblies of AßpE3 and studied the potential involvement of oligomeric AßpE3 in vivo using transgenic mouse models as well as human brains from sporadic and familial AD cases. 9D5 showed an unusual staining pattern with almost non detectable plaques in sporadic AD patients and non-demented controls. Interestingly, in sporadic and familial AD cases prominent intraneuronal and blood vessel staining was observed. Using a novel sandwich ELISA significantly decreased levels of oligomers in plasma samples from patients with AD compared to healthy controls were identified. Moreover, passive immunization of 5XFAD mice with 9D5 significantly reduced overall Aß plaque load and AßpE3 levels, and normalized behavioral deficits. These data indicate that 9D5 is a therapeutically and diagnostically effective monoclonal antibody targeting low molecular weight AßpE3 oligomers.

# NEW MARKERS FOR EARLY DIAGNOSIS OF ALZHEIMER DISEASE: THE FIRST STEP TOWARDS EFFICIENT TREATMENTS.

Stefania Averaimo[1], Laura Gasparini[2], Simona Gornati[1], Michele Mazzanti[1]

[1]University of Milan, Dept. Biomolecular Sciences and Biotechnology, Via Celoria 26, 20133 Milan, Italy

[2] Dept. of Brain Technology, IIT, Genova, Italy

Alzheimer disease (AD) is the most common form of dementia in the elderly. From a clinical point of view it is characterized by cognitive impairments due to neurodegeneration mainly localized in the hippocampus and in the cortex. From a histological point of view it is characterized by amyloid beta (Aß) deposits in the extracellular milieu, surrounded by activated microglia, and intraneuronal accumulation of the hyperphosphorilated form of the cytoskeletal protein tau. Today an effective treatment for this invalidating disease is yet to be identified, mainly due to the absence of a certain and early diagnosis. The pathological state is identified only after the appearance of the first symptoms, which usually means that the disease is in a late stage and the damage is already broad and thus irreversible. The disease is definitely diagnosed only post mortem, with a histological exam of the brain tissue.

For this reason the search for an early diagnosis is imperative and must be parallel to the research of an effective treatment. The early diagnosis of the neurodegenerative process must be early, specific and easily performed in living subjects.

Many studies show a correlation between AD symptoms and retinal dysfunctions, due to degeneration of retinal ganglion cells (RGCs) in the deepest retina layers. Previous studies have demonstrated altered redox conditions preceding neurodegenerative events of RGCs. CLIC1 (Chloride Intracellular Channel 1) is a recently identified protein, whose involvement in oxidative processes triggered by AB has been extensively described. In microglia cells this protein is usually soluble in the cytoplasm and inserts into cell membranes upon specific oxidative stimuli initiated by Aß interaction.

We decided to explore the possibility that CLIC1 could be involved in the degenerative process of RGCs, and immunolocalization studies on isolated RGCs and retina slices from AD mouse models strongly support this hypothesis.

CLIC1 is mainly localized in the ganglion cell layer and in Muller cells. Moreover, its expression appeared to increase in the eye tissue before the manifestation of the first symptoms of cognitive impairment, and before the appearance of cerebral AB deposits and the activation of brain microglia. In isolated mouse RGCs, stimulation with low concentrations of AB peptide causes a cell depolarization and an apparent subcellular relocalization of CLIC1.

Thus, CLIC1 is a protein which tightly feels the effect of AB stimulation and whose expression is increased in the eye, the most accessible part of the central nervous system, before the clinical onset of the disease, in both acute and chronic AD mouse models.

In conclusion, these data propose CLIC1 as a valuable tool for an early diagnosis of AD. In vivo confocal laser scanning microscopy (CLSM) combined with fluorescent probes specifically interacting with CLIC1, enabling it to be visualized with CLSM, could represent a promising tool in the diagnosis of AD, making the actual and future treatments more successful.

# Impaired cerebellar GABAergic feedforward inhibition in CLN3 knockout mice

Christian Werner[1], Benedikt Grünewald[1], Klaus Viktor Toyka[1], Claudia Sommer[1], Christian Geis[1]

[1] Department of Neurology, Universitätsklinikum Würzburg

Juvenile neuronal ceroid lipofuscinosis (JNCL) is caused by mutations in the CLN3 gene. It is the most prevalent form of a group of autosomal recessive disorders referred to as neuronal ceroid lipofuscinosis (NCL). Together they are the most common neurodegenerative disorders in childhood. Disruption of the CLN3 gene leads to loss of vision, frequent seizures, impaired motor learning, progressive cognitive decline and death in the early twenties. CLN3 knockout mice (CLN3-/-) show a similar phenotype including atactic behavior. Additionally, both, afflicted patients and CLN3-/- mice, develop spontaneously autoantibodies against the glutamate decarboxylase 65 (GAD 65), the rate limiting enzyme for vesicular GABA synthesis. Since GAD 65 antibodies are thought to be pathogenic in another CNS disorder, stiff-person syndrome, we examined their potential role in CLN3 mice.
To correlate the atactic motor deficits with possible functional defects on the cellular level, we analyzed cerebellar molecular layer interneuron–mediated GABAergic feedforward inhibition by performing single unit ex-vivo recordings from cerebellar Purkinje cells of CLN3-/- mice and control littermates. To analyze a pathogenic impact of GAD65 autoantibodies, CLN3-/- mice and their wild type (WT) littermates were treated with lipopolysaccharide (LPS) to disrupt the blood brain barrier (BBB) and facilitate the access of autoantibodies to the CNS. In cerebellar slices of treated animals, loose-patch recordings from floccular Purkinje cells were used for measurement of inter spike intervals (ISI) of simple spikes. Peristimulus histograms (PSTH) were calculated from recordings after stimulation of parallel fibres at 200µm distant from recorded Purkinje cells. Coeffecient of variation and coefficient of variance of adjacent intervals were calculated as a measure of regularity of Purkinje cell firing.
Ex vivo experiments revealed a significant decrease of mean ISI of spontaneous cerebellar simple spike discharges in CLN3-/- mice after BBB opening in comparison to WT animals. Furthermore, we found a significant increase of variability of simple spike firing in Purkinje cells after parallel fiber stimulation in LPS treated CLN3-/- mice, pointing toward a defective GABAergic modulation of the finescale patterns of Purkinje cell function. There were no significant changes in LPS untreated animals (WT versus CLN3-/- mice). The coefficient of variance and coefficient of variation of inter spike intervals were similar between groups.
Taken together, we found a disturbed interneuron–mediated GABAergic feedforward inhibition onto Purkinje cells in CLN3-/- mice. Defective Purkinje cell function may contribute to the behavioural changes seen in the animal model and patients with JNCL. Since symptoms in the CLN3-/- mice were increased and GABAergic inhibition deficits were pronounced after BBB opening, autoantibodies against GAD 65 may have direct pathophysiological implications on GABAergic cerebellar function.

# Impairment of N-cadherin function accelerates the effects of amyloid beta peptide (Aß) on glutamatergic synapses.

Aksana Andreyeva[1], Katharina Horstmann[1], Kurt Gottmann[1]

[1]Heinrich Heine University Düsseldorf, Institute of Neuro- and Sensory Physiology, Universitätsstraße 1, 40225 Düsseldorf, Germany

Synaptic dysfunction is thought to have a strong impact on the progression of Alzheimer's disease. Alterations of the pre- and postsynaptic structures lead to synapse loss, changes in behaviour and appearance of typical symptoms of dementia. However, the molecular mechanisms of synapse loss, as well as of reorganization in diseased synapses, remain poorly understood. For instance, the role of synaptic adhesion molecules – key elements in the maintenance of synaptic structure and functionality – is not known.

N-cadherin is a well-known synaptic adhesion molecule that modifies synapse function and spine morphology. To test the potential impact of N-cadherin function on the synaptotoxicity of AB, we used patch-clamp recordings and immunocytochemical stainings for the synaptic marker VAMP2. Primary cultured cortical mouse neurons were treated with the supernatant from constantly Aß secreting cells. As a control we used the same supernatant after immunodepletion with an antibody specific to Aß. To block N-cadherin function, we overexpressed a dominant-negative N-cadherin construct in neurons. Indeed, the block of N-cadherin function accelerated the synaptotoxicity of Aß. We observed a reduction in AMPA-receptor-mediated miniature EPSC frequency as well as a reduction in VAMP2-positive puncta in response to Aß application that occurred much earlier in neurons expressing the N-cadherin dominant-negative construct. To exclude unspecific effects of other components of the Aß-containing supernatant, we repeated the experiments with synthetic Aß. The effect of impairing N-cadherin function on Aß synaptotoxicity did not depend on the type of Aß.

To confirm our results, we also blocked N-cadherin function with the specific N-cadherin antagonistic peptide INPISGQ. Again, the neurons treated with the blocking peptide responded faster to Aß than the neurons treated with a scrambled peptide.

Our results indicate that N-cadherin function might have a strong impact on the vulnerability of synapses in Alzheimer's disease.

# Induced pluripotent stem cell derived neurons: a human model for Alzheimer's Disease?

Katja Nieweg[1], Aksana Andreyeva[1], Kurt Gottmann[1]

[1]Universität Düsseldorf, Institut für Neurophysiologie, Universitätstr,1, 40225 Düsseldorf, Germany

Disease modeling in cultured human neurons represents an emerging in vitro technology, which may have a strong impact on the development of therapeutic approaches for neurological disorders. A major prerequisite for this is the availability of human neurons that exhibit functional properties, i.e. excitability and synaptic transmission, crucial for the formation of functional neuronal networks. Recently, the establishment of reprogramming techniques has led to the availability of human induced pluripotent stem (iPS) cells, that show a similar differentiation potential as embryonic stem cells. Efficient in vitro differentiation protocols to obtain human neurons from these iPS cells now need to be developed.
We used iPS cells generated from human umbilical cord blood stem cells by reprogramming with viral factors (4-factor protocol; Zaehres et al., 2010) to develop an in vitro differentiation protocol and to study the functional properties of the obtained human neurons. iPS cells were cultured according to standard protocols and were tested for pluripotency using standard assays, e.g. OCT- 4 expression. In vitro differentiation was performed via formation of embryoid bodies, which upon attachment gave rise to neuronal rosettes. These rosette structures were characterized immunocytochemically for expression of Pax6 and Sox1. Further in vitro differentiation gave rise to human neurons that formed a large number of neuritic processes and, finally, a neuronal network. Neurons were characterised immunocytochemically and functionally. Patch-clamp recordings from individual neurons revealed the expression of large (>100 pA) voltage-activated sodium and potassium currents that enabled these cells to generate action potentials upon depolarization. Furthermore, strong spontaneous synaptic activity was observed consisting of both miniature postsynaptic currents and large bursts of PSCs driven by network activity.
In summary, we have developed an efficient protocol that enables the differentiation of fully functional human neurons from iPS cells. These neurons will be used for studying the molecular mechanisms of neurological diseases in a human in vitro model.

Zaehres et al. (2010) Induction of pluripotency in human cord blood unrestricted somatic stem cells. Exp Hematol 38: 809-818.

# Intrastriatal Botulinum toxin A injection ameliorates some motor abilities of hemiparkinson rats and leads to morphological changes in the brain

Alexander Hawlitschka[1], Veronica Antipova[1], Eilhard Mix[2], Oliver Schmitt[1], Martin Witt[1], Reiner Benecke[2], Andreas Wree[1]

[1]University of Rostock, Institute of Anatomy, Gertrudenstraße 9, 18055 Rostock, Germany
[2]University of Rostock, Department of Neurology, Gehlsheimer Straße 20, 18147 Rostock, Germany

Parkinson's disease is one of the most frequent neurodegenerative diseases. In Germany 300000 – 400000 people are affected.
Systemic application of anticholinergic drugs ameliorates symptoms of Parkinson's disease (PD), but is hampered by adverse side-effects. Botulinum neuro toxin A (BoNT-A) blocks cholinergic transmissions locally. To test the potential of an intracerebral BoNT-A treatment as a possible therapeutic option of PD we have investigated the consequences of intrastriatal injections of BoNT-A on brain morphology and on motor functions in the 6-hydroxydopamine (6-OHDA) animal model of hemi PD. The rational of intrastriatal BoNT-injection is to decrease the hypercholinism of the striatum known to occur in PD.
In naive young adult rats different doses of BoNT-A (100 pg, 1 ng and 2 ng) were applicated stereotacticilly into the right striatum and the brains were investigated 1 and 3 month post injection. In a second series rats firstly got 6-OHDA into the right medial forebrain bundle for lesioning dopaminergic neurons of the substantia nigra (hemiparkinson model) and 4 weeks later BoNT-A into the right striatum.
In addition to Nissl stainings, immunohistochemical visualisation of cholinergic neurons via choline acetyle transferase (ChAT) and dopaminergic axons and terminals via tyrosine hydroxylase (TH) and visualisation for synaptic proteins and cytoskeleton proteins were done.
Furthermore, we performed stereological investigations on BoNT-A treated brains and counted cholinergic cells to check for possible cytotoxic effects of BoNT-A.
Animal behaviour due to 6-OHDA and/ or BoNT was investigated by following tests: 1) apomorphine induced rotation test, 2) cylinder test (forelimb preference), 3) activity test in open field, and 4) RotaRod test.
Morphologically, we showed that it is possible to inject BoNT-A in the striatum in a narrow dose range. The number of ChAT-immunoreactive neurons in the left and in the right striatum remains unchanged after right-side striatal BoNT-injection. Interestingly, in the BoNT-injected striata - in contrast to controls – many small round structures were detectable, which were immunoreactive for ChAT or TH, which we called botulinum toxin induced varicosities (BiVs) at the moment. It can be supposed, that the observed BIVs are formed by a BoNT-induced retention of the synaptic vesicles in the boutons of the treated striata.
Behaviourally, hemiparkinsonian rats showed more than 8 apomorphine induced rotations per minute in anticlockwise direction. Following ipsilateral injection of 1 ng and 2 ng BoNT, these rotations were completely abolished, the effect lasting for at least 3 months. Six months after BoNT application a slight reappearance of pathological apomorphine rotations was noted, 9 months after BoNT-injection the original rotation behaviour reappeared.
It can be speculated that BoNT reduced the inhibitory cholinergic signals of the striatum, which annuls the apomorphine-induced rotations in 6-OHDA lesioned rats and thereby reversed the 6-OHDA induced motor deficit.

Till now we could prove, that the cholinergic system and also the dopaminergic system are affected after local intrastriatal BoNT-injections. The results of the behaviour studies speak for an involvement of further systems.
Theoretically intrastriatal BoNT application could be useful in new therapeutic strategies of PD avoiding undesirable side-effects and adverse pathogenetic effects of systemically administered anticholinergics.

# In-Vivo Mouse Brain Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) and Immunohistochemistry (IHC) Reveals Gender Specific Pathology induced by Cuprizone

Neele Hübner[1], Laura-Adela Harsan[1], Alexandru Parlog[1,2], Nicoleta Baxan[1], Jürgen Hennig[1], Dominik von Elverfeldt[1]

[1]Department of Radiology, Medical Physics, University Medical Center Freiburg, Germany
[2]Institut de Génetique et de Biologie Moleculaire et Cellulaire, University Louis Pasteur, Strasbourg, France

*Introduction*

Clinical observations in multiple sclerosis patients (MS) show that the course of the disorder is gender dependent, women reach disability milestones at older ages than males and the male sex is associated with a more progressive and severe outcome than female sex. In the present study we addressed the question of gender dimorphism in the development of the white matter disorders by using the cuprizone demyelinated mice in a longitudinal in vivo DT-MRI investigation. We aimed to compare the pattern of microstructural changes overtime in male and female mouse brains.

*Material and Methods*

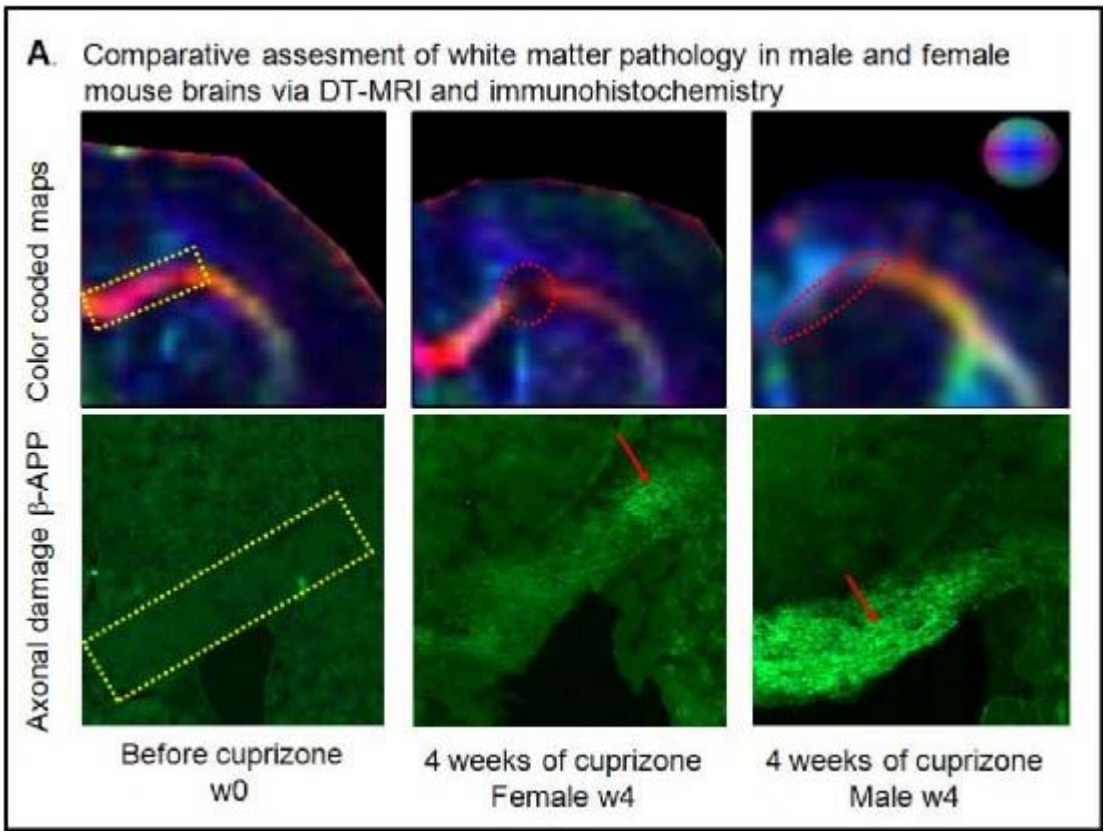
Male and female 8-week old C57BL/6 mice were treated with 0.2% cuprizone for 12 weeks, underlining the progression of active demyelinating processes towards the chronic state. In vivo brain DT-MRI exams were performed at different time points (week 0 – before cuprizone treatment, w4, w8 and w12 – 4, 8 and 12 weeks after starting the cuprizone diet). Duplicates for each group were kept in the same conditions of housing and treatment and used for the histopathological examination.

DT-MRI: Mice were scanned under isofluorane anesthesia using a 9.4T small bore animal scanner (Biospec 94/20, Bruker). DT -MRI was performed using a 4- shots DT -EPI sequence with 45 non- collinear directed diffusion gradients (d.g.), b factor of 1000 s/mm2, TR/TE=5000/30 ms, time between d.g. application $\Delta$=17ms, d.g. duration d=7ms. The obtained image resolution was of 156x156x500µm³. Using house developed DT -MRI software, different diffusion tensor parametric maps (fractional anisotropy (FA), mean ($D_{mean}$)/ radial ($D_{perpendicular}$) / axial ($D_{parallel}$) diffusivity) were generated and the values obtained in different ROIs were correlated with the histological investigation.

IHC: Myelin staining was performed on mouse brain cryosections using an anti- myelin basic protein (MBP) antibody. The axonal damage, gliosis and the astrocytosis were detected in cuprizone treated animals using anti ß-APP, anti- IBA1 and anti- GFAP antibodies. Fluorescence images were obtained using a Zeiss microscope (Axio Imager.A1). Comparative DT -MRI and histological analyses were focused on corpus callosum, external capsule, anterior commissure and fornix of male and female animals.

*Results and discussion*

Cuprizone treatment induced marked white matter (WM) pathology: region specific oligodendrocyte depletion, gliosis, demyelination and axonal damage. These pathologic alterations, observed starting with the early phase (week 4) of the disease, caused changes of DT -MRI derived parameter values. DT -MRI evidenced high vulnerability of the corpus callosum, the values of the axial diffusivity drastically decreasing at w4. The great decrease of $D_{parallel}$ was correlated with the histological findings of axonal damage evidenced with ß APP. The axonal damage was more pronounced in the male white matter, with statistically significant differences between males and females observed in the genu and the splenium of the cc. The increase in the radial diffusivity values was associated with progressive myelin loss from w4 to w12 in cuprizone mice. Constant higher values of radial diffusivity were assessed in the male animals, suggesting a faster and more severe demyelinating phenotype and course of the disease. The existence of a gender dimorphism in demyelination processes implies also a gender specific response to different therapeutic strategies, opening interesting perspectives for remyelinating treatments.


A. Comparative assesment of white matter pathology in male and female mouse brains via DT-MRI and immunohistochemistry
Color coded maps
Axonal damage β-APP
Before cuprizone w0
4 weeks of cuprizone Female w4
4 weeks of cuprizone Male w4

# Localization and subcellular distribution of the amyloid precursor family in the adult mouse brain

Kristine Gampe[1], Christine Altmann[1], Sebastian Czaplinski[1], Susanne Walter[1], Walter Volknandt[1]

[1]J.W.Goethe-University Frankfurt, Institute of Cell biology and Neuroscience, Max-von-Laue-Str.9/N210, 60438 Frankfurt, Germany

There is a wealth of literature describing the role of amyloid precursor protein in Alzheimer disease albeit our knowledge about the physiological function remains rudimentary. APP belongs to the amyloid precursor family, which in mammals includes the amyloid precursor-like protein 1 (APLP1) and amyloid precursor-like protein 2 (APLP2). All three members contain highly conserved regions in particular within the ectodomain and the intracellular tail. All three proteins are similarly cleaved by the a-, ß-, γ-secretase although APLP1 and APLP2 lack the expression of the Aß region. APP and APLP2 mRNAs are ubiquitously expressed in a variety of developing and adult mouse tissues, in contrast, the APLP1 mRNA is restricted to the nervous system. Several loss of function studies of APP mutants revealed a perinatally lethal phenotype in simultaneously depleted APP/APLP2 and APLP1/APLP2 double-knockout mice assigning them essential functional roles in mouse development, whereas APP/APLP1 double-knockouts and single knockouts for each of the three proteins exhibited milder phenotypes. These data might be suggestive of compensatory mechanisms of the three proteins with partially redundant physiological roles thus prompting the question of the specific endogenous physiological role of each of the three related proteins. We determined the expression of APP, APLP1 and APLP2 proteins in the adult mouse brain by Western blot and immunohistochemical analysis. We observed the expression of all three APP family members including their corresponding splice variants in homogenates of the hippocampus, the cortex, the olfactory bulb and the cerebellum by Western blotting. Moreover applying confocal laser scanning microscopy we evaluated the localization and the subcellular distribution of all three amyloid precursor proteins. Immunohistochemical stainings revealed high expression levels for all three proteins in the mitral cells of the olfactory bulb, the purkinje cells of the cerebellum, and in cell populations of the medulla oblongata and the Ammon's horn. In a first series of experiments none of the proteins could be colocalized with macroglial cell type markers (oligodendrocytes and astrocytes). Immunohistochemical data indicate that all APP family members are contained in membranes of intracellular organelles with a frequently perinuclear localization. In order to provide additional information on the physiological role of amyloid precursor proteins we show here a detailed immunohistochemical analysis of the cellular localization and the subcellular distribution of APP, APLP1 and APLP2 alone and in combination with each other in central nervous system of the adult mouse.

# Longitudinal study of the effect of traumatic brain injury on lateral ventricle and hippocampal volumes using fully automated MRI volumetry

Veronika Brezova[1], Alexander Olsen[1], Toril Skandsen[2], Anne Vik[2], Asta Håberg[2]

[1]NTNU, Department of Medical Imaging and Circulation, Norway

[2]Department of Neuroscience, NTNU, Trondheim, Norway

Aim: Atrophy of both grey and white matter is well documented following traumatic brain injury (TBI) in the clinic and in experimental models. The pathological processes leading to cell death are initiated at the moment of TBI, and subsequently perpetuated by secondary injury mechanisms. The period over which these pathological changes take place is extended. Our aim was to monitor the time -course of the structural changes in lateral ventricle and hippocampal volumes from the acute to chronic phase following moderate to severe TBI.

Methods: 109 patients (15-66 years, 27 females) with acute GCS<16 were scanned on a 1.5 T Siemens MRI scanner with the T1W ADNI volume at 4 different time points: acute (0-28 days after the injury), subacute (3 months after the first scan) and chronic (12 months and 2-3 years after the first scan). Fully automated segmentation was performed using NeuroQuant (CoreTechs Labs, SD, CA, US).

Results: Normalized structure volumes (% of intracranial volume) were used for statistical analysis using the mixed linear model. The relative lateral ventricle volumes (rLVV) in acute phase (1.480±0.104) and subacute (1.877±0.107) phase were significantly lower than in the chronic phases (2.047±0.109 and 2.102±0.112) ($p<0.05$). The relative hippocampal volume (rHV) was significantly increased 2-3 years after injury (0.484±0.06) compared to acute (0.466±0.005), subacute (0.460±0.006) phase and 12 months (0.460±0.006) ($p<0.05$). No significant difference was found between females and males in neither rLVV (1.686±0.197 and 1.843±0.115) nor rHV (0.474±0.09 and 0.464±0.05).

Conclusions: The time course of structural change was different between the normalized lateral ventricle and hippocampal volumes of TBI patients. Relative enlargement of lateral ventricles appeared already during the first months after injury, while the relative change in hippocampal volumes first became evident more than one year after TBI. No significant differences between normalized volumes in females and males were found in our study, which allows for performing gender-independent analysis of volumetric data from TBI patients.

# Mismatch in network dynamics in a model of temporal lobe epilepsy

Ulrich Paul Froriep[1], Arvind Kumar[1,3], Delphine Cosandier-Rimélé[1], Ute Häussler[4], Carola A. Haas[1,4], Ulrich Egert[1,2]

[1] Bernstein Center Freiburg
[2] Dept. of Microsystems Engineering - IMTEK, Faculty of Applied Sciences
[3] Neurobiology and Biophysics, Faculty of Biology
[4] Experimental Epilepsy Group, Neurocenter, Albert-Ludwigs-University, Freiburg, Germany

Mesial temporal lobe epilepsy (MTLE), the most common form of focal epilepsies in adults, is often accompanied by histological changes within the hippocampal formation, summarized as hippocampal sclerosis (HS). In many cases, surgical removal of the sclerotic parts does not result in a seizure-free outcome. In the intrahippocampal kainate mouse model of MTLE, recurrent epileptiform activity (EA) and HS are observed after focal injection of kainic acid (KA) into the dentate gyrus (DG) [1]. In this animal model, the sclerotic parts close to the injection site have been found to be unable to generate or sustain EA [2]. It has therefore been suggested that parahippocampal structures could also be involved in EA generation.
As a major source of direct input to the DG, the superficial layers of the entorhinal cortex (EC) could be a key candidate. However, so far, most studies on the role of the EC in animal models of MTLE have been performed after a systemic, not focal, application of pharmacological agents and therefore, a specific role of the EC in the generation of EA could not be isolated.
Thus, we performed simultaneous in vivo local field potential (LFP) recordings at the injected DG and the EC to investigate the relation between these two structures in the focal kainate model. We found a phase shift in EA-free baseline activity between the DG and the EC in kainate mice which was never observed in controls. This suggests that the mutual coupling between the EC and the DG in ongoing activity between EA periods is impaired. To investigate the neural mechanisms underlying this observed delayed synchrony we used a computational model of the entorhinal-hippocampal network. Consistent with cell loss throughout the septal hippocampus, our simulation results suggest that asymmetric coupling between the DG and the EC may underlie the observed temporal shift in the entorhinal-hippocampal loop.

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(DFG, SFB TR3 and SFB780).

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# Modifying alpha-synuclein dimerization in living cells

Susana Alexandra Gonçalves[1], Tiago Fleming Outeiro[1, 2]

Instituto de Medicina Molecular, Cell and Molecular Neuroscience Unit,Lisbon, Portugal

Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisbon, Portugal

A common hallmark in several neurodegenerative disorders (ND) is the accumulation of misfolded proteins. These altered proteins enable aberrant protein-protein interactions, the formation of aggregates, and the disruption of several essential cellular functions.
The misfolding and aggregation of alpha-synuclein (a-syn) is the pathological hallmark of Parkinson's disease (PD), the second most common age related ND. Recently, it has been postulated that oligomeric species of a-syn represent the toxic genus, rather than the complex aggregated forms of the protein.
We have been investigating the molecular mechanisms underlying the initial steps involved in a-syn oligomerization through a novel system we established to monitor a-syn dimerization/oligomerization in living cells, using bimolecular fluorescence complementation (BiFC). Using this approach as a readout for a-syn dimerization/oligomerization, we started to identify and characterize genetic modifiers of a-syn oligomeric precursors, through an unbiased genome-wide lentiviral RNAi screen.
We identified 17 genetic modifiers of a -syn oligomerization, including genes involved in intracellular transport/trafficking, and in signal transduction pathways.
The identification of genetic modifiers of a -syn oligomerization, possible early events in the protein aggregation cascade, will represent a significant advance for the development of novel therapeutic strategies for PD and related diseases.

# Nervous system and general toxic effects in rats after subacute intratracheal application of nanosized lead oxide

Gábor Oszlánczi[1], Edina Horváth[1], Andrea Szabó[1], András Papp[1], Péter Pusztai[2], Mária Szabó[2], Gábor Kozma[2], András Sápi[2], Zoltán Kónya[2], Tünde Vezér[1]

[1]University of Szeged Faculty of Medicine, Department of Public Health, Dóm tér 10., H-6720 Szeged, Hungary
[2]University of Szeged Faculty of Science and Informatics, Department of Applied Chemistry, Rerrich Béla tér 1., H-6720, Szeged, Hungary

Lead (Pb), a harmful heavy metal, used to be applied as anti-knock additive in petrol. Nowadays the main sources of lead exposure are the emissions of Pb processing and reprocessing industries, as well as solders and paints. In the processes of Pb industry, airborne particles are emitted exposing the workers by inhalation. The nervous system is a main target of lead, the consequences being occupational neuropathy and CNS disorders. Delayed mental development of children due to lead exposure is well known. Inhalational exposure may result in massive internal doses whereby the size of particles entering the airways is crucial. In this study, submicroscopic (mean diameter ca. 20 nm) PbO particles were suspended in distilled water and were instilled into the trachea of male Wistar rats (2 and 4 mg/kg b.w.), 5 times a week for 6 weeks. To check general toxicity, the animals' body weight was checked weekly, and other symptoms of toxicity were also observed and noted. At the end of treatment, the rats were prepared for electrophysiological recording in urethane anesthesia. Spontaneous (electrocorticogram, ECoG) cortical activity and sensory evoked potentials (EPs) was recorded from the primary somatosensory, visual and auditory areas. The rats were finally overdosed with urethane and dissected. Organs were weighed, and Pb levels and oxidative stress parameters (superoxide dismutase, SOD; and reduced glutathione, GSH) were measured from samples of brain, lungs, liver and blood.

The treated rats' body weight gain was significantly lower than in the controls from the 3rd week on. Among the organ weights, there was significant increase in the lungs, mild increase in the kidneys and some decrease in the brains (high dose vs. control). The ECoG was moderately shifted to higher frequencies in the treated rats. The cortical EPs in the treated rats had mostly increased latency, sometimes also increased duration. In the somatosensory EP there was also an extra latency lengthening on increasing stimulation frequency (at 2 and 10 Hz vs. 1 Hz). SOD activity was higher in the treated rats' brains. The level of GSH was decreased in the brain but increased in the liver of the treated rats. The Pb level was dose-dependently increased in the treated rats' organs, and there was fair correlation between the brain or blood Pb levels and body weigh gain, frequency distribution of the electrocorticogram, and latency of the somatosensory evoked potential.

The results showed that Pb in nanosuspension form had access to the brain and caused several functional alterations. The CNS and other effects of inhalation of lead nanoparticles in humans can thus be modelled in rats this way.

# Neurological and molecular biological characterization of the mutant mouse line Tom40, the protein that comprises the general import pore of mitochondria

Ramona Maria Zeh[1], Lore Becker[1,2], Andreas Bender[2], Thomas Floss[3], Anja Schrewe[1,4], Julia Calzada-Wack[6], Frauke Neff[6], Helmut Fuchs[1], Valérie Gailus-Durner[1], Raffi Bekeredjian[4], Thomas Meitinger[5], Wolfgang Wurst[5], Martin Hrabé de Angelis[1], Holger Prokisch[3], Thomas Klopstock[2]

[1]Institute of experimental genetics/German Mouse Clinic, Helmholtz-Zentrum München, Ingolstädter Landstr.1, German Research Center for Environment and Health, 85764 Neuherberg, Germany

[2]Department of Neurology, Friedrich-Baur-Institute, Ludwig-Maximilians-Universität, Ziemssenstr.1a, 80336 Munich, Germany

[3]Institute of Human Genetics, Helmholtz Zentrum München, German Research Center for Environment and Health, 85764 Neuherberg, Germany

[4]Department of Medicine III, Otto-Meyerhof-Zentrum, University of Heidelberg, Heidelberg, Germany

[5]Institute of Developmental Genetics, Helmholtz Zentrum München, German Research Center for Environment and Health, 85764 Neuherberg, Germany

[6]Institute of Pathology, Helmholtz Zentrum München, German Research Center for Environment and Health, 85764 Neuherberg, Germany

The vast majority of mitochondrial proteins is encoded by nuclear genes and then imported into the organelle. The TOM (translocase of outer mitochondrial membrane) complex mediates the import of all proteins of mitochondria into the intermembrane space and additional the insertion of proteins into the outer membrane. TOM40 comprises the main component of the TOM complex as it forms the general import pore. Recent reports showed that in human AD brains amyloid precursor protein (APP) accumulates in the Tom40 import channel and thereby inhibiting the entry of various nuclear -encoded proteins. Moreover, a variant in the *Tom40* gene has been associated with age of onset in AD. We generated the *Tom40* mutant mouse line by genetrap mutagenesis using a non-retroviral pT1ßgeo vector. Homozygous *Tom40*-/- mice are not viable, embryos die before E9,5. Heterozygous *Tom40* +/ - mice showed no differences in basic neurological functions like locomotor activity, muscle force and motor coordination. Real -time quantitative PCR revealed a 50%-reduction of *Tom40*mRNA in heart tissue. In the electrocardiogram (ECG) measurement under anesthesia we detected a decreased P-wave duration and prolonged Q-T and S-T intervals in the mutants indicating conduction impairments. Electron microscopic images of heart tissue illustrate several alterations concerning structure and arrangement of mutant mitochondria. Despite these alterations, mitochondrial function as tested by oxygen consumption was normal in young and only compromised in old mutant mice. However, enzyme activities measured in the respiratory chain seemed to be normal in all tested animals.

Our next steps will include more detailed investigation of the respiratory chain complexes and mitochondrial proteome alterations, using for example blue native PAGE and DIGE. Furthermore, we want to perform different challenges in order to stress mitochondria and eventually trigger a phenotype in young animals. Since *Tom40* is strongly associated with Late-Onset Alzheimer's disease, follow-up studies with aged mice will be necessary.

# Neurons associated with aggrecan-based perineuronal nets are protected against tau pathology in subcortical regions in Alzheimer's disease.

Markus Morawski[1], Gert Brückner[1], Carsten Jäger[1], Gudrun Seeger[1], Thomas Arendt[1]

[1]Paul Flechsig Institute for Brain Research, Department of Molecular and Cellular Mechanisms of Neurodegeneration, Jahnallee 59, 04109 Leipzig, Germany

The biological basis for the selective vulnerability of neurons in Alzheimer's disease (AD) is elusive. Aggrecan-based perineuronal nets (PNs) of the extracellular matrix have been considered to contribute to neuroprotection in the cerebral cortex. In the present study, we investigated the organization of the aggrecan-based extracellular matrix in subcortical regions known to be predominantly affected by tau pathology in AD. Immunocytochemistry of aggrecan core protein was combined with detection of neuronal markers and hyperphosphorylated and aggregated tau indicating neurofibrillary tangles and neuropil threads. The results showed that many regions showing severe tau pathology in AD, such as the basal nucleus of Meynert, the dorsal thalamus, the raphe nuclei, and the locus coeruleus were devoid of a characteristic aggrecan-based extracellular matrix. Regions composed of nuclei with clearly different intensity of tau pathology, such as the amygdala, the thalamus and the oculomotor complex, showed largely complementary distribution patterns of neurofibrillary tangles and PNs. Quantification in the reticular interstitial nucleus of the longitudinal fascicle potentially affected by tau pathology in AD revealed that tau pathology was not accompanied by loss of aggrecan-based PNs. Fibrillary tangles in net-associated neurons extremely rarely occurred in the pontine reticular formation. Distinct tau pathology was observed in the subthalamic nucleus in neurons devoid of PNs but contacted by fibers associated with axonal coats of aggrecan-based extracellular matrix. We conclude that the low vulnerability of neurons ensheathed by PNs previously described for cortical areas in AD also exists in subcortical regions. The aggrecan-based extracellular matrix of PNs may be involved in neuroprotection.

# Neuroprotective effects of hematopoietic stem cells in the G93A animal model of ALS

Sarah Knippenberg[1], Nadine Thau[1], Ralf Hass[2], Reinhard Dengler[1], Susanne Petri[1]

[1]Medical School Hannover, Department of Neurology, Carl-Neuberg Str. 1, 30615 Hannover, Germany
[2]Medical School Hannover, Department of Obstetrics and Gynecology, Carl-Neuberg Str. 1, 30615 Hannover, Germany

Amyotrophic Lateral Sclerosis (ALS) is a devastating adult-onset motor neuron disorder with marginal therapeutic options. The disease is characterized by progressive degeneration of motor neurons in spinal cord and motor cortex.
Adult stem cells such as human umbilical cord blood cells (hUCBCs) have recently come into focus as promising therapeutic approach in neurodegenerative diseases. While a direct replacement of degenerating neurons by stem cells does currently not appear feasible in motor neuron disorders, they could possibly protect motor neurons by release of neurotrophic factors. HUCBCs delayed symptoms in a SOD1 mouse model of ALS after intravenous administration. Because of the blood-brain-barrier, the establishment of local administration of the cells appears to be beneficial.
Therefore we administered hUCBCs intraspinally in a G93A transgenic mouse model of ALS at presymptomatic (day 40) and symptomatic (day 90) disease stages.
HUCBCs were isolated via Ficoll density gradient centrifugation and CD34 or CD133 associated MACS beads. The stem cell enriched population then was expanded in presence of stem cell growth factors for 7-14 days. 100.000cells/µl in a volume of 1µl per side were administrated bilaterally into the ventral horn of the lumbar spinal cord.
The effects of the transplantation were assessed by behavioural and survival analyses as well as by histological examinations.
Treatment at a presymptomatic stage led to significant improvements in behavioural tests and to a significant increase in survival as well as to reduced motor neuron loss and astrogliosis in spinal cord. Thereby females seemed to be respond better to treatment than males.
This study confirms the neuroprotective potential of human umbilical cord blood cells. Further studies will investigate whether pre -differentiation of the cells before transplantation, repeated injections and/ or multiple injection sites can increase the observed effects.

# Neurotoxicity of nanosized manganese by subchronic exposure

Andrea Szabó[1], Szabolcs Takacs[1], Zsuzsanna Máté[1], Edina Horváth[1], Gábor Oszlánczi[1], András Papp[1]

[1]University of Szeged, Department of Public Health, Dóm tér 10., 6720 Szeged, Hungary

Manganese (Mn) is an essential trace element, but overexposure (mainly of occupational origin by inhalation) leads to impairment in energy metabolism, finally manifested in neurological disorders. The health risk of airborne Mn-containing particles is well-known, however dissimilar sized particles can cause different alterations, therefore nano-Mn may has special effects, though literature data are still incomplete.

To model airborne exposure, adult male Wistar rats were intratracheally instilled with a suspension of Mn-oxide nanoparticles for 9 weeks in daily doses of 2.63 and 5.26 mg Mn/b.w. Effects of exposure were studied by electrophysiological (spontaneous and evoked cortical and peripheral evoked potentials), open field activity and analytical measurements (detection of Mn level, MnSOD and GSH from different organs); besides general toxicity (body and organ weights) were also investigated.

Weekly body weighing revealed that control rats had normal weight gain but the treated rats' body weight failed to increase from the 6th week on. Analysis of organ weight data resulted in dose- and time-dependently increased relative lung weights. Mn level was significantly elevated in brain and blood samples. The treated rats' open field behaviour showed decreased ambulation and rearing, and increased local activity and immobility. Electrophysiological dataset indicated a shift of the spontaneous cortical activity to higher frequencies, lengthened cortical evoked potential latency, and slowed nerve conduction. The deleterious effect of Mn on antioxidant system was also clearly detected by changes of enzyme parameters.

These results indicate that the instilled nanosized Mn had reached the brain from the airways, and the resulting damage could be investigated by the applied exposure model by using neuro-functional, biochemical and general toxicological endpoints. Majority of these general and neuro-functional parameters were more significantly correlated to the Mn levels of the brain than to the Mn levels of the blood. Moreover, this means that certain functional parameters can be useful indicators of nervous system effects of Mn exposure.

# Nigral injection of AAV-mediated overexpression of alpha-synuclein: A Parkinson-like model in the marmoset monkey

Yi-Fang Cui[1], Enrique Garea-Rodriguez[1], Sebastian Kuegler[2], Christina Schlumbohm[1], Eberhard Fuchs[1]

[1]Deutsches Primatenzentrum GmbH, Clinical Neurobiology Laboratory, Kellnerweg 4, 37077 Goettingen, Germany

[2]Center of Molecular Physiology of the Brain (CMPB), Department of Neurology, University Medicine Goettingen, Goettingen, Germany

Parkinson's disease (PD) is a slowly progressive, degenerative illness, with a long term pre-symptomatic period and severe motor dysfunction in the later stage resulting from the loss of dopaminergic neurons in the substantia nigra. The current medications can only ameliorate the symptoms but not cure. Non-human primates have been used to model PD. In most preclinical studies in non-human primates the species-of-choice is the macaque monkey. However, there is an increasing trend to investigate the common marmoset monkey (*Callithrix jacchus*) in biomedical research programs because this species has clear advantages over the macaque in terms of animal welfare and practicality. The marmoset models of PD that are currently available (MPTP; unilateral 6-OHDA lesion) have limitations. First, the application of neurotoxins results in a rapid onset of neurological symptoms and an acute neurodegeneration. Second, the affected dopaminergic neurons do not develop a progressive a-synucleinopathy. To overcome these limitations, we establish in marmoset monkeys a model of idiopathic PD using adeno-associated virus (AAV) mediated a-synuclein overexpression. AAV, a stable and efficient viral vector, is used to carry the a-synuclein gene and transfect dopaminergic neurons after injection into the substantia nigra. The existing atlases for the marmoset brain are - at least at the level of the substantia nigra - not precise. In a pilot study we therefore determined reliable and reproducible coordinates to inject AAVs into the substantia nigra. Gene transfer to the marmoset substantia nigra and caudate/putamen by AAV vectors has been achieved. The selective overexpression of a-synuclein in midbrain dopaminergic neurons should result in a chronic and progressing disease, a long pre-symptomatic period and should allow to study the etiological role of a-synuclein. In our model, we will investigate the development of the disease (presumably degeneration of dopaminergic terminals in the striatum) using behavioral analysis and single photon emission computed tomography (SPECT) with the dopamine transporter ligand iodine-123 FP-CIT to monitor in vivo the degeneration of dopaminergic terminals.

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# Non-local impairment and therapeutic restoration of visual plasticity mechanisms after a localized cortical stroke

Franziska Greifzu[1], Silvio Schmidt[2], Karl-Friedrich Schmidt[1], Otto W. Witte[2], Siegrid Löwel[1,3]

[1]Friedrich-Schiller-Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstr. 1, 07743 Jena, Germany

[2]Hans-Berger Klinik für Neurologie, Universitätsklinikum, Erlanger Allee 101, D-07747 Jena, Germany

[3]present address: Bernstein Focus Neurotechnologie (BFNT) and Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Georg-August-Universität Göttingen, Berliner Str. 28, D-37073 Göttingen, Germany

We tested the influence of a photothrombotic (PT) lesion in the somatosensory cortex on plasticity in the adult mouse visual system and the efficacy of anti-inflammatory treatment to rescue compromised learning. To challenge plasticity mechanisms, we induced monocular deprivation (MD), a well established model for experience-dependent plasticity in the visual system, in 3-month-old mice. Sensory learning was analyzed behaviourally with a virtual-reality optomotor system measuring both visual acuity and contrast sensitivity. In addition, visual cortical maps were recorded using intrinsic signal optical imaging. In control animals, 7 days of MD induced an increase of visual acuity and contrast sensitivity of the open eye and an ocular dominance (OD) shift towards this eye. In contrast, after PT, there was neither an enhancement of visual acuity or contrast sensitivity nor an OD -shift. OD -plasticity was however present in the hemisphere contralateral to the lesion indicating that the vanished plasticity was not caused by global mechanisms affecting the entire brain. Since stroke is associated with inflammation, we next tested the therapeutic effect of the anti-inflammatory drug ibuprofen: Daily intraperitoneal injections of ibuprofen restored sensory learning but not OD -plasticity in the lesioned hemisphere. In agreement with this, a delay of two weeks between photothrombosis and MD also restored the enhancement of both visual acuity and contrast sensitivity, but not OD-plasticity. Thus inflammation was responsible for reductions in sensory learning but lesion-induced impairment of OD-plasticity was mediated by a different cellular mechanism, supporting the view that distinct mechanisms underlie sensory learning and the classical OD -plasticity. We conclude that i) both sensory learning and cortical plasticity are compromised in the surround of a cortical lesion, ii) transient inflammation is responsible for impaired sensory learning suggesting anti-inflammatory treatment as a useful adjuvant therapy to support rehabilitation following stroke and iii) OD-plasticity cannot be conceptualized solely as a local process since non- local influences are more important than previously assumed. Supported by the BMBF.

# Overexpression of glutaminyl, the enzyme responsible for pyroglutamate Abeta formation, cyclase induces behavioral deficits and glutaminyl cyclase knock-out rescues the behavioral phenotype in 5XFAD mice

Sadim Jawhar[1], Oliver Wirths[1], Stephan Schilling[2], Sigrid Graubner[3], Hans-Ulrich Demuth[2], Thomas A. Bayer[1]

[1]Department of Molecular Psychiatry, University Medicine Goettingen, Goettingen, Germany
[2]Probiodrug, Halle, , Germany
[3]Ingenium, Munich, Germany

Pyroglutamate-modified Abeta (AßpE3-42) peptides are gaining considerable attention as potential key players in the pathology of Alzheimer's disease (AD) due to their abundance in AD brain, high aggregation propensity, stability and cellular toxicity. Overexpressing AßpE3-42 induced a severe neuron loss and neurological phenotype in TBA2 mice. In vitro and in vivo experiments have recently proven that the enzyme glutaminyl cyclase (QC) catalyzes the formation of AßpE3-42. The aim of the present work was to analyze the role of QC in an AD mouse model with abundant AßpE3-42 formation. 5XFAD mice were crossed to transgenic mice expressing human QC (hQC) under the control of the Thy1 promoter. 5XFAD/hQC bigenic mice showed significant elevation in TBS, SDS and formic acid soluble AßpE3-42 peptides and aggregation in plaques. In 6-month-old 5XFAD/hQC mice, a significant motor and working memory impairment developed compared to 5XFAD. The contribution of endogenous QC was studied by generating 5XFAD/QC-KO mice (mouse QC knockout). 5XFAD/QC-KO mice showed a significant rescue of the wild-type mice behavioral phenotype demonstrating the important contribution of endogenous mouse QC and transgenic overexpressed QC. These data clearly demonstrate that QC is crucial for modulating AßpE3-42 levels in vivo and prove on a genetic base the concept, that reduction of QC activity is a promising new therapeutic approach for AD.

# Proteome analysis of a detergent insoluble fraction from spinal cords of SOD1 transgenic mice by label-free LC-MSE mass-spectrometry

Martina Liebl[1], A. Murat Kaya[1], Stefan Tenzer[2], Susanne Petri[3], Jörg Kuharev[2], Hansjörg Schild[2], Christian Behl[1], Albrecht M. Clement[1]

[1]Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg University Mainz, Germany
[2] Institute for Immunology, University Medical Center, Johannes Gutenberg University Mainz, Germany
[3] Department of Neurology, Hannover Medical School, Hannover, Germany

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disease affecting primarily motor neurons. The presence of protein aggregates is a common pathological hallmark of sporadic as well as familial forms of the disease, although it has not been resolved yet if their appearance is a determinant for toxicity. In mouse models of ALS expressing mutant variants of Cu/Zn-superoxide dismutase (SOD1) aggregates (i.e. the biochemical detection of SDS -resistant SOD1 -multimers) are detectable shortly before symptom onset and increase over time. Immunohistological studies revealed that other proteins beside SOD1 are associated with aggregates. In fact, one hypothesis of aggregate toxicity indicates that proteins entrapped in aggregates might be missing to maintain protein homeostasis and cellular integrity. Following this line we have investigated the composition of detergent insoluble fractions from SOD1-transgenic mice. We established a differential centrifugation protocol to enrich SOD1-containing aggregates. The efficiency of this procedure was monitored by the selective enrichment of mutant SOD1 versus SOD1(WT) and endogenous mouse SOD1 in the pellet fraction. Subsequent label-free quantitative proteomic analysis on a Waters nanoUPLC- ESI-QTOF instrument platform using LC-MSE acquisition method revealed that about 20 out of 450 proteins were enriched in two mutant SOD1 mouse lines compared to SOD1(WT) over-expressing mice and non-transgenic littermates in two independent experiments. Proteomic data as well as Western blot analysis confirmed the presence of ubiquitinated proteins and heat shock proteins in the aggregate enriched fraction. Data have been confirmed in preparations from sporadic ALS patients.

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# Rat models of Huntington's disease – what can we learn about the neurodegenerative process and its impact on the adult SEZ niche?

Yvona Mazurova[1], Ivana Guncova[1], Ivan Latr[2], David Astapenko[1]

[1]Fac. of Medicine in Hradec Kralove, Charles Univ. in Prague, Dept. of Histology and Embryology, Simkova 870, P.O. Box 38, 500 38 Hradec Kralove, Czech Republic

[2]University Hospital, Neurosurgery Clinic, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Although Huntington's disease (HD) occurs only in humans, the use of animal models is crucial for HD research. This study is aimed to the histopathological alterations (detected particularly by immunofluorescent methods) in brains of rats with neurotoxic (quolinic acid – QA) lesion, surviving 7 days, 1, 6 and 12 months after the lesion, and 6- , 12- , 18- and 24- month-old rats transgenic for HD (Dept. of Medical Genetics, Univ. of Tübingen, Germany).

A hallmark of HD in a human brain is successive neurodegenerative process (NDP) within the striatum. The development and character of NDP in both compared rat models of HD substantially differ. In rats with the QA lesion, the genuine neurodegeneration and typical concomitant reactive gliosis develop very quickly (over 1 week), then the process turns (at about 1 month after the lesion) and its extent and severity slowly decrease with survival time of animals. On the contrary, a very slow development of atypical NDP, in which the first indication of striatal atrophy occurs at 6 months of age and typical reactive astrocytes appear only in 12-month-old rats, is significant for tgHD rats. On the other hand, genetically induced mutation of huntingtin (mhtt), roughly comparable with human HD brains, occurs exclusively in transgenic animals.

Furthermore, the most affected region, the caudate nucleus, is adjacent to the subependymal zone (SEZ) neurogenic region. The ongoing process of neurogenesis in the adult mammalian SEZ indicates the possible capacity for limited self-repair not only in healthy but also in damaged brain. Therefore, we were interested in patterns in which the endogenous neural stem/progenitor cells react to the development of striatal NDP. This process represents completely specific irritation of the adult SEZ niche. Our data also strongly indicate that the intensity and character of the neurogenic niche reaction correspond to the extent and severity of the brain injury, which determines the patterns of the SEZ response. Accordingly, significant neurodegeneration within the striatum of rats in the acute stage of the QA lesion promotes the significant proliferation and differentiation of cells within the SEZ niche. On the contrary, a reaction of the SEZ to discreet slowly developing neuronal degeneration in tgHD rats is only inconspicuous. However, slight but continual increase in proliferation of cells was detected in 12- and 24-month-old tgHD rats in comparison with decreased proliferative activity in the SEZ of their age-matched counterparts.

Morphological alterations in cells of astrocytic phenotype (in both striatum and SEZ neurogenic region) in acute stage of the QA lesion and during the progression of HD known from autopsies show significant features which allow drawing of important parallels. These similar patterns, however, were not found in relation to tgHD rats. On the other hand, the presence of mhtt seems to play an important role in the initiation and progression of NDP of HD phenotype. Our observations also indicate that the shortcomings of animal models can be diminished using a combination of outcomes gained from models of different backgrounds, e.g. the neurotoxin-induced rat model of HD and tgHD rats in this case.

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# Read-through of a nonsense mutation as a treatment option for Usher type 1C

Tobias Goldmann[1], Nora Overlack[1], Fabian Möller[1], Igor Nudelman[2], Timor Baasov[2], Uwe Wolfrum[1], Kerstin Nagel-Wolfrum[1]

[1]JoGu-Mainz, Institute of Zoology, Cell & Matrix Biology, Mainz, Germany

[2]Edith and Joseph Enzyme Inhibitors Laboratory, Schulich Faculty of Chemistry, Technion-Israel Institue of Technology, Haifa, Israel

The Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. It is clinically and genetically heterogeneous, assigned to three clinical USH types of which the most severe type is USH1. The USH1C gene encodes the PDZ containing scaffold protein harmonin which is expressed in form of numerous alternatively spliced variants. Hamonin binds directly to all USH1 und USH2 proteins and is a key organizer in the USH protein network. So far no effective treatment for the ophthalmic component of USH exists. Aminoglycosides are known to facilitate read-through of nonsense mutations, but their clinical use is limited due to their toxicity. Synthetic redesign of a clinical approved aminoglycoside to reduce toxic side-effects resulted NB30 and NB54. In addition, PTC124 is a new promising compound for translational read-through of nonsense mutations leading to a premature termination stop. It is currently gauged in clinical phase II for nonsense mutations in non-ocular diseases.

Here we investigated the potential of PTC124, NB30 and NB54 as a treatment option for patients carrying a nonsense mutation in the USH1C gene (p.R31X) causing the USH1 disease. We demonstrated translational read-through of the p.R31X mutation in USH1C not only in cell culture and in retinal explants, but also in mice in vivo. Our assays concerning the molecular function of the restored protein showed that the recovered harmonin expression restored harmonin´s scaffolding function and F-actin bundling activity in the cell. Furthermore, we compared the biocompatibility of PTC124, NB30 and NB54 with the clinically approved read-through inducing aminoglycoside gentamicin. In this evaluation, PTC124, NB30 and NB54 showed a much better biocompatibility in murine and human retinal explants.

High retinal compatibility of PTC124, NB30 and NB54 combined with their transcriptional read-through efficacy emphasize the high potential of these molecules as therapeutic agents for the p.R31X nonsense mutation in USH1 as well as in other retinal genetic conditions.

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# Reduced olfactory bulb volumes in patients with Parkinson Disease

Jochen Klucken[1], Johannes Kahlis[2], Nina Mallog[2], Beate Winner[3], Mark Greenlee[4], Gerhard Schuierer[3], Juergen Winkler[1]

[1]Division of Molecular Neurology, University Hospital Erlangen, Erlangen, Germany [2]Institute of Neuroradiology, University of Regensburg, Germany [3]Nikolaus-Fiebiger-Center for Molecular Medicine, University Hospital Erlangen, Germany [4]Institute for Experimental Psychology, University of Regensburg, Germany

Olfactory deficit is an important premotor symptom in Parkinson's disease (PD). Neuropathological findings report early degenerative changes in the olfactory bulb (OB) in PD. Thus, impaired olfaction and associated neuronal structures might serve as a biomarker to identify subjects with an increased risk to develop PD. In order to assess the structure of the OB we tested the hypothesis whether OB volume is reduced in PD and correlates with smell deficiency.

Using structural magnetic resonance tomography we quantified the OB volume in 20 PD patients and 20 controls. We observed a gender related difference in OB volumes with smaller OBs in females compared to males irrespectively of the disease status. Furthermore, a significant reduction of the mean volume of both OBs could be observed both in male and female PD patients. In addition, a significant correlation to L-Dopa equivalent dose was observed. No correlation could be found to the Hoehn&Yahr stage, UPDRS motor score or disease duration. As expected reduced olfaction was present in PD patients, however with a weak correlation to the OB volumes.

These data suggest that reduced OB volumes in PD patients might correspond to the disease progression reflected by L -Dopa equivalent doses, but not necessarily correlate with global measures of motor symptoms such as Hoehn&Yahr stages or UPDRS motor score. Our finding suggest that measurements of OB volumes may be an additional biomarker in the diagnosis of premotor stages in PD and might be easily implemented in routine acquisition of MR imaging procedure in early PD.

# Regulation of the processing of Amyloid Precursor Protein (APP) by GGA transport proteins

Anke Hellrung[1], Björn von Einem[1], Daniel Schwanzar[1], Christine von Arnim[1]

[1]Ulm University, Experimental Neurology, Helmholtzstraße 8/1, 89081 Ulm, Germany

Alzheimer's disease (AD) is characterized by the extracellular senile plaques, which main component is a set of insoluble amyloid-beta (Aß) peptides. Aß is generated by sequential processing of the amyloid precursor protein (APP) by ß-secretase BACE1 and the γ-secretase enzyme complex. The formation of Aß requires complex sorting mechanisms through the cell that cause co-localization of APP and the secretases in the same subcellular compartment, especially to the acidic compartments of the secretory pathway. A few years ago, several laboratories identified the Golgi-localized ã-ear containing Arf-binding (GGA) proteins as new monomeric clathrin adaptor proteins involved in specific membrane trafficking events at the trans-Golgi network. The GGAs were already shown to interact with BACE1 via their VHS domain facilitating its intracellular transport to endosomes. Besides, a potential modulation of the APP processing by the GGAs was previously hypnotized. Therefore, the role of the GGA proteins on the trafficking and the processing of APP was investigated. HEK293 cells were transfected with GGA1/2/3-myc constructs or GGA1/2/3 siRNAs and the levels of the sAPP's and Aâ were analyzed by Western Blot and ELISA. Furthermore, the cellular localization the GGAs was investigated and analyzed by fluorescence microscopy. Additionally cell organelle marker stainings were performed to identify the subcellular compartments where the localization occurred. Our data indicate that all three GGA proteins affect the APP processing in a similar manner. While overexpression of the GGAs lead to significant reduction of the amyloidogenic processing, knock down facilitate the amyloidogenic processing. However, simultaneous expression of more than one GGA protein or siRNA showed no additive effect, suggesting that the GGA proteins operate independently within the cell. The localization studies revealed that all three GGA proteins mainly occur at the Golgi apparatus, but they are different distributed within the other cell organelles. The different distribution within the cell confirmed the proposal that the single members of the protein family act independently of each other within the cell and fulfil different function in traffic processes.

# RET mediates the neuroprotective and neuroregenerative effects of GDNF in the MPTP model of Parkinson's disease

V.V Durga Praveen Meka[1], Karsten Sollich[1], Anja Drinkut [2], Jorg Schulz [2,3,5], Sebastian Kugler [2,4], Edgar R. Kramer [1]

[1]Centre for Molecular Neurobiology Hamburg (ZMNH), Development and maintenance of the nervous system, Falkenried 94, 20251 Hamburg Hamburg , Germany

[2]DFG Research Center Molecular Physiology of the Brain (CMPB) at University Medicine Gottingen, Germany

[3]Department of Neurodegeneration and Restorative Research, Waldweg 33, 37073 Gottingen, Germany

[4]Department of Neurology, University Medicine Gottingen, Waldweg 33, 37073 Gottingen, Germany

[5]present address: Department of Neurology, University Hospital, RWTH Aachen, Pauwelstrasse 30, 52074 Aachen, Germany

Glial cell-line derived neurotrophic factor (GDNF) is a potent neurotophic factor for dopaminergic neurons required for postnatal development and survival, regeneration and placticity. GDNF holds great promise as a therapeutic molecule to treat diseases affecting the dopaminergic system, such as Parkinson's disease. The signaling receptors mediating the benefical effects of GDNF in dopaminergic neuron are however not yet defined. GDNF binds with high affinity to the GPI-linked GDNF family receptor a1. This receptor-ligand complex can activate the canonical GDNF receptor RET or alternative receptors such as the neuronal cell adhesion molecule or integrins. To address the question, if the receptor tyrosine kinase RET is essential for mediating the neuroprotective and regenerative effect of GDNF in dopaminergic neurons in vivo, we used a viral vector to overexpress GDNF in the striatum of mice deficient for RET in dopaminergic neurons and subsequent challenged these mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). In this MPTP induced Parkinson's disease mouse model, exogenous GDNF could only in the presence of RET protect dopaminergic neurons in the substantia nigra pars compacta, the dopaminergic innervation of the striatum, and the levels of dopamine. In addition, exogenous GDNF required the RET receptor for mediated regeneration of dopaminergic fibers and terminals in mice investigated 90 days after MPTP treatment. We therefore conclude that so far all GDNF mediated beneficial effects on the dopaminergic system, such as adult maintenance, protection and regeneration, absolutely depend on the presence of the RET receptor.

# Corticosteroid modulation of status epilepticus: the role of GRs and MRs

Nicola Maggio[1], Menahem Segal[1]

[1]The Weizmann Institute of Science, Neurobiology, Herzl St., 76100 Rehovot, Israel

[2]Talpiot Medical Leadership Program, Department of Neurology and Joseph Sagol Neuroscience Center, The Chaim Sheba Medical Center.

Stress is among the most frequently self-reported precipitant of seizures in patients with epilepsy. To date, it has not yet been clarified how stress and specifically the stress hormone, corticosterone, (cortisol in humans) modulates seizures.
Two types of receptors, the glucocorticoid (GRs) and mineralocorticoid (MRs), are in charge of the corticosterone effects in the body. Both GRs and MRs can be expressed either at the cell nucleus or at the membrane and therefore play a different roles in cellular functions.
We have studied the effects of stress and the targeted activation of GRs and MRs in the modulation of seizures in vivo by using an animal model of status epilepticus.
Briefly, kainic acid (10 mg/kg) was. injected i.p. in 1 month old male Wistar rats either 30s or 1hr after their exposure to a 15 min. forced swim stress (FSS). Seizures latency was shorter in the animals that received kainic acid 30s following FSS compared to control and to those treated with kainic acid 1 hr after the FSS. In addition, seizures reached a higher Racine's score in shorter time in the former group compared with controls. Interestingly, injection of diazepam halted seizures faster in the animals that received kainic acid 1hr after FSS than in those that were treated 30s following FSS.
Surprisingly, blocking MRs or GRs receptors prior to the stressful exposure showed that MRs activation by stress enhanced the effects of kainic acid injection following FSS, while GRs activation suppressed these effects.
In an effort to understand the mechanism of the differential MRs and GRs activation on seizures modulation, we performed whole cell patch-clamp recordings in acute slices of the hippocampus. We found that GRs and MRs differently regulate GABA -A currents. Respectively through membrane bound receptors, MRs reduce the frequency of IPSCs while GRs increase IPSCs amplitude.
We conclude that stress and activation of MRs and GRs modulate seizures by differential regulation of GABAergic synapses.

# Role of inhibition in unleashing and quenching oscillations in the basal ganglia

Stefano Cardanobile[1], Arvind Kumar[1], Stefan Rotter[1], Ad Aertsen[1]

[1]Bernstein Center Freiburg, Faculty of Biology, University of Freiburg

Hansastrasse 9/a 79104 Freiburg, Germany

Neural mechanisms underlying slow oscillations and increased synchrony in the basal ganglia associated with various motor dysfunctions of Parkinson's disease[1,2] are poorly understood. Using a minimal model of basal ganglia, validated by biologically realistic simulations, we show that the strength of the inhibitory inputs from the striatum to the globus-pallidus-external is the key parameter that controls the oscillatory behavior of the basal ganglia.

In fact, experimentally observed increase in the striatal activity[3,4] is sufficient to unleash the oscillations in the basal ganglia. This theoretical framework provides a possibility to understand and optimize the deep-brain-stimulation protocols. We show that broadband stimulation of the subthalamaic nucleus could be more efficient than the conventional periodic stimulation in quenching oscillations. Finally, this unified explanation of both emergence and quenching of oscillations naturally leads to novel therapeutic suggestions for electrical and chemical intervention of oscillations in the dopamine-depleted basal ganglia.

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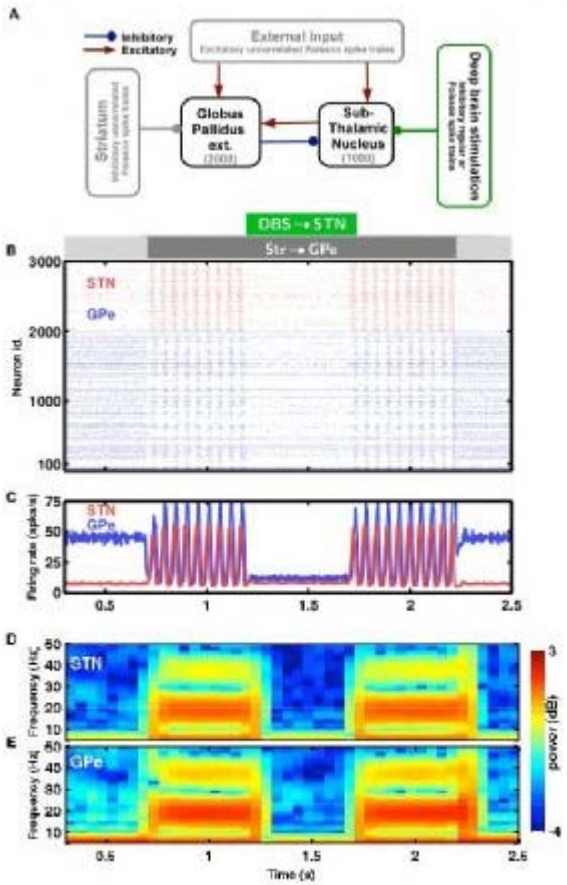
A
Inhibitory
Excitatory
External Input
Striatum
Globus Pallidus ext.
(2000)
Sub-Thalamic Nucleus
(1000)
Deep brain stimulation
B
DBS → STN
Str → GPe
STN
GPe
Neuron id.
3000
2000
1000
100
C
STN
GPe
Firing rate (spk/s)
75
50
25
0
0.5
1
1.5
2
2.5
D
STN
E
GPe
Frequency (Hz)
60
40
30
20
10
3
-4
power (dB)
Time (s)

# Screening for cone neuroprotective substance using 661W cells

Stine Mencl[1], Dragana Trifunovic[1], Francois Paquet-Durand[1]

[1]University of Tübingen, Institute for Ophthalmic Research, Cell death mechanisms, Röntgenweg11, 72076 Tübingen, Germany

Purpose:
Human vision is strongly depending on cone photoreceptors. However cone degeneration is taking place in many eye diseases like age -related-macula-degeneration (AMD), diabetic retinopathy, retinitis pigmentosa, achromatopsia, and cone -rod-dystrophies. At present no satisfactory treatment options are available for these diseases. Searching for a neuroprotective compound requires a reliable, high-throughput model system. The cone-photoreceptor-function-loss-1 (*cpfl1*) mouse is an animal model for cone degeneration. It is characterized by a mutation in the cone specific phosphodiesterase 6 (PDE6) which is consequently non-functional leading to an increase in intracellular cGMP. cGMP acts on cGMP -dependent protein kinase (PKG) which appears to be involved in cone degeneration.
The currently available animal models, such as the *cpfl1* mouse, or retinal explant cultures are only poorly suited for large scale drug screening purposes. As an alternative, we want to establish a cell culture based screening system using the cone-like 661W cell line (Al-Ubaidi et al. 1992). Advantages of a cell culture model system are increased possibilities for large scale testing that does not require use of live animals.
Method:
The cone -like nature of 661W cells was validated using different cone specific markers. The expression of these markers and also of PKG was verified with immunohistochemistry. To test for the suitability of 661W cells as a cone-degeneration model, we first established corresponding cell cultures in a 96- well culture plate format. We then emulated a situation similar to the cpfl1 mouse degeneration by pharmacological treatment with the highly specific PDE6 inhibitor zaprinast. As a second cone degeneration paradigm the 661W were treated with the selective PKG activator 8-pCPT-PET-cGMP. After different points in time, cGMP accumulation and cell viability was analyzed.
Results:
The expression of cone opsins, glycogen phosphorylase, cone PDE6 and also PKG1 and 2 was positive in 661W cells. After treatment with zaprinast for 24h, cell viability was significantly reduced and cGMP levels were altered. Similarly, cell viability was reduced 24h after PKG activation with 8-pCPT-PET-cGMP.
Conclusions:
The 661W cell line shows PKG and cone marker expression. Inhibition of cone PDE6 or activation of PKG leads to reduced viability in 661W cells, suggesting that the degenerative mechanisms in these cells are similar to the metabolic processes active in degenerating cpfl1 cone photoreceptors. This in turn implies that this cell line may be well suited for the testing of cone neuroprotective substances.

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# Seeding effect of pyroglutamate amyloid beta 3-42 promotes plaque deposition and behavioral deficits in a novel bigenic mouse model of Alzheimer's disease

Jessica Wittnam[1], Oliver Wirths[1], Thomas Bayer[1]

[1]University of Goettingen, Department of Molecular Psychiatry, Von Siebold Strasse 5, 37075 Goettingen, Germany[1]

N-terminally truncated amyloid beta (Aß) peptides form a major fraction of Aß within the brains of Alzheimer's disease (AD) patients. Recent studies have suggested that one isoform of N -terminally modified Aß, pyroglutamate Aß (AßpE3-42), may have particular relevance to the pathogenesis of AD. AßpE3-42 has a higher aggregation rate, increased resistance to proteolytic degradation and enhanced toxicity *in vitro* relative to N-terminally-intact Aß. Furthermore, it was demonstrated in the TBA2 mouse that expression of Aß3Q-42 resulted in AßpE3-42 formation and neurodegeneration.

In this study, we tested the hypothesis that AßpE3 -42 is capable of seeding plaque formation in vivo and augmenting behavioral deficits in an AD mouse model. To accomplish this, we crossed a mouse model expressing five familial AD mutations (5XFAD) to a mouse line exclusively expressing Aß3Q -42 (TBA42) in order to produce a new bigenic mouse (FAD42). Both the 5XFAD and TBA42 mice displayed age-dependent behavioral impairments which were enhanced in the FAD42 mice. In addition, the plaque pathology in the FAD42 mice was noticeably increased relative to the 5XFAD animals. Taken together, these data suggest that AßpE3-42 may be an important contributor to the progression of AD.

# Structural and functional interactions between wildtype and amyotrophic lateral sclerosis-causing mutant SOD1: A study with obligate SOD1 dimers

Anna Weichert[1], Anna Besemer[1], Nadja Hellmann[2], Elmar Jaenicke[2], Heidrun Witan[1], Heinz Decker[2], Christian Behl[1], Albrecht M. Clement[1]

[1]Institute for Pathobiochemistry, University Medical Center, Johannes Gutenberg-University, Mainz, Germany
[2]Institute for Molecular Biophysics, Johannes Gutenberg-University, Mainz, Germany

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease leading to the progressive loss of upper and lower motoneurons. Due to this loss, patients suffer from a proceeding paralysis which eventually results in patient death caused by respiratory failure. In 90 % of all cases patients exhibit no genetic background (sporadic ALS, sALS), while in 10 % the disease is inherited (familiar ALS, fALS). More than 15 years ago it has been discovered that point mutations in the gene for Cu/Zn superoxide dismutase (SOD1) cause disease in about 20 % of fALS cases. SOD1 is an enzyme of the cellular oxidative defense and acts as a dimer consisting of two identical SOD1 monomers. Since nearly all of the SOD1 mediated fALS are inherited dominantly patients carry one mutated and one wildtype allele resulting in the expression of mutant as well as wildtype SOD1 proteins. Enzymatic active dimers might therefore consist of two wildtype, two mutant subunits or one wildtype and one mutant subunit. Double transgenic mice overexpressing mutant and wildtype SOD1 show an accelerated disease compared to mice overexpressing mutant SOD1 only, suggesting that wildtype SOD1 is involved in disease pathogenesis.

To investigate the biochemical and biophysical properties of defined SOD1 dimers we generated constructs where two SOD1 subunits are moleculary attached to each other by a polypeptide. SOD1 dimers containing a wildtype and mutant subunit are herein referred to as SOD1 heterodimers while dimer constructs containing two mutant subunits are referred to as SOD1 homodimers.

Bacterially expressed and purified SOD1 dimers displayed dismutase activity indicating that proteins are folded correctly even under non- permissive expression conditions. Secondary structure analysis by CD spectrometry revealed that the overall secondary structure of wildtype SOD1 fusion proteins was similar to those published for monomeric SOD1. To analyze the stability of SOD1 dimers, purified proteins as well as extracts of transiently transfected HEK293 cells were treated with proteinase K. In agreement with earlier studies, we could demonstrate that in HEK293 expressed SOD1 mutant proteins were more sensitive to proteinase K digest than the wildtype homodimer. Nonetheless, heterodimers were less sensitive to proteinase K treatment compared to mutant homodimers suggesting that a wildtype subunit stabilized the dimeric conformation for SOD1 heterodimers. Interestingly, purified SOD1 dimers (homo- and heterodimers) show no distinctions in sensitivity to treatment with proteinase K.

# Substantia nigra pars reticulata neurons projecting to the dorsal raphe nucleus appear to receive afferents from the subthalamic nucleus

Henrike Hartung[1], Sonny Tan[3,4], Peter J Magill[2], Yasin Temel[3,4], Tervor Sharp[1]

[1]University of Oxford, Department of Pharmacology, Mansfield Road, Oxford, OX1 3QT, UK

[2]MRC Anatatomical Neuropharmacological Unit, University of Oxford, Mansfield Road, Oxford, OX1 3TH, UK;

[3]Department of Neurosurgery, University Hospital Maastricht, 6202 AZ, Maastricht, The Netherlands;

[4]Department of Psychiatry and Neuropsychology, Division of Cellular Neuroscience, Maastricht University, 6226 ER, Maastricht, The Netherlands;

High-frequency stimulation (HFS) of the STN is an established surgical therapy for movement disability in advanced Parkinson's disease (PD). However, despite beneficial motor effects this procedure often causes debilitating psychiatric side-effects including depression. There are strong links between depression and 5-hydroxytryptamine (5-HT), and previously we reported that HFS of the STN inhibited the activity of identified 5-HT containing neurons in the dorsal raphe nucleus (DRN) *in vivo* (Hartung et al. 2010, 7th Forum or European Neuroscience, Abstract 197.53, p236). However, to date no direct anatomical connections between the STN and DRN have been shown. Here we aimed at firstly, identifying a brain region that could function as a relay for this effect i.e. that receives afferents from the STN and sends projections to the DRN. Secondly, within this region we sought to investigate whether STN afferents establish contacts with neurons projecting to the DRN.

While under deep isoflurane anaesthesia, rats received a single iontophoretic tracer injection (7 μA, 7 s on/ 5s off, 30 s-7 min) of the retrograde tracer Fluorogold (FG) into the DRN and either uni- or bilateral discrete injections of the anterograde tracer Biotinylated dextranamine (BDA) into the STN. After a survivial period of 7-10 days, animals were perfused and the brains fixed and cut into 30-40 μm coronal brain sections. Sections were processed for FG immunocytochemistry and the tracer revealed with slate grey. BDA was visualized by the avidin–biotin–peroxidase complex and 3,3-diaminobenzidine tetrahydrochloride (DAB) labelling.

FG labelled cell bodies were found in brain regions previously reported to project to the DRN including the lateral habenula, medial prefrontal cortex, anterior hypothalamic areas and preoptic areas but not in the STN. BDA labelled fibres were found in the well-known STN target regions substantia nigra pars reticulata (SNr), globus pallidus and entopeduncular nucleus. Furthermore, a moderate number of FG labelled cell bodies was found in the dorsal SNr, SN pars compacta and medial SN. Within the SNr, BDA labelled fibres were found in close apposition to FG labelled cell bodies and dendrites. We are currently investigating by electron microscopy whether these appositions also establish synaptic contacts.

These data suggest that the SNr provides an anatomical link between the STN and DRN and gives insights into a potential mechanism of the inhibitory effect of STN HFS on DRN 5-HT neuronal activity.

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# Synaptic and neurophysiological deficits in a fly model of Parkinson's disease (PD) with reduced locomotion.

Christopher Elliott[1], Amanda Vincent[1], Laura Briggs[1], Elizabeth Emery[1], Matt Oswald[1], C. Adam Middleton[1], Rose Tomlins[1], Sean Sweeney[1]

[1]University of York, Biology, Heslington, YO10 5DD York, United Kingdom

The PD-related gene parkin is known to affect muscle mitochondria in adult flies. Since parkin is associated with juvenile PD, we examined larvae. We find parkin mutants have overgrown neuromuscular junctions: the number of synaptic boutons increases 51% (ANOVA, $P<0.001$). This is completely rescued by global, neuronal or muscular expression of wild-type parkin (Act5c, elav, G14). Larval velocity is also less in parkin mutants, at 64% of wild-type (ANOVA, $P<0.001$). The length change during peristalsis is not affected by parkin, but the frequency of contractions and the rate of contractile movement both reduce, indicating bradykinesia. Suction electrode recordings of motoneuronal activity show reduced numbers of bursts in the parkin mutants (44%, $P<0.05$). The resting potential of the larval body wall muscles (6/7/12/13) is 10mV more positive in the parkin mutants (ANOVA, $P<0.001$). At all membrane potentials, the excitatory junction potentials (EJPs) are reduced significantly in parkin mutants.

A common feature associated with PD is oxidative stress. Overexpression of genes that scavenge reactive oxygen species with the global driver partially rescue synaptic overgrowth phenotype, but have little impact on locomotion.

Our results provide the first physiological model of PD, with parkin affecting synaptic function both in the periphery and CNS. We suggest that it is harder to rescue locomotion than neuromuscular junction overgrowth because locomotion depends on multiple synapses.

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# Synaptic proteome alterations in patients with sporadic Creutzfeldt-Jakob disease

Julie Carimalo[1], Martin Nowak[1], Joanna Gawinecka[1], Barbara Ciesiekczykl[1], Walter Schulz-Schaeffer[2], Abdul R. Asif[3], Franco Cardone[4], Maurizio Pocchiari[4], Inga Zerr[1]

[1]National Reference Center for TSE Surveillance

[2]Department of Neuropathology

[3]Department of Clinical Chemistry, Medical Center Georg-August University, Göttingen, Germany

[4]Department of Cell Biology and Neurosciences Istituto Superiore di Sanita, Rome, Italy.

Sporadic Creutzfeldt-Jakob disease (sCJD) is the most common form of transmissible spongiform encephalopathy affecting humans. Disease phenotype is mainly influenced by the methionine/valine (M/V) polymorphism at codon 129 in the PRNP gene and by the presence of two major types of protease-resistant form of PrP leading to 2 different profiles in Western blot (types 1 and 2). MM1 and VV2 represent the most frequent sCJD subtypes. Recent data strongly suggest that synaptic degeneration is a critical step of prion neuropathogenesis and is responsible for the onset of the clinical manifestations. However, the precise mechanism sustaining this process is still not completely understood. In this study, we have aimed to provide an overview of the synaptic alterations occurring in the brain from MM1 and VV2 sCJD patients by using a proteomics approach. For this purpose, synaptosome fractions were isolated from the frontal cortex from 5 MM1 and 5 VV2 sCJD patients as well as from 5 age-matched non-demented controls and subjected to 2-D Fluorescence Difference Gel Electrophoresis (2D-DIGE). Gel plugs containing protein spots displaying up- or down-regulation in sCJD patients were then excised from Coomassie blue-stained gels and subjected to in-gel digestion. Subsequently, the digested peptides were identified by ESI-Q-TOF mass spectrometry. Dysregulation of protein of interests was confirmed by western-blot. Densitometric and statistical analysis of the 2-D maps revealed a significant more than 1.5-fold change in the abundance of 35 (14 up- and 21 down-regulated) and 18 (7 up- and 11 down-regulated) different protein spots in MM1- and VV2-sCJD patients, respectively, when compared to non-demented controls. Among them, we could identify 39 protein spots by mass spectrometry. When protein biological functions were taken into consideration, the major alterations affected proteins belonging to the functional blocks 'synapse structure and intracellular trafficking' and 'mitochondria and energy metabolism'. However, heat shock proteins as well as proteins involved in apoptosis and oxidative stress were also well represented. Our results show the dysregulation of newly identified synaptic proteins in sCJD neuropathology. We strongly believe that our results will give clues for a better understanding of the mechanisms underlying synaptic degeneration in sCJD.

# The alpha-synuclein A30P-mutation negatively affects regeneration of dopaminergic neurons

Lars Toenges[1], Patrizia Roszak[1], Eva Szegoe[2], Jan C. Koch[1], Christoph P. Dohm[1], Pawel Kermer[1,3], Sebastian Kuegler[1,3], Mathias Baehr[1,3], Paul Lingor[1,3]

[1]University Medicine Goettingen, Dept. of Neurology, Goettingen, Germany
[2]University of Goettingen, Neurodegeneration and Restorative Research, Goettingen, Germany
[3]Deutsche Forschungsgemeinschaft Research Center for Molecular Physiology of the Brain, Goettingen, Germany

Alpha-synuclein is one of the most important proteins to be associated with inherited Parkinson´s disease (PD). Here, the A53T- and A30P-mutations have been found to result in an early-onset phenotype. As axonal degeneration is one of the earliest features of PD pathology and this may precede the degeneration of dopaminergic neurons in the substantia nigra we examined whether expression of either wild-type human alpha-synuclein or the two mutations have a differential effect in *in vitro* and *in vivo* paradigms of PD for neuronal survival and axonal regeneration.

*In vitro*, we infected midbrain dopaminergic (DA) neurons with AAV encapsuled expression plasmids for either EGFP, human wild-type alpha-synuclein or the A30P or A53T alpha-synuclein mutations. After transection of neuritic processes using the scratch technique we evaluated survival and neuritic outgrowth of lesioned DA neurons. Here, the regenerative response in A30P alpha-synuclein expressing neurons was significantly impaired.

*In vivo*, we performed a unilateral 6-OHDA striatal lesion in mice and optimized the lesion dose to generate a suitable model for the study of DA neuron survival and regeneration. Then we injected in parallel to the 6-OHDA striatal lesion AAV encapsulated expression plasmids for EGFP, wild-type alpha-synuclein or the A30P or A53T alpha-synuclein mutations into the substantia nigra (SN). Here, we present data on the number of surviving TH-positive DA neurons in the SN and density of TH-positive and GAP43-positive fibres in the striatum as measure for the regenerative response in the nigrostriatal system. The role of alpha-synuclein and its disease-relevant mutants for these mechanisms is discussed.

# The brain region-specific effect of MPP+ on the expression of cytochrome c oxidase subunit IV isoforms and viability of astrocytes

Marion Barbara Victor[1], Syama Sundar Boyalla[1], André Roemgens[1], Cordian Beyer[1], Susanne Arnold[1]

[1]RWTH Aachen University, Faculty of Medicine, Institute for Neuroanatomy, Wendlingweg 2, 52074 Aachen, Germany

The neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administered systemically to mammals induces a relatively specific degeneration of mesencephalic dopaminergic neurons and a marked parkinsonian syndrome. The highly active toxin 1-methyl-4-phenylpyridinium (MPP+) which is produced by conversion of MPTP in neural cells is known to inhibit mitochondrial complex I and to cause cell death of primarily dopaminergic neurons. With respect to astrocytes being a major regulator of neuronal cell death and survival we investigated the effect of MPP+ on mesencephalic vs. cortical astrocytes from female vs. male mice.

Real time PCR and Western blot analyses of MPP+-treated astrocytes demonstrated a toxin concentration-dependent decrease of astrocyte viability which was accompanied by an increase of cytochrome c oxidase subunit isoform IV-2 (COX IV-2) in mesencephalic male astrocytes, whereas female astrocytes as well as cortical astrocytes from both genders showed no change in COX IV-2 expression. The up-regulation of COX isoform IV-2 was paralleled by an increase of intracellular ATP and reactive oxygen species levels.

Taken together, our data suggest a crucial role for the regulation of COX isoform IV -2 expression and its functional consequences on energy production, oxidative stress, and viability of astrocytes supporting a brain region- and gender-specific effect of MPP+.

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# The co-layer method as an efficient way for neurotrophic factor release by transplanted genetically modified neuronal progenitor cells in a rat model of Parkinson's disease

Ieva Kalve[1], Andreas Ratzka[1], Meltem Özer[1,2], Maike Wesemann[1], Julia Jungnickel[1], Christiane Köster-Patzlaff[1], André Nobre[1,2], Claudia Grothe[1,2]

[1]Hannover Medical School, Institute of Neuroanatomy, Germany
[2]Center for Systems Neuroscience (ZSN), Hannover, Germany

Parkinson's disease (PD) is a neurodegenerative disorder resulting from the loss of dopaminergic (DA) neurons in the substantia nigra. Exogenous cell replacement represents a potent treatment option for this condition. But there are several limitations still to overcome, such as lack of donor tissue and low survival of grafted cells, when elaborating this therapeutic strategy.

To improve the transplantation outcome, we established a method to combine transient genetic modification of neuronal progenitor cells (NPCs) with an optimized cell culture protocol prior to intrastriatal transplantation into 6-OHDA unilateral lesioned rats. NPCs obtained from the ventral mesencephalon of E12 rat embryos were in vitro proliferated, nucleofected and differentiated as previously described (Timmer et al. Neurobiol. Dis. 2006; Cesnulevicius et al. Stem Cells 2006). Brain-derived neurotrophic factor (BDNF) and enhanced green fluorescence protein (EGFP) were expressed with a C-terminal 3xFLAG epitope tag to allow a sensitive detection. Western blot analysis confirmed the functionality of the BDNF-FLAG protein by phosphorylation of BDNF-receptor TrkB after NPCs were incubated with BDNF -FLAG conditioned media. Further, plasmid -based delivered BDNF-FLAG increases the number of tyrosine hydroxylase positive (TH+) neurons by 25% in vitro compared to EGFP transfected controls. However, the nucleofection procedure itself, especially the cell detachment, decreases the number of TH+ neurons to 40% compared to non-transfected sister cultures. To circumvent this drawback we established the co -layer method, which contains a mix of detached and nucleofected cells reseeded on top of an adherent sister culture in a ratio 1:3. In this setup TH+ neuron number remained high and was 25% increased after BDNF-FLAG transfection in vitro.

Comparison of both cell culture procedures (standard and co -layer) after intrastriatal transplantation revealed similar DA neuron survival as in vitro. Two weeks after grafting TH+ neuron number was strongly reduced in the standard group (271 ± 62) compared to 1723 ± 199 TH+ neurons in the co-layer group. In contrast to the in vitro results, no differences in the number of grafted TH+ neurons were observed between BDNF-FLAG, EGFP-FLAG and non- transfected co -layers, neither 2 nor 12 weeks after transplantation. Likewise, amphetamine induced rotation behaviour improved similarly over time in all groups. Interestingly, even 13 weeks after transplantation EGFP-FLAG expression was still detectable in few neurons and an abundant neurite branching inside the grafts could be visualized by anti-FLAG staining, whereas axons of TH+ neurons innervated also the host striatum.

Nevertheless, the co -layer protocol provides an efficient way for neurotrophic factor release by transplanted progenitor cells and will be used to study the effects of promising factors on survival and integration of transplanted dopaminergic neurons.

# The gender- and brain region-specific role of cytochrome c oxidase in neurodegeneration

Susanne Arnold[1], Marion Victor[1], Syama Sundar Boyalla[1], Shilpee Singh[1], Magdalena Misiak[1], Cordian Beyer[1]

[1] RWTH Aachen University, Faculty of Medicine, Institute for Neuroanatomy, Wendlingweg 2, MTI-I, 52074 Aachen, Germany

The selective loss of neural cells from a particular brain region is a common feature of neurodegenerative diseases. A failure of mitochondrial function seems to be a causative factor. Toxins, such as 3-nitropropionic acid (3-NPA) and 1-methyl-4-phenylpyridinium (MPP+), affect mitochondrial function as well as neural cell viability and are known as inducers of Huntington's and Parkinson's disease, respectively.

We demonstrated that these toxins affect mitochondrial gene expression, ATP and reactive oxygen species (ROS) production and that cytochrome c oxidase (COX), the terminal enzyme of the mitochondrial respiratory chain, appears to play a crucial role. The application of 3-NPA and MPP+ to cultured primary astrocytes and neurons from different brain regions of female and male mice caused an increase of COX isoform IV-2 transcription and protein expression in female/male striatum and male midbrain, respectively. A siRNA against COX IV-2 revealed that COX IV-2 is causally related to elevated intracellular ATP levels at the expense of increased mitochondrial ROS production and neural cell death.

Our data suggest that cell death in response to 3-NPA and MPP+ is primarily caused due to increased oxidative stress in neural cells in a brain region-specific and in case of MPP+ also in a gender-specific way. COX appears to take center stage of metabolic and cell survival control in neurodegeneration.

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# The impact of Cytoplasmic Polyadenylation Element Binding Protein (CPEB)-mediated translational regulation on development and progression of temporal lobe epilepsy: Evidence from mice expressing a dominant negative CPEB in forebrain neurons

Peter Bedner[1], Pavel Dublin[1], Sada Turimella[1], Vamshidhar Vangoor[1], Kerstin Hüttmann[1], Eric Kandel[2], Christian Steinhäuser[1], Martin Theis[1]

[1]University of Bonn, Institute of Cellular Neurosciences, Sigmund-Freud-Str. 25, 53105 Bonn, Germany
[1]HHMI, Center for Neurobiology & Behavior, Columbia University, New York, USA[2]

Protein synthesis is required for long lasting enhancement of synaptic efficacy. Mechanisms regulating activity-induced protein synthesis in neurons include the Cytoplasmic Polyadenylation Element Binding (CPEB) Proteins. In mice with impaired CPEB function, we found that several protein synthesis-dependent forms of long term potentiation were disrupted. In order to test whether inhibition of protein synthesis might be beneficial under conditions of neuronal hyperactivity, we subjected mice expressing a dominant negative CPEB in forebrain neurons to an animal model of temporal lobe epilepsy, i.e. unilateral intrahippocampal kainate injection. By telemetric EEG and video monitoring, we investigated severity of status epilepticus (SE), duration of the latent phase and the frequency of repeated spontaneous seizures characteristic for the chronic phase. We found DN-CPEB mice exhibited a weaker SE compared to controls, yet they show more post SE seizures and a higher frequency of spontaneous seizures in the chronic phase. Morphologically, control animals exhibited typical granule cell dispersion which was barely noticeable in DN -CPEB mice even though the decrease in reelin immunoreactivity was similar. Astrogliosis and microglia activation was likewise altered in DN CPEB mice. When we applied doxycycline to prevent DN-CPEB expression until kainate injection, DN-CPEB mice exhibited SE similar to control animals, indicating that acute CPEB activity is crucial for seizure activity during SE and for post SE seizures. Application of doxycycline also restored granule cell dispersion.
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# The influence of the NKCC1-inhibitor bumetanide on alterations in seizure susceptibility after status epilepticus in mice

Manuel Töpfer[1], Kathrin Töllner[1,2], Claudia Brandt[1,2], Wolfgang Löscher[1,2]

[1]University of Veterinary Medicine, Department of Pharmacology, Toxicology, and Pharmacy, AG Löscher, Bünteweg 17, 30559 Hannover, Germany

[2]Center for Systems Neuroscience, Hannover

The development of epilepsy, particulary temporal lobe epilepsy (TLE), can be induced by a variety of brain insults, including status epilepticus (SE). During this process, named epileptogenesis, a variety of molecular and functional changes in the central nervous system progress, but the mechanisms underlying epileptogenesis are still poorly understood. Recent studies indicate that the development of hyperexcitability of neurons and neuronal circuits as a result of expression changes of cation -chloride-co-transporters might play a crucial role in epileptogenesis. In this context the K+-Cl- co-transporter KCC2 is downregulated, while the Na+-K+-2Cl- co-transporter NKCC1 is upregulated. In consequence, this leads to a GABA-shift from an inhibitory to an excitatory action caused by an accumulation of intracellular chloride.

Several studies indicate that NKCC1 is upregulated in models of TLE and that the diuretic drug bumetanide, a selective NKCC1 -inhibitor, is a useful tool to counteract the upregulation of NKCC1, and so, may prevent neuronal hyperexcitability during epileptogenesis.

The aim of this study is to investigate in the pilocarpine model of TLE whether bumetanide has an effect on alterations of seizure susceptibility developing after a pilocarpine-induced SE. Seizure susceptibility is determined by timed i.v. infusion of the GABAA receptor antagonist pentylenetetrazole (PTZ).

NMRI-mice were used for inducing SE with pilocarpine. In a first experiment, the seizure threshold was determinded by PTZ infusion 48 h after SE. Animals were divided in two groups and were pretreated with bumetanide or vehicle, respectively, eight minutes before starting the PTZ infusion. Two other groups without SE received bumetanide or vehicle, respectively, and served as control.

Unexpectedly, the PTZ threshold was increased 48 h after SE, indicating enhanced GABAergic transmission at this time point following the brain insult. Treatment with bumetanide normalized the PTZ threshold to control level. In mice without SE, bumetanide did not alter the PTZ seizure threshold, indicating that the effects of bumetanide after SE were due to a specific action on SE-induced alterations. Further experiments are under way, to study the time-course of the alterations in PTZ threshold and bumetanide's effects on these alterations after SE. Furthermore, the consequences of bumetanide's action on development of spontaneous seizures after SE will be evaluated.

This study is supported by the DFG (Lo 274/11-1; Forschergruppe 1103).

# THE NUCLEOLUS AS A SOURCE OF OXIDATIVE DAMAGE AND NEURODEGENERATION.

Rosanna Parlato[1], Claus Rieker[1], Grzegorz Kreiner[1], Holger Bierhoff[2], Maria Armentano[3], David Engblom[1], Andreas Schober[4], Luca Bonfanti[3], Ingrid Grummt[2], Günther Schütz[1]

[1]German Cancer Research Center, Molecular Biology of the Cell I, In Neuenheimer Feld 581, 69121 Heidelberg, Germany[2]Dept. Molecular Biology of the Cell II, German Cancer Research Center[3]Dept. of Veterinary Morphophysiology, University of Turin, Italy[4]Institute of Anatomy and Cell Biology II, Department of Molecular Embryology, University of Freiburg, Germany

The nucleolus is an essential stress sensor that tightly regulates its activity in favorable or adverse conditions to optimize the metabolic cellular resources. Nucleolar malfunction contributes to the pathology of several human diseases, in particular multiple genetic disorders. However, the impact of nucleolar damage on neuronal survival has not been explored. To this end, we generated mutant mice in which the transcription factor TIF-IA, essential for the regulation of ribosomal RNA (rRNA) synthesis, is genetically ablated in specific neurons by the Cre-loxP system. Loss of TIF-IA in neuronal progenitors results in mice born without a brain, but when lost in mature neurons, the major features of the neurodegenerative process are recapitulated (Parlato, R. et al., 2008).

Here we report that mutants characterized by inhibition of rRNA synthesis and nucleolar disruption in dopaminergic (DA) neurons or in medium spiny neurons of the striatum show cardinal features of neurodegenerative disorders, such as severe oxidative damage, progressive neuronal loss and typical motor dysfunctions. Inactivation of the TIF- IA gene in adult DA neurons leads to a phenotype closely resembling Parkinson´s disease (PD), characterized by depletion of dopamine in the striatum, progressive loss of DA neurons, and marked deficiency in motor performance - reproducing the temporal sequence of clinical onset of PD. Interestingly, increased levels of oxidative stress is detected in DA midbrain neurons of mice lacking TIF-IA prior to any loss of these neurons. Moreover, DA neurons located in the substantia nigra are more vulnerable to TIF-IA loss than those of the neighboring ventral tegmental area, mimicking a major but mechanistically still not well understood characteristic of dopaminergic neurodegeneration in PD. We found that the nucleolar damage is also present in PD brain autopsies. The molecular mechanisms downstream of the TIF- IA mutation indicate that nucleolar damage leads to overexpression of the proapoptotic transcription factor p53 and downregulation of the PI3K/mTOR signaling. In medium spiny neurons of the striatum, the neuronal population affected in Huntington´s disease, the ablation of the TIF-IA gene causes increased oxidative damage and progressive degeneration associated with impaired locomotor behavior. Gene expression profiling and biochemical assays accompanied with electron microscopy analysis, indicate the activation of neuroprotective responses, such autophagy, prior to cell death.

These analyses highlight the crucial role of the nucleolus as mediator of the stress response and indicate a novel key player for neurodegenerative processes and diseases.

# The perivascular clearance of neuronal apolipoprotein E is modulated by amyloid ß-protein in mouse models of Alzheimer's disease

Dietmar Rudolf Thal[1], Tom van Dooren[2], Christian Haass[3], Fred van Leuven[2], Harshvardhan Rolyan[1]

[1]University of Ulm, Laboratory of Neuropathology, Helmholtzstrasse 8/1, D-89081 Ulm, Germany
[2]Experimental Genetics Group - LEGTEGG, Department of Human Genetics, Catholic University Leuven, Leuven, Belgium
[3]Adolf Butenandt Institute, Ludwig Maximilians University and German Center for Neurodegenerative Diseases, Munich, Germany;

The deposition of the amyloid-ß protein (Aß) is a histopathological hallmark of Alzheimer's disease (AD). Apolipoprotein E (apoE) is involved in the clearance of Aß and the APOE e4 allele is a major risk factor for sporadic AD. ApoE is drained into the perivascular space (PVS), where it co-localizes with Aß. To further clarify the role of apoE in perivascular clearance of Aß, we studied apoE-transgenic mice over-expressing human apoE4 either in astrocytes (GE4) or in neurons (TE4). These animals were crossbred with amyloid precursor protein (APP)-transgenic mice and with APP-presenilin-1 (APP-PS1) double transgenic mice. Using an antibody that specifically detects human apoE (h-apoE), we found that astroglial expression of h-apoE in GE4 mice leads to its perivascular drainage whereas neuronal h-apoE expression in TE4 mice does not, indicating that neuron-derived apoE is usually not subject of perivascular drainage. However, h-apoE was observed not only in the PVS of APP-GE4 and APP-PS1-GE4 mice but also in that of APP-TE4 and APP-PS1-TE4 mice. Immunofluorescence staining showed colocalization of neuron-derived h-apoE and Aß in the PVS as well as in the cytoplasm of perivascular astrocytes of these animals indicating that astrocytes take up the neuron-derived apoE bound to Aß presumably prior to its clearance into the PVS. Thus, it is tempting to speculate that neuronal apoE-Aß complexes but not neuronal apoE alone are substrate for astroglial uptake and subsequent perivascular drainage. The production of Aß and its intraneuronal interaction with apoE, thereby, leads to pathological perivascular drainage of neuronal apoE. This Aß-related modulation of neuronal apoE metabolism may represent a potential pathomechanism for Aß neurotoxicity.

# The role of astrocytes in the Parkinson´s Disease pathology

Sonja Mendritzki[1], Hermann Lübbert[1, 2]

[1]Ruhr-University Bochum, Department of Animal Physiology, Universitätstr. 150, 44780 Bochum, Germany

[2] Biofrontera Bioscience GmbH, Hemmelrather Weg 201, 51377 Leverkusen, Germany

Introduction: Mitochondria play a central role in the pathogenesis of Parkinson`s disease (PD). In accordance to PD patients transgenic mice carrying PD-inducing gene mutations develop severe region-, age- and genotype-dependent mitochondrial abnormalities (Stichel et al., 2007). These structural and functional alterations were not only restricted to neurons but also affected glial cells in the adult transgenic mice (Schmidt et al., 2010).
In our present study we focused on two main aspects:
1. Characterization of mitochondrial morphology and function in transgenic astrocytes.
2. Elucidation of the impact of transgenic astrocytes on neuronal differentiation.

Results: Ultrastructural studies revealed severe structural changes of mitochondria in primary astrocytes from all mouse lines. In addition, transgenic astrocytes showed an upregulation of mitochondria related proteins as well as a deficit in mitochondrial Ca2+-storage. Furthermore, cortical neurons cocultured with transgenic astrocytes in an indirect coculture system developed shorter processes and smaller neuronal areas compared to neurons cocultured with non-transgenic astrocytes.

Conclusion: Taken together, transgenic astrocytes display morphological and functional alterations and fail to influence neuronal differentiation. These data implicate that astrocytes are prominent contributors to the pathophysiology of Parkinson`s disease.

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# The role of BAG1 in tau pathology

Sandra C. Signore[1], Mathias Bähr[1,3], Fred S. Wouters[2,3], Pawel Kermer[1,3]

[1]University of Göttingen, Department of Neurology, 37075 Göttingen, Germany
[2]University of Göttingen, Department of Neuro- and Sensory Physiology, 37075 Göttingen, Germany
[3]DFG-Research Center for Molecular Physiology of the Brain (CMPB), 37075 Göttingen, Germany

Protein accumulation and aggregation is one of the pathological hallmarks in many neurodegenerative diseases. In Alzheimer's disease, the intracellular microtubule -associated tau protein becomes hyperphosphorylated and aggregates into tangles being associated with neuronal cell death. Chaperones and co-chaperones are essential instruments for re-folding and detoxifying processes in the cell. In the recent years it has been shown that the co-chaperone BAG1 is able to ameliorate pathology in models of Huntington's or Parkinson's disease.
For the present experiments, several mutant tau isoforms were created which show different propensity for aggregation and toxicity. BAG1 co -transfection resulted in increased aggregate formation indicating that BAG1 stabilizes high molecular tau. At the same time, tau cleavage into small fragments (43 and 26 kDa) was delayed resulting in decreased tau toxicity. This effect was dependent on the integrity of the C-terminal BAG known to be essential for interaction with Hsp70 and the proteaosomal system. In summary, these results show an important role for BAG1 in tau pathology and could also provide therapeutic potential.

# The Role of Striatum and Hippocampus in Sequential Learning: Interaction, Dissociation or Competition?

Moritz Thede Eckart[1], Moriah Hülse-Matia [1], Rebecca McDonald[1], David Loer[1], Rainer Schwarting[1]

[1]Philipps-Universität Marburg, Psychologie, Gutenbergstraße 18, 35037 Marburg, Germany

Sequential behaviour, a type of procedural behaviour, has been intensively investigated in humans using the so-called serial reaction time task (SRTT), which was first introduced by Nissen and Bullemer (1987).In the SRTT, subjects have to respond to visual stimuli by key pressing. Decreases in reaction times to sequential – compared to random – stimulus presentation are taken as an indicator of sequential learning.
Theories that postulate a crucial role of dopaminergic processes in the basal ganglia in sequential learning are widely accepted and find substantial empirical support from human and animal research.
The role of the hippocampus in sequential learning however remains unclear. There are three conflicting major hypotheses: interaction, competition or dissociation between hippocampus and striatum. Evidence for each of these theories is found in human and animal experiments.
In our research we used an analogous rat model of the human SRTT which was recently developed by Domenger and Schwarting (2005). In the rat SRTT, the animals have to respond to visual stimuli by nose poking. As in the human SRTT, stimuli are either presented randomly or in a sequential order.
Rats with striatal 6-OHDA lesions showed inferior sequential learning while rats with dorsal hippocampal ibotenic acid lesions showed superior SRTT performance in terms of reaction time and accuracy, as compared to sham-lesioned and control animals.

# The role of voltage gated sodium channels in the pathogenesis of glaucomatous optic neuropathy

Birte Könnecke[1], Shai Sandalon[2], Hani Levkovitch-Verbin[3], Katharina Hein[1], Muriel Sättler[1], Mathias Bähr[1], Mikael Simons[1,4], Ron Ofri[2]

[1]Georg-August-University Göttingen, Department of Neurology, Göttingen, Germany
[2]Hebrew University of Jerusalem, Koret School of Veterinary Medicine, Rehovot, Israel
[3]Tel-Aviv University, Sackler Faculty of Medicine, Tel-Hashomer, Israel
[4]Max-Planck-Institute of Experimental Medicine, Göttingen, Germany
E-mail: bkoenne@gwdg.de

Glaucoma, as an optic neuropathy, is the second major cause of blindness in the world. Since progressive degeneration of retinal ganglion cells (RGCs) and loss of their axons in the optic nerve (ON) can result in blindness, it has become accepted that there is a need to develop neuroprotective therapies to prevent RGC death and preserve vision of glaucoma patients.
Previous studies in animal models of glaucoma and multiple sclerosis (MS) revealed mechanistical similarities of neuronal apoptosis like an increased expression of the pro-apoptotic protein Bax and a downregulation of the anti-apoptotic protein Bcl -2. Changes in voltage -gated sodium ($Na_v$) channel expression and activation could be possible early "upstream" events preceding this activation of the pro-apoptotic signaling cascade that leads to RGC and axon degeneration like indicated by studies in animal models of glaucoma and other ON and CNS diseases. Furthermore it has been demonstrated that inhibition of $Na_v$ channels by lamotrigine treatment has neuroprotective properties in various animal models of neurodegenerative diseases like cerebral ischemia, Parkinson's disease and MS.

In this study we tested the hypotheses that in two animal models of glaucoma up-regulated $Na_v$ channel expression plays a role in RGC apoptosis and that treatment with the sodium channel blocker lamotrigine may be neuroprotective.
In the first phase of this study we induced unilateral glaucoma in rats through anterior chamber cannulation (acute model) or laser cyclophotocoagulation (chronic model). Immunohistochemical staining for $Na_v$ channels supported our first hypothesis. We could show increased expression of $Na_v1.2$ channels, and decreased expression of $Na_v1.6$ channels in both models. These changes appear within early and late timepoints after the induction of the disease and were correlated with substantial infiltration of inflammatory cells, axonal loss and retinal atrophy.
In the second phase of this study, animals were treated with intraperitoneal injections of the $Na_v$ channel blocker lamotrigine. We couldn't observe significant differences in the parameters investigated between lamotrigine and vehicle treated animals. In spite of the fact, that $Na_v$ channel expression is affected in these animal models, the block of $Na_v$ channels by lamotrigine hasn't any efficacious or protective effect on the progress of the disease in our study.

# The transport of neurotrophins in neurodegenerative diseases

Bianca Seifert[1], Volkmar Leßmann[1], Tanja Brigadski[1]

[1]Otto-von-Guericke Universität Magdeburg, Institut für Physiologie, Leipziger Str. 44, 39120 Magdeburg, Germany

Neurotrophins are important growth factors supporting the developing and adult central nervous system. The most prominent members of this protein family in CNS neurons are NGF and BDNF. Depending on the cell type and the expression pattern they coordinate a multitude of biological functions, like the organisation of neurites, synaptogenesis and the survival of neurons. The efficiency of anterograde and retrograde transport, as well as of exo- and endocytosis of neurotrophin vesicles critically determine the capacity of BDNF and NGF in performing these functions. Recent studies suggest that changes in neurotrophin transport could underlie different neurodegenerative diseases. Despite extensive research concerning the neuroprotective function of neurotrophins, less is known about the mechanisms of neurotrophin transport. Investigation of disease related changes in neurotrophin transport might provide insights into the cellular mechanism underlying e.g., Alzheimer's and Huntington's disease.

In order to analyse the role of neurotrophin transport in Alzheimer's disease, dissociated cultures of mouse hippocampal neurons were cotransfected with fluorescent protein-labelled BDNF and amyloid precursor protein (APP), which plays a crucial role in the pathogenesis of Alzheimer's disease. Vesicles containing fluorescent-labelled BDNF were analysed by live cell imaging and immunocytochemical staining. Using hemagglutinin (HA)-tagged and GFP-labelled BDNF (HA-ProBDNF-GFP) we could show colocalisation of proBDNF and mature BDNF with the N-terminal domain of APP (soluble APP) in vesicular structures. Cotransfection of BDNF-mCherry and C-terminally tagged APP, which marks the intracellular domain of APP (AICD), revealed a colocalisation of both proteins in endoplasmic-reticulum-like structures. A colocalisation of APP-YFP and BDNF-mCherry was infrequently observed in vesicular structures. In addition, we have analysed the kinetics of BDNF transport using live cell imaging of BDNF-mCherry containing vesicles. Hippocampal neurons were cotransfected with BDNF-mCherry and APP-YFP to mimic a pathological overexpression of APP. One day after cotransfection, a dynamic transport of BDNF-mCherry containing vesicles was observed in hippocampal neurons. A reduction in transport velocity and an increase in pause frequency were observed three days after cotransfection with APP-YFP. These data suggest a colocalisation of soluble APP, proBDNF and mature BDNF in secretory granules and a reduction in BDNF vesicle transport in cells overexpressing APP.

This study was supported by NeuroNetworks NN11 from the CBBS/EFRE program

# Transcriptional regulators in the pathogenesis of Amyotrophic Lateral Sclerosis (ALS) – Histopathological and biochemical studies in the G93A ALS mouse model and in ALS post mortem tissue

Nadine Thau[1], Sarah Knippenberg[1], Reinhard Dengler[1], Susanne Petri[1]

[1]Medical School Hannover, Department of Neurology, Carl-Neuberg Str.1, 30615 Hannover, Germany

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease characterized by selective motoneuron loss in brain and spinal cord. Mutations in the superoxide dismutase (SOD) 1 gene are detected in 10-20% of familial ALS patients. The ALS-mouse model over-expressing a mutant human SOD1 (G93A) closely mimics human ALS. The cause for the selective death of motoneurons is still unclear, but many different pathomechanisms are discussed including oxidative stress, inflammation and mitochondrial dysfunctions.
The transcriptional co-activator peroxisome proliferator-activated receptor-gamma co-activator 1alpha (PGC-1alpha) plays a central role in the regulation of mitochondrial metabolism and biogenesis via activation of transcription factors such as nuclear respiratory factor-1 and -2 (NRF-1/-2). NRF-1 and NRF-2 are targets of PGC-1alpha and stimulate the expression of mitochondrial transcription factor A (Tfam), a mitochondrial matrix protein essential for the replication and transcription of mitochondrial DNA. Alterations in PGC-1alpha expression and function have previously been described in models of Huntington's and Alzheimer's disease.
In the present study, we investigated the RNA and protein expression of PGC-1alpha and downstream factors (NRF1, NRF2, Tfam, mnSOD) in human post mortem brain, spinal cord and muscle tissue of ALS patients and controls as well as in spinal cord, muscle and brown fat tissue of G93A-ALS mice and non-transgenic controls in presymptomatic and late symptomatic disease stages. In addition, we are analyzing the expression of Utrophin and AChRepsilon in muscle because it is known that PGC -1alpha levels correlate with the number of acetylcholine receptor (AChR) clusters and there is evidence that neurodegeneration in ALS could be a dying back process. RNA-expression was quantified by realtime PCR. Immunohistochemistry was performed on frozen paraformaldehyde-fixed sections using anti-PGC-1alpha, anti-NRF-1, anti-mnSOD and anti-Tfam antibodies.
Up to know we have detected a reduction of PGC -1alpha and mnSOD mRNA levels in spinal cord and muscle tissue in G93A transgenic mice at disease endstage while they were not differentially expressed at the presymtomatic stage. We therefore conclude that PGC -1alpha could represent an interesting therapeutic target in ALS.

# Treatment of 5XFAD transgenic mice with Ibuprofen

Antje Hillmann[1], Oliver Wirths[1], Thomas A. Bayer[1]

[1]University of Goettingen, Department of Molecular Psychiatry, Von-Siebold-Str. 5, 37075 Göttingen, Germany

In Alzheimer disease (AD), patients show a chronic inflammatory pathology characterized by activated mircoglia, reactive astrocytes, complement factors and increased inflammatory cytokine expression.
Studies have shown that treatment with non-steroid anti-inflammatory drugs (NSAID) leads to a decreased risk of AD. Moreover, it has been published that a subset of NSAIDs acts as selective amyloid lowering agents by reducing the levels of Aß1-42. Consequently, the anti-inflammatory activity of NSAIDs, combined with their Aß modulatory capabilities, seemed to be a promising AD therapy. However, so far the results of pharmacological studies in both mice and humans are inconsistent.
In the present study the 5XFAD mouse model was used representing a double transgenic APP/PS1 mouse line co-expressing five familial Alzheimer disease (5XFAD) mutations. 5XFAD mice on a C57Bl6/J genetic background show early plaque formation, increased levels of Aß-42 and a variety of behavioural deficits such as working memory impairment, motor deficits and reduced anxiety.
5XFAD mice were treated with Ibuprofen for a period of 3 months starting at the age of 3 months. At 6 months of age mice were tested for changes in working memory and motor performance. In addition, immunohistochemical, biochemical and RT-PCR analysis were performed, addressing plaque deposition, Abeta levels and inflammatory markers.

# Visualizing dopamine transporter activity with [123I]FP-CIT SPECT in 6-OHDA lesioned marmoset monkeys : A non-human primate model of Parkinson's disease

Enrique Garea-Rodríguez[1], Yi-Fang Cui[1], Christina Schlumbohm[1], Eberhard Fuchs[1]

[1]German Primate Center, Clinical Neurobiology Laboratory, Kellnerweg 4, 37077 Göttingen, Germany

Suitable animal models are a prerequisite for the evaluation of new therapeutic targets of Parkinson's disease (PD). Preclinical research often involves non- human primates e.g. common marmosets (Callithrix jacchus) to overcome the limitations of rodent studies regarding complex brain function and drug safety. The widely used 6-hydroxydopamine (6-OHDA) lesion model nicely resembles motor dysfunctions observed in PD patients. As mild lesions are required to evaluate the neurorestorative capacity e.g. of neurotrophic factors (*Lindholm et al., 2007, Nature*), we tested different 6-OHDA lesion protocols in order to investigate the neurorestorative potential of novel therapeutic approaches. We induced unilateral lesions in the caudate or the nigrostriatal projection pathway of common marmosets using different numbers of injections and concentrations of 6- OHDA. The effects of 6-OHDA on dopamine transporter (DaT) activity were visualized using [123I]FP-CIT single photon emission computed tomography (SPECT). First results reveal a complete loss of striatal DaT activity accompanied by overt behavioural deficits in an animal with nigrostriatal bundle lesions. In contrast, animals at a subclinical disease stage and with a relatively small striatal lesion showed no obvious alterations of DaT activity 3 weeks after 6-OHDA injection. Combined analysis of behavioural and *in-vivo* imaging techniques is important for the assessment of disease progression in the current model. This approach can be used in the future to evaluate new therapeutic treatments in the context of neuroprotection and neuroregeneration.

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# Zebrafish: A new model of Parkinson's disease

Tomás Ribeiro da Silva Lopes da Fonseca[1], Ana Dulce Correia[1,2], Tiago Fleming Outeiro[1,2]

[1]Instituto de Medicina Molecular, Cell and Molecular Neuroscience Unit, Avenida Professor Egas Moniz , 1649-028 Lisboa, Portugal

[2]Instituto de Fisiologia, Faculdade de Medicina da Universidade de Lisboa, Lisboa, Portugal

Parkinson's disease (PD) is the second most common neurodegenerative disease. To unveil the mechanisms associated with the disease, several in-vivo models have been generated. Although the existing models have provided invaluable information about the molecular mechanisms underlying the disease, they do not fully recapitulate all the key features of the disease.

In invertebrate models such as Caenorhabditis elegans and Drosophila melanogaster, the overexpression of human alpha-synuclein (a-syn) causes the loss of dopaminergic neurons, resulting in motor coordination defects. Nevertheless, there is a great disadvantage in invertebrate models of PD which is the different nervous system complexity compared to higher species. Moreover, these models lack endogenous a-syn.

To overcome this issue, Danio rerio, commonly designated as zebrafish, is now being used as an intermediate model between flies and mice. This model has the advantage of being a vertebrate and therefore is phylogenetically closer to humans than flies, easier to maintain than mice, with a short life cycle and, importantly, presents a nervous system similar to that in humans.
The dopaminergic system in zebrafish is homologous to the human one and is located in the ventral diencephalon.

The main goal of this project was to generate a-syn transgenic zebrafish lines using human wild-type a-syn. We chose the Tyrosine Hydroxylase (TH) promoter to drive expression of the gene specifically in dopaminergic neurons in an attempt to mimic the pathology found in PD patients. The constructs were injected in the one-cell stage of zebrafish development using the Tol2 transposase technique which guarantees a single-copy mode insertion.

Currently, we are in the process of validating these models in the F1 generation by immunohistochemistry, western blot and behavior analysis.

We expect this new model of PD might prove useful to further understand the molecular basis of PD and may lead to the development of novel strategies for therapeutic intervention.

# A severe epileptic phenotype due to a moderate loss of M-current in the KCNQ2$^{Nmf134}$ mouse model

Daniel Milkereit[1], Axel Neu[1], Fabio Morellini[1], Quyen Le[1], Dirk Isbrandt[1]

[1]Center for Molecular Neurobiology Hamburg (ZMNH), Experimental Neuropediatrics, Falkenried 94, 20251 Hamburg, Germany

Mutations in the human *KCNQ2* and *KCNQ3* genes encoding M-channel subunits are associated with an epilepsy syndrome of newborns (BFNC). The KCNQ2$^{Nmf134}$ (Nmf134) mouse line (Jackson lab) that carries a V182M-mutation in the S3-helix of KCNQ2 was identified by a reduced seizure threshold in response to electrical stimulation. We used the Nmf134 mouse line as novel M channel-deficient model complementary to our dominant negative KCNQ2 transgenic mouse line (Peters et al., 2005).

Homozygous Nmf134 mice were smaller and lighter than their wildtype littermates and died from spontaneous seizure activity during the first postnatal weeks with a mortality rate of about 70% after 6 weeks. No gross morphological changes were observed in hippocampal brain sections. However, in preliminary immunostains, augmented c-fos immunoreactivity pointed to increased neuronal activity, and the presence of reactive astrocytes indicated an ongoing pathological process.

Electrophysiological analysis of the V182M mutation in *Xenopus leavis* oocytes showed a 50% reduction in KCNQ2/KCNQ3 channel-mediated current amplitudes and a significant shift of voltage-dependent gating to more positive membrane potentials. Similar results with respect to M-channel amplitudes were obtained from CA1 pyramidal neurons of Nmf134 mice, leading to increased cellular excitability upon current injection. The sensitivity of mutant M-channels to the anticonvulsive drug and M-channel mimetic retigabine was unaffected, both in oocyte and brain slice recordings.

Our experiments demonstrate the generation of a severe epileptic phenotype by a moderate shift in voltage dependence of the KCNQ2 channel activation. We now use the Nmf134 mouse line as a model for neonatal seizures to evaluate treatment strategies during neonatal brain development.

# The role of the C-C chemokine CCL17 in Alzheimer's disease

Kim Neitzert[1], Onder Albayram[1], Sathish Kumar[2], Jochen Walter[2], Irmgard Förster[3], Andras Bilkei-Gorzo[1], Mira Kron[5], Wolfgang Maier[4], Andreas Zimmer[1], Judith Alferink[1,4]

[1]University of Bonn, Institute of Molecular Psychiatry, Bonn, Germany
[2]Department of Neurology, Laboratory of Molecular Cell Biology, University of Bonn, Bonn, Germany
[3]Molecualr Immunology, Institut für Umweltmedizinische Forschung at the Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany
[4]Department of Psychiatry, University of Bonn, Bonn, Germany
[5]Department of Dermatology, University of Bonn, Bonn, Germany

Alzheimer's disease (AD) is characterized by a progressive loss of cognitive abilities and the deposition of accumulated amyloid-beta (Aß) peptides, so-called senile plaques, in the CNS of AD patients. Inflammatory processes, such as microglial activation and production of proinflammatory cytokines and chemokines have also been suggested to contribute to neurodegeneration associated with AD. The CC-chemokine CCL17, which signals through the chemokine receptor CCR4, is produced by a subpopulation of mature dendritic cells (DCs) recruited to parenchymal brain regions during neuroinflammation. We found that CCL17 expression is upregulated in brain tissues in an AD mouse model, suggesting the functional impact of this chemokine in AD. To investigate the role of CCL17 in AD pathogenesis a mouse model for AD (double transgenic APPswe/PSEN1ΔE9 mice) was crossed with CCL17-deficient mice (APP/PS1xCCL17$^{E/E}$). As expected, APP/PS1 mice exhibit impaired long-term and spatial memory functions at the age of 14 months. In contrast, APP/PS1 mice on a CCL17-deficient background show a significantly improved working memory, indicating that CCL17 controls the cognitive decline during neurodegeneration. Additionally, in APP/PS1xCCL17$^{E/E}$ mice soluble Aß42 brain levels remain constant between 6 and 14 months of age, in contrast to the observed Aß42 increase in APP/PS1 brains during aging. Furthermore, CCL17-deficient APP/PS1 mice exhibit an upregulation of the astrocyte marker GFAP (glial fibrillary acidic protein) as verified by immunofluorescence, indicating enhanced glial responses in the absence of CCL17. Altogether, these data suggest, that CCL17 affects inflammatory CNS responses during AD and is a critical mediator of the cognitive decline in disease pathogenesis.

# Poster Topic

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*Gholamreza Hassanzadeh , Azami Tameh Abolfazl, Bayat Mohammad*

# Beta-adrenergic stimulation suppresses phagocytosis in microglia via EPAC

Tanja Steininger[1], Hubert Kerschbaum[1]

[1]University of Salzburg, Department Cell Biology, Hellbrunnerstraße 34, 5020 Salzburg, Austria

Microglia is the immunocompetent system within the central nervous system. Despite their phagocytic significance in neurodegenerative diseases, trauma and infection, little is known about the regulatory cellular mechanisms underlying this process. A successful phagocytosis of particles is accompanied by local changes in cell volume and ion conductance. Microglia expresses chloride as well as potassium channels and furthermore alpha- and beta-adrenergic receptors for neurotransmitters. The role of microglia in a given neurodegenerative process or brain injury condition might be determined by the balance between neurotoxic versus neuroprotective and ani- versus pro-inflammatory microglial factors. Recent findings that noradrenaline reduces inflammatory cytokine expression in microglial, astroglial, and brain endothelial cells in vitro, suggest that neurotransmitters act as local endogenous modulators of brain inflammatory responses. Mediator-modulated intracellular cAMP level may affect phagocytosis. The present study investigated the impact of the cAMP cascade and of the beta-adrenergic agonist, isoproterenol, on the phagocytotic capacity of microglial cells.

The uptake of hydrophobic polystyrenic microspheres in the microglial cell line BV -2, was visualized using confocal laser microscopy and scanning electron microscopy. Microspheres were used instead of apoptotic cells because in scanning electron microphagic images, engulfed particles were clearly discriminated from attached ones.

Beta-adrenoreceptor stimulation is known to result in an activation of adenylylcyclase (AC) which leads to an increase in cAMP. Exposure of BV-2 cells to the nonselective beta-agonist isoproterenol inhibited phagocytosis. The uptake of particles was also suppressed by forskolin, which activates AC to elevate the intracellular cAMP, IBMX, a nonselective phosphodiesterases blocker, and the membranepermeable cAMP analogue 8-Br-cAMP.

Increase in cAMP activates PKA and exchange proteins activated by cAMP (EPAC). EPACs are specific guanine nucleotide exchange factors for the Ras GTPase homologues, Rap1 and Rap2. To evaluate whether PKA or Epac conveys the cAMP-dependent effect on phagocytosis, we used the PKA inhibitor, H-89, and the Epac-specific cAMP analogue 8- pCPT-2`-O-Me-cAMP. Our results suggest that beta -adrenoreceptor stimulation suppresses phagocytosis in microglia by elevating intracellular cAMP. Elevated cAMP levels inhibit phagocytosis in microglial BV-2 cells through EPAC.

# Brain hypoxia causes early increase in vesicular glutamate release in hippocampal area CA1 but not in hippocampal area CA3

Christine Gebhardt[1], Christoph Behrens[1], Marlene Jarosch[1], Uwe Heinemann[1]

[1]Universitätsmedizin Charité, Institut of Neurophysiology, Oudenarder Str. 16, 13347 Berlin, Germany

The release of glutamate during brain hypoxia is considered to be an important factor in generating anoxic depolarisation and subsequent neuronal damage. In CA1 pyramidal cells that are killed preferentially during brain hypoxia in vitro brain slice studies have determined that an early consequence of acute hypoxia is a marked increase in the frequency of miniature excitatory postsynaptic currents (mEPSCs). This early increase which was also found in neonatal pyramidal cells might contribute to selective impairment of hypoxia sensitive neurons. Using the electrophysiological patch clamp technique here we compare hypoxia induced changes in CA1 and CA3 pyramidal neurons in horizontal brain slices from adult rats. Recordings were performed in an interface-type chamber at 34 °C. Hypoxic conditions were produced by switching the gas flow over the slices from 95 % O2/5 % CO2 to 95 % N2/ 5% CO2. In contrast to CA1 neurons, frequency of mEPSCs recorded from CA3 neurons is decreased within the first three minutes of hypoxia. That decrease is reversible after reoxygenation. The decrease in mEPSC frequency was not affected by blocking ATP -sensitive K channels using Tolbutamide. Indeed, application of the A1 adenosine antagonist 8-CPT led to a hypoxia induced increase in mEPSC frequency similar to that recorded in CA1 neurons. Our data suggest that i) the sensitivity of hippocampal CA1 neurons to hypoxia might be related to an increase in spontaneous presynaptic glutamate release which not occurred in area CA3 and ii) that adenosine which is known to act neuroprotective when exogenously applied contribute physiologically to protection of CA3 neurons against hypoxic impairment.

# Calcium-independent phospholipase $A_2$ ($iPLA_2$) protects astrocytes and their mitochondria against rotenone-induced oxidative stress.

Caroline Nordmann[1], Mikhail Strokin[1], Georg Reiser[1]

[1]Otto-von-Guericke-Universität Magdeburg, Institut für Neurobiochemie, Leipzigerstraße 44, 39120 Magdeburg, Germany

In the central nervous system, calcium-independent phospholipase $A_2$ ($iPLA_2$) has been shown to be associated with various physiological and pathological processes, including cellular growth, cell death and cell membrane homeostasis. The enzymes of the phospholipase $A_2$ family control the release of polyunsaturated fatty acids (PUFAs) from the sn-2 position of cellular phospholipids (Strokin et al. 2007), thereby initiating pathways of signal transduction. The $iPLA_2$ family consist of two isoforms, VIA $iPLA_2$ ($iPLA_2$ß) and VIB $iPLA_2$ ($iPLA_2\gamma$). Both are present in substantial amounts in mitochondria. We suppose that the $iPLA_2$ important for mitochondria functions and suppression of $iPLA_2$ will evoke oxidative stress, lipid peroxidation and cell death. However, the role of iPLA2 in the mechanism of mitochondria dysfunction remains unclear. Here we investigated the role of $iPLA_2$ in development of oxidative stress and cell survival in primary rat astrocytes by pharmacological inhibition of $iPLA_2$ with bromoenol lactone (BEL). We measured the mitochondrial potential using fluorescence microscopy and specific fluorescence dyes rhodamine 123, ROS (reactive oxidative species) generation was detected by oxidation of 2'-7'-dichlorofluorescein diacetate, lipid peroxidation was measured by TBARS-assay (Thiobarbituric Acid Reactive Substance) and cell death was estimated by release of LDH (lactate dehydrogenase). Treatment of rat astrocytes with different concentrations of BEL (2.5 µM, 5 µM and 10 µM) induced lipid peroxidation and oxidative stress. These effects of BEL were clearly concentration-dependent. Additionally, we could detect reduction of mitochondria membrane potential after treatment with BEL. The rotenone-induced loss of mitochondria potential was concentration-dependently amplified after inhibition of $iPLA_2$ with BEL (from 0.5 µM to 5 µM BEL). Finally, inhibition of $iPLA_2$ activity with BEL under oxidative stress induced by rotenone (24 h preincubation) evoked enhanced cell death. Our results indicate that members of the $iPLA_2$ might play a critical role for maintenance of mitochondria functions under oxidative stress. Consequently, it was presumed that $iPLA_2$ plays a cytoprotective role under oxidative stress conditions but the details of pathway are still not clear. However, our findings highlight the importance of further elucidation of precise role of individual VIA $iPLA_2$ and VIB $iPLA_2$ isoforms in protection of mitochondria and whole cells against oxidative stress. The results of our study would be beneficial for understanding of the molecular mechanism of diseases linked to the mutations in the human gene of the VIA $iPLA_2$ like “infantile neuroaxonal dystrophy” (INAD) and “neurodegeneration with brain iron accumulation” (NBIA)

M. Strokin, M. Sergeeva and G. Reiser. Prostaglandin synthesis in rat brain astrocytes is under the control of the n-3 docosahexaenoic acid, released by group VIB calcium-independent phospholipase $A_2$. J. Neurochem, 2007, 102, 1771-1782

# CD14 and TRIF govern distinct responsiveness and responses in mouse microglial TLR4 challenges by structural variants of LPS

Uwe-Karsten Hanisch[1], Tommy Regen[1], Denise van Rosssum[1], Jörg Scheffel[1], Maria-Eleni Kastriti[1], Natalia H. Revelo[1], Marco Prinz[2], Wolfgang Brück[1]

[1171]University of Göttingen, Institute of Neuropathology, Robert-Koch-Straße 40, 37075 Göttingen, Germany[2]University of Freiburg, Department of Neuropathology, Breisacherstraße 64, 79106 Freiburg

Toll-like receptor (TLR) 4 responds to a range of agonists in infection and injury, but is best known for the recognition of bacterial lipopolysaccharides (LPS). Assembly in heterologous receptor complexes as well as signaling through both MyD88 and TRIF adaptor proteins, as unmatched by other TLRs, could underlie its versatile response options, probably also in a cell type-dependent manner. We show that microglia, the CNS macrophages, react to diverse LPS variants, including smooth (S) and rough (R) LPS chemotypes, with cytokine/chemokine induction, MHC I expression and suppression of myelin phagocytosis. The TLR4 co-receptor CD14 was shown in peritoneal macrophages to be essential for S-LPS effects and the link of both S- and R-LPS to TRIF signaling. In contrast, *cd14*$^{-/-}$ microglia readily respond to S- and R-LPS, suggesting an a priori high(er) sensitivity to both chemotypes, while CD14 confers increased S- and R-LPS potencies and compensates for their differences. Importantly, CD14 controls the magnitude and shapes the profile of cyto/chemokine production, this influence being itself regulated by critical LPS concentrations. Comparing reactive phenotypes of microglia with deficiencies in CD14, MyD88 and TRIF (*cd14*$^{-/-}$, *myd88*$^{-/-}$, *trif* $^{lps2}$), we found that distinct signaling routes organize for individual functions in either concerted or non-redundant fashion and that CD14 has contributions beyond the link to TRIF. Modulation of response profiles by key cytokines finally reveals that the microglial TLR4 can differentiate between the class of LPS structures and a self-derived agonist, fibronectin. It thus proves as a sophisticated decision maker in infectious and non-infectious CNS challenges.

# Cholinergic markers are altered in two different models of traumatic brain injury.

Cornelius Kurt Donat[1], Peter-Georg Hoffmeister[1], Bernd Walter[2,5], Winnie Deuther-Conrad[1], Martin U. Schuhmann[3,4], Cornelia Voigt[4], Reinhard Bauer[5], Jürgen Meixensberger[4], Peter Brust[1]

[1]Forschungszentrum Dresden - Rossendorf e. V., Forschungsstandort Leipzig -Interdisziplinäre Isotopenforschung Abteilung Neuroradiopharmaka, Permoserstr. 15, 04318 Leipzig, Germany

[2]Department of Neurosurgery, Zentralklinik Bad Berka, Germany

[3]Department of Neurosurgery, Eberhard Karls University Tübingen, Germany

[4]Department of Neurosurgery, University of Leipzig, Germany

[5]Institute of Molecular Cell Biology, Friedrich-Schiller-University Jena, Germany

Traumatic brain injury (TBI) is the leading cause of death and disability in childhood, adolescence and early adulthood and often results in cognitive impairments. There is evidence from behavioural animal experiments and patient studies that those cognitive deficits are related to functional alterations within the cholinergic system. The present study was performed to investigate cholinergic markers in two different animal models of TBI with emphasis on the time-course of posttraumatic events and critical brain regions. The identification of sensitive targets within the cholinergic system is a precondition for the development of radioligands for neuroimaging of TBI patients with Positron-Emission-Tomography (PET).

Male Sprague -Dawley rats were randomized into three groups (post- TBI survival time: 2 h, 24 h and 72 h), anaesthetized and subjected to sham injury (control, n = 8) or controlled cortical impact injury (CCI) (n = 8) with 2 mm depth of impact at a velocity of 4 m/sec.
Thirteen newborn piglets (post- TBI survival time: 6 h) underwent fluid percussion (FP) injury (n = 7) or sham operation (n = 6) with an impact pressure of 3.8 ± 0.3 atmospheres.
For both species, cryostat brain sections were cut (rat 12 µm, pig 20 µm) and density of nicotinic (nAChR; a7, a4*/a3*), muscarinic (mAChR; M1 -M5) acetylcholine receptors and the vesicular acetylcholine transporter (vAChT) were assessed with in vitro autoradiography. Additionally, histochemical staining of the acetylcholinesterase (AChE) was performed.

A significant decline in the receptor density of the a4*/a3* nAChR up to -33% was found in injured rats within brain regions critical for cognitive processes (thalamus, basal forebrain). In contrast, the brains of injured piglets did not reveal significant changes in receptor density.
The a7 nAChR density was drastically reduced (up to -47%) in injured rats at all time points and in piglets. Rats showed early decline of receptor density in 14 of 15 investigated brain regions (including hippocampus, thalamus, basal forebrain and cortex), while in piglets impairment was found especially in the hippocampus.
The mAChR showed smaller (~-20%), time-dependent reductions in injured rats and in piglets. However, almost identical brain regions were affected as found for a4*/a3* nAChR.
Histochemical staining indicated region-dependent increases and decreases in AChE activity in rats after TBI (~+/-20%) in contrast to injured piglets where only increased AChE-activity (+20%) was found.

In conclusion, cholinergic markers are significantly altered after experimental TBI independently of species, age and model. Even though differences in methodology do not allow direct comparisons between both models, results indicate common mechanisms of cholinergic changes after TBI. Considering the role of cholinergic markers for cognition in the brain, it seems likely that these alterations contribute to attention and memory deficits. Identifying the underlying mechanisms, for instance with PET, could help to ameliorate deficits in TBI-patients.

# Crucial role of CB1 receptors on hippocampal GABAergic neurons in brain aging

Önder Albayram[1], Judith Alferink[1, 2], Anastasia Piyanova[1], Karola Poppensieker[1], Krisztina Monory[3], Beat Lutz[3], Andreas Zimmer[1]

[1]University of Bonn, Institute of Molecular Psychiatry, 53127 Bonn, Germany
[2] University of Bonn, Department of Psychiatry, 53127 Bonn, Germany
[3]Johannes Gutenberg University Mainz, Department of Physiological Chemistry, 55099 Mainz , Germany

Brain aging is associated with cognitive decline accompanied by progressive neuroinflammatory changes. It is suggested that the age-dependent increase in neuroinflammation substantially contributes to age-related memory deficits. However, the molecular and cellular mechanisms that might contribute to these processes are still unknown. The endocannabinoid system is involved in the regulation of glial activity therefore we asked whether the early cognitive decline in animals lacking cannabinoid 1 receptors (Cnr1 -/-) is associated with enhanced neuroinflammation. The age-dependent increase in astrocyte and microglia cell numbers, the ratio of CD40 expressing activated microglia and the interleukin-6 levels were elevated in the Cnr1-/- mice. This enhanced glial activity was accompanied by a loss of principal neurons in the hippocampus and deficits in spatial learning. A principal role of enhanced neuroinflammation in the aging phenotype of Cnr1-/- animals is suggested by the fact that the neuronal loss and learning deficits correlated with the location and onset of the inflammatory changes. Surprisingly, deletion of CB1 receptors from the forebrain GABA-ergic, but not from the glutamatergic neurons, led to similar age-related changes as observed in the constitutive knockout animals. In the present study, we show that reduced CB1 receptor signaling impairs the glial control by GABA-ergic cells. The resulting enhanced neuroinflammation, elevation of IL-6 levels further aggravate GABA-ergic neuronal activity in the hippocampus leading to further deficits in glial control, progression of pathological changes and thus to an early onset of age-related changes in the brain. Our results suggest that CB1 receptor activity on hippocampal GABA-ergic neurons is necessary for glial activity regulation, for protection against neuroinflammation and thus against age-dependent cognitive deficits.

# Cyclosporine protects RGC-5 cells against excitotoxicity

Maximilian Schultheiss[1], Tomasz Mlynczak[2], Sven Schnichels[1], Johanna Hofmann[1], Peter Szurman[1], Karl Ulrich Bartz-Schmidt[1], Martin Stephan Spitzer[1]

[1]Centre of Ophthalmology, University Eye Hospital Tuebingen, Tuebingen - Germany
[2]Wroclaw Medical University, Eye Hospital, Wroclaw - Poland

Purpose: Cyclosporine (CSA) is widely used in clinical practise as an immunosuppressive drug. Furthermore, CSA showed neuroprotective properties in several neurologic disorders. However, nearly no data exists about CSA and its possible neuroprotective effect on retinal ganglion cells (RGC).
Methods: Cells of an immortalized RGC cell line (RGC -5) were stressed with 10mM glutamate for 24h with or without adding 1- , 3- , 6- or 9Âµg/ml of CSA to the culture medium. Cell morphology and cell death, via propidium iodide staining, were investigated with phase contrast and fluorescence microscopy. Cell viability and cell density were analyzed by MTS assay and crystal violet staining, respectively. Apoptosis was determined by analyzing caspase 3/7 activity and Annexin V+/ PI- flow cytometry, respectively.
Results: The incubation of RGC-5 cells with 10mM glutamate for 24h induced cell-death ($p<0.0005$), decreased overall cell viability ($p<0.0005$) and cell density ($p<0.0005$) compared to controls. The coincubation of 9Âµg/ml CSA with 10mM glutamate for 24h led to an increase in cell viability ($p<0.0005$) and cell density ($p<0.0005$) compared to RGC-5 cells incubated only with 10mM glutamate. Furthermore, the addition of 9Âµg/ml CSA to 10mM glutamate reduced caspase 3/7 activity ($p<0.0005$) and significantly reduced the amount of Annexin V+/ PI- cells compared to RGC -5 cells incubated with 10mM glutamate without the addition of CSA. The neuroprotective effect was dose-dependent and decreased with lower concentrations of CSA.
Conclusion: CSA can effectively protect RGC-5 cells against glutamate induced excitotoxicity and cell death. This neuroprotective effect seems to be mainly attributed to the antiapoptotic properties of CSA.

# Deep hypothermia affects morphological changes and decreases the Il-6 and MCP-1 release in LPS stimulated BV-2 Microglial cells

Annemarie Krauß[1], Sonja Wollersheim[1], Poya Soltani[1], Felix Berger[1,2], Katharina R.L. Schmitt[1]

[1]Deutsches Herzzentrum Berlin, Department of Congenital Heart Diseases, Augustenburger Platz 1, 13353 Berlin, Germany[2]Department of Pediatric Cardiology, Charité-Universitätsmedizin Berlin, Campus Virchow, Berlin

Background:
Deep Hypothermia is a standard method for neuroprotection during cardiac surgery in children. With major advances in diagnostic, surgical, anesthetic and cardiopulmonary bypass technology, the mortality rate for repair of congenital heart diseases has been reduced to values under 5%. Today the most serious risk factors affecting the long-term neurological outcome are neurological complications during and after corrective surgery. However, the cellular mechanisms which are induced by deep hypothermia have not been clearly understood. Therefore we investigated the effects of deep hypothermia and rewarming on BV -2 microglial cells which retain most of the morphological and functional properties seen in primary microglial cells.
Methods:
BV-2 microglial cells were exposed to 17°C for 2 hours, slowly rewarmed to 37°C within 2 hours and were observed under normothermic conditions for additional 24 hours. In order to simulate the cardiopulmonary bypass we stimulated the microglial cells for 4 hours with 1µg/ml Lipopolysaccharid (LPS), a major constituent in the outer membrane of gram-negative bacteria. To detect morphological changes during cooling and rewarming cells were stained with 4',6 -Diamidin-2-phenylindol and isolectin B4. The viability of BV -2 microglial cells was quantified using MTT assay (3-(4,5-Dimethylthiazol-2-yl)-2,5-iphenyltetrazoliumbromid).
The pro-inflammatory cytokine IL-6 and chemokine MCP-1 release was investigated by ELISA.
Results:
Cell viability was temperature independent during the observation period (24 hours). However deep hypothermia led to morphological changes from a ramified and resting status under 37°C to amoeboid shaped cells under 17°C even without LPS stimulation.
The release of IL-6 was significantly decreased 4 hours after the start of the experiment in comparison to the normothermic control. Interestingly, after 24 hours the IL-6 release assimilated for both groups. Moreover, MCP-1 release was significantly decreased after 4 and 24 hours in the hypothermic group.
Conclusion:
Deep hypothermia had no influence on the cell viability but led to morphological changes in BV-2 microglial cells. The pro -inflammatory cytokine and chemokine release of IL -6 and MCP -1 decreased under hypothermic conditions. Interestingly the MCP-1 release resisted down regulated until 24 hours. However the IL-6 release assimilated over time in both groups. Hypothermia differently modulated the inflammatory respons of BV-2 microglial cells.

# Detecting the neurodegenerating effects of oxidative stress induced by microinjection of iron in the mouse brain

Anne Suttkus[1], Markus Morawski[1], Gert Brückner[1], Thomas Arendt[1]

[1]Universität Leipzig, Paul Flechsig Institute for Brain Research, Jahnallee 59, 04109 Leipzig, Germany

Oxidative stress is one of the key-factors in the development and progression of neurodegenerative diseases like Alzheimer`s (AD) and Parkinson`s disease (PD). It is known that free radicals produce oxidative damage, particularly on neuronal lipids, proteins, nucleic acids and sugars in brains of AD patients. Iron is believed to contribute to oxidative stress in AD brains by catalyzing the generation of free radicals. Especially the highly reactive hydroxyl radical (OH-) leads to neurodegeneration by damaging cell membranes and DNA. Here, we investigate the degenerative effects of iron in the mouse brain. Therefore, wildtype mice were microinjected with 0,2µl of a 20mM solution of ferric chloride into the left barrel field. Mice belonging to the control group received an equal volume of 0,9% NaCl with the pH adjusted to the ferric solution. After 24h alternatively 72h the mice were perfused intracardially under deep anesthesia. Brains were removed, sectioned and analyzed using Fluorojade-staining as well as H2AX-labelling to reveal neuronal degeneration. These methods help us to assess the degree of damage, caused by iron-induced oxidative stress. Surprisingly, the range of neurodegeneration in the brain was smaller after 72h in comparison to 24h. This suggests an early start of regenerating processes that will be analyzed in future projects.

# Effect of cladribine on primary rat microglial cells

Vikramjeet Singh[1], E.V. Voss[1], M. Stangel[1]

[1]Hannover Medical School, Department of Neurology, Carl-Neuberg Strasse 1, 30625 Hannover, Germany

Cladribine (2-CdA) is an immunosuppressant that has recently been shown to modulate the clinical course of multiple sclerosis (MS). Its mode of action is thought to involve the long lasting leukopenia that is achieved after the administration of 2-CdA. It is well-known to induce apoptosis in leukemic and several other neoplastic cells. Furthermore, 2-CdA crosses the blood-brain-barrier and thus may also exert its effect on cells of the central nervous system (CNS), and in particular on microglia.

In this study, we have tested the effect of 2- CdA on proliferation and induction of apoptosis in primary rat microglial cells. Proliferation of microglia was assessed by immunostaining of incorporated BrdU in dividing cells and apoptosis was measured via flow cytometric analysis of Annexin-FITC binding to phosphatidylserine (PS) proteins present on the outer cell membrane of apoptotic cells. 2- CdA (0.1 -10 μM) inhibited proliferation of rat microglial cells and this effect was not affected by adding dipyridamole (DP), a nucleoside transporter inhibitor which suggests another mode of 2-CdA entry into the cells. 2-Deoxycytidine, a competitive substrate for cytidine kinase completely reversed the inhibitory effect of 2- CdA on microglia proliferation, revealing importance of 2-CdA phosphorylation for its action. Furthermore, we observed a concentration and time dependent effect of 2-CdA (1- 10 μM) in the induction of microglia apoptosis which was significant after 48 h of drug treatment. The most pronounced effect was observed at 96 h of incubation.

These results demonstrate that 2-CdA has also profound effects on microglia, the resident immune cells in the CNS. Further work is required to explore these effects also in vivo.

# Tenascin-C-activated signalling pathways in the migration of human glioma cells

Nicole Brösicke[1], Björn Scheffler[2], Andreas Faissner[3]

[1]Department of Cellmorphology and Molecular Neurobiology, Ruhr-University Bochum, 44801 Bochum, Germany
[2]Institute for Reconstructive Neurobiology, LIFE and Brain, University of Bonn, Bonn, Germany
[3]Department of Cellmorphology and Molecular Neurobiology, Ruhr-University Bochum, 44801 Bochum, Germany

The WHO class IV tumour glioblastoma multiforme corresponds to the most malignant form of glial tumours. Patients suffering from this fast progressing neoplasia mostly die within 14 months after diagnosis. On the molecular level, these tumours show a notable overexpression of the glycoprotein tenascin-c (TN-C). Produced by the tumour cells themselves TN-C could be ascertained as a migration-enhancing protein. It displays a multimodular composition and alternative splicing of fibronectin type III domains. In our study, the function of different domain combinations has been analyzed with regard to several biological parameters.
We could determine the epitopes of newly generated monoclonal antibodies against TN-C by using recombinantly expressed TN-C fragments. Immunohistological analysis of tumour specimen confirmed the high concentration of TN-C within glioma tissue and identified distinct expression patterns for each antibody.
To elucidate the effects of the TN-C fragments on migration behaviour and migration associated structures (stress fibres and focal adhesion sites), immunocytochemical stainings of cell lines growing on TN-C substrates was examined. Furthermore, migration assays were performed. The TN-C fragments individually influenced the morphology and the migration behaviour of human glioma cells via different signalling pathways. Beyond the effect on the cytoskeleton, some domain combinations influenced the migration of glioma cells. The addition of antibodies against the TN-C domains TNfnD, TNfnA2 as well as against the ß1-subunit of integrin receptors significantly decreased the migration. Therefore, it seemed plausible to assume that some fragments modulated migration by signalling pathways that include TN-C and ß1-Integrins. Significant differences could be detected between the effects of large splice variant TNfnALL and the intact TN-C-molecule. While cells of the WHO IV cell lines U-373-MG and U-251-MG showed a complete loss of stress fibres and focal adhesion sites combined with a minimized migration activity after being cultured on TNfnALL, WHO III U-87-MG cells developed distinct stress fibres and focal adhesion sites on the same substrate. Blocking the ß1-subunit of integrins inversed these effects and demonstrated the importance of this integrin-subunit for the morphology and migration of glioma cells.
Besides this the migration behaviour on the large splice variant TNfnALL as substrate in combination with the blocking of different proteins (ß1-Integrin, TNfnA2, TNfnD) suggested the involvement of many different pathways implicated in the migration processes of human glioma cells. Blocking the ß1-Integrin-subunit on TNfnALL led to an increase of migration. This effect might be associated with signalling pathways including the heparansulfate proteoglycans Glypican-1 and Syndecan-4 or the integrin avß3. Furthermore, it could be demonstrated that TNfnALL causes a decrease in the activity of the small GTPase RhoA that could result from a changed activation state of integrin receptors.
We revealed a strong influence of the glycoprotein TN-C on the migration of glioma cells and contributing structures like stressfibres and focal adhesion sites. Furthermore it could be elucidated that many different signalling pathways are probably involved in these processes and that activation degrees of these pathways could differ between tumours, even when they exhibit the same pathological grade.

# Effect of laquinimod on cuprizone-induced demyelination in mice

Ramona Pförtner[1], Wolfgang Brück[1], Christiane Wegner[1]

[1]University Medical Center, Neuropathology, Robert-Koch-Str. 40, 37081 Göttingen, Germany[1]

Introduction: Laquinimod is a new oral immunomodulatory substance that has been shown to suppress active lesions in multiple sclerosis (MS) patients. Laquinimod inhibited the development of EAE through modulation of Th1/Th2 response.
Aim: The aim of this study is to assess the effects of laquinimod on demyelination, inflammation and axonal damage in the cuprizone model in C57BL/6 mice.
Methods: 10-week-old male C57BL/6 mice were fed 0.25% cuprizone for 6 weeks and were treated daily with laquinimod (25 mg/kg). The body weight of mice was assessed once per week. Brains were fixed for paraffin embedding after 6 weeks of cuprizone feeding. Paraffin sections were used for standard histology and immunohistochemistry. LFB-PAS staining was performed to assess demyelination in the corpus callosum semi-quantitatively. Immunohistochemistry using antibodies for amyloid precursor protein (APP), MAC3 and CD3 was performed to obtain densities of APP-positive axons, MAC3-positive macrophages and CD3-positive T cells. Mann-Whitney U tests were performed to test for statistical significance.
Results: Laquinimod-treated mice showed significantly less weight loss under cuprizone in comparison to controls. Myelin appeared relatively intact in treated mice, whereas control animals showed marked demyelination in the corpus callosum. Acute axonal damage as well as scattered macrophages and T cells were detected in the white matter of control animals. Less inflammation and axonal damage was observed in treated mice.
Conclusions: Our results show that laquinimod also has beneficial effects in the cuprizone model, where it might protect from myelin and secondary axonal damage. These results further support the premise that laquinimod may have a role in the future treatment of MS.

# Effects of proteasome inhibition on macrophages and on myelin degradation during peripheral nerve degeneration in vivo and in vitro

Heike Siebert[1], Stefan Denninger[2], Brigitte Maruschak[1], Wolfgang Brück[1]

[1]University Medical Centre, Institute of Neuropathology, Robert-Koch-Str. 40, 37075 Göttingen, Germany
[2]Dept. Anesthesiology, University Hospital Regensburg, 93095 Regensburg, Germany

The proteasome-ubiquitin system plays an important role during cellular recycling processes of proteins. It comprises several degradation steps that can be inhibited by different synthetic inhibitors. In peripheral nerves, proteasomal degradation also appears to be an essential part of Wallerian degeneration which takes place after toxic or traumatic injury. During axon and myelin degradation an increasing number of peripheral blood cells (mainly macrophages) ensure the phagocytosis of the accruing cell debris.
Here we investigate whether in vivo systemic application of proteasome inhibitors after peripheral nerve axotomy affects macrophage migration or phagocytosis on the one hand and how it influences axon degeneration or myelin degradation on the other. In addition we observe the direct influence of these inhibitors in a co-culture system of nerve tissue and macrophages. In co-cultures the application of the reversible proteasome inhibitor MG132 was found to have significant effects on axon preservation (8.6fold attenuation) and myelin preservation (3fold). MG132 also diminished the number of invading macrophages (2.6fold) and reduced dramatically the phagocytic capacity of these cells. No effect on macrophage parameters was found after application of Lactacystin in equimolar concentrations, but a distinct positive effect on axon (3.3fold) and myelin (1.5fold) preservation. The application of MG132 in vivo resulted also in distinctly preserved axons, but at lower magnitudes: 2.6fold after intraperitoneal injection, 1.9fold with gelatine sponge). Less conspicuous effects were observed for myelin preservation and mitochondria occurrence. On macrophage parameter the effect of MG132 in vivo was the opposite of that in co-culture data: intraperitoneal application of the inhibitor had no effect on macrophage number or size nor on the amount of the incorporated myelin, however the latter parameters were slightly increased after applying the inhibitor by means of a gelatine sponge. The total number of invading macrophages was not influenced by either application method.
However, we propose that the proteasome inhibitor MG132 in the nervous system affects axons and myelin directly and that it can be directed to act protectively on axons. From the distinct results in vitro and the less conspicuous effects in vivo we conclude that in live situations some compensating mechanisms diminish the effects of the proteasome inhibitor whereas it acts directly on the cells in vitro. The continuation of in vivo experiments might reveal a possible therapeutic relevance for proteasome inhibitors in models for peripheral nerve degeneration.

# Expression Analysis of Native and Cultured Microglia from Conditional NF-κB RelB Knockout Mice

Robert Wilke[1], Ronny Haenold[1], Christian Günschmann[1,2], Anne Gompf[1], Marc Riemann[1], Falk Weih[1]

[1]Leibniz-Institute for Age Research - Fritz-Lipmann-Institute (FLI), Immunology, Beutenbergstr. 11, 07745 Jena, Germany

[2]present address: University of Cologne, Center for Molecular Medicine Cologne (CMMC), Departments II of Internal Medicine, Surgery and Dermatology, Robert-Koch-Straße 21, 50931 Cologne, Germany

Because of their myeloid origin and immunological function in the central nervous system (CNS) microglia are designated as "brain macrophages". Their characteristic feature is the (patho-)physiological reaction to acute CNS injuries and chronic neuronal diseases. In this context, primary resting microglia are activated by molecules released from affected or injured cells, leading to morphological and physiological phenotypes of dead cell scavenging and chemokine-segregating immune-effector cells. In numerous studies the classical NF-κB pathway (RelA-p50 dimer activation) has been identified as a major signaling cascade for microglial activation [1,2]. However, neither putative functions nor the expression of components of the alternative NF-κB pathway (RelB-p52) has been investigated in microglia so far. This project focuses on the expression of the NF-κB subunit RelB in cultured primary mouse microglia as well as in microglial cells freshly isolated from postnatal and adult brain. We also analyze the efficiency of *relB* gene deletion in microglia of conditional RelBflox mice with myeloid cell-restricted Cre recombinase activity (RelBflox;LysM-Cre line).

For *in vitro* expression studies, postnatal (P4) brain hemispheres were dissociated and glial cells were cultured for three weeks, followed by FACS analysis of F4/80-positive microglia. *Ex vivo* isolation of native microglia from postnatal and adult brains was performed by magnetic cell separation (MACS) using CD11b-tagged microbeads. Microglia populations were analyzed by Western blotting for the expression of RelB in wild-type cells and its depletion in cells derived from conditional knockouts.

Our results show that RelB and its dimerization partner p52 are expressed in primary cultured as well as in native microglia derived from postnatal brain. Analysis of cultivated microglia from myeloid -specific RelB mutants revealed a significant reduction of RelB protein levels. We are currently investigating the expression profile of microglia freshly isolated from adult brain. Thus, RelBflox;LysM-Cre mice provide an attractive tool to study neuro-immunological functions of the alternative NF-κB pathway in microglia.

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# Galanin-receptors in microglia

Marlena Beyreis[1], Sandra Wintersteller[2], Barbara Kofler[2], Hubert H. Kerschbaum[1]

[1]University of Salzburg, Department Cell Biology, Hellbrunnerstrasse 34, 5020 Salzburg, Austria
[2]Department of Pediatrics, Laura Bassi Centre of Expertise THERAPEP, Paracelsus Medical University, Salzburg, Austria

The neuropeptide, galanin, is synthesized in different brain areas and co-localizes with acetylcholine. It participates in nociception, wake-sleep rhythm, feeding, control of blood pressure, and cognition. At the cellular level, galanin modulates neuronal excitability as well as the activity of macroglia and microglia. Microglial cells are representatives of the immune system in the central nervous system. Because galanin is up-regulated during inflammatory processes, this could indicate that it is involved in the communication between nervous and immune system during neuro-inflammatory processes. Interestingly, microglial cells and galanin have been linked to neurodegenerative diseases, like Alzheimer's disease. Microglial cells are activated either by on-signals, like bacterial compounds, or off-signals, like withdrawal of suppressive factors. We evaluated whether the murine microglial cell line, BV-2, expresses the galanin peptide family members - galanin, alarin, galanin message-associated peptide - and the known galanin receptors (GalR1-3).
Using real time PCR, we identified expression of galanin, GalR2 and GalR3, but not of alarin and galanin-like peptide in BV-2 cells. Whereas galanin and GalR2 expression has been reported previously in BV-2 cells, the expression of GalR3 is new and BV-2 cells are up-till know one of the few cell lines which express this receptor endogenously. Preliminary experiments indicate that galanin modulates phagocytosis in BV-2.
Our data indicate that the galanin system plays a role in microglial function.

# Histochemical characterization of the neurovascular unit and a novel quantification of blood-brain barrier damage after embolic stroke in rats

Wolfgang Härtig[1], Jens Grosche[1], Johann Pelz[1,2], Dietmar Schneider[2], Christopher Weise[2], Ute Bauer[1], Johannes Kacza[3], Ulrich Gärtner[1], Carsten Hobohm[2], Dominik Michalski[2]

[1]University of Leipzig, Paul Flechsig Institute for Brain Research, Pathophysiology of Neuroglia, Jahnallee 59, 04109 Leipzig, Germany
[2]University of Leipzig, Department of Neurology, Leipzig, Germany
[3]University of Leipzig, Faculty of Veterinary Medicine, Department of Anatomy, Histology and Embryology, Leipzig, Germany

Currently, treatment strategies in acute ischemic stroke are limited despite numerous efforts. Many approaches of preclinical-to-clinical translation failed until now. Therefore, several attempts are focused on human-like animal models and consider a more-complex perspective of tissue salvaging involving endothelial, glial and neuronal components according to the neurovascular unit (NVU) concept. Among the main consequences of ischemia, blood-brain barrier (BBB) alterations affect NVU components, lead to brain edema and hemorrhagic transformation.

This study aims on a novel quantification method of BBB damage and affected tissue following experimental cerebral ischemia, closely to the human condition. Right-sided middle cerebral artery occlusion was induced in male Wistar rats using an embolic stroke model, followed by an intravenous application of fluorescein isothiocyanate (FITC)-tagged albumin and/or biotinylated rat IgG at 4 or 24 hours as BBB permeability markers with physiological significance.

FITC-albumin and biotinylated rat IgG displayed similar leakage and allowed the quantification of BBB permeability by fluorescence microscopy and - after immunohistochemical conversion into a permanent diaminobenzidine label - at light microscopic level. The reliable detection of NVU components with confocal laser-scanning microscopy was achieved by the following markers: rat endothelial cell antigen-1 (RECA) and laminin for vessels, the lectins from tomato (*Lycopersicon esculentum* agglutinin; LEA), potato (*Solanum tuberosum* lectin; STL) and *Griffonia simplicifolia* agglutinin (GSA) for vessels and microglial subpopulations, ionized calcium binding adaptor molecule 1 (Iba1), CD68 and CD11b for microglia, neutrophils and macrophages, S100ß for astroglia, as well as NeuN and HuC/D for neurons.

In conclusion, this study shows for the first time the usefulness of simultaneously applied FITC-albumin and biotinylated rat IgG as BBB permeability markers in experimental stroke, and specifies appropriate antibodies and lectins for multiple fluorescence labeling of NVU components. The presented newly elaborated protocols might facilitate a more-complex outcome measurement in drug development for cerebral ischemia.

# In vivo microglia depletion modifies short term plasticity in the CA1 Schaffer collateral pathway of mouse hippocampus.

Ismini Papageorgiou[1], Gina Eom[2], Frank L Heppner[2], Oliver Kann[3]

[1]Institut für Neurophysiologie, Charité - Universitätsmedizin Berlin, Campus Charité Virchow, Oudenarderstr. 16, Haus A, 13347 Berlin
[2]Institut für Neuropathologie, Charité - Universitätsmedizin Berlin,Campus Charité Mitte, Charitéplatz 1 | Virchowweg 15, 10117 Berlin
[3]Institut für Physiologie, Ruprecht-Karls-Universität, Im Neuenheimer Feld 326, 69120 Heidelberg

Microglia are resident macrophages, which comprise the innate immune system of the CNS. In the physiological brain environment "surveying" microglial cells constantly scan their surroundings and confirm homeostasis. Upon homeostatic imbalance, microglial cells switch their activity towards a reactive phenotypic spectrum, known as "activation" state.

Microglial cells are equipped with sensors for neuronal activity and they are potent to modify neurotransmission. It has been shown that the proinflammatory cytokines TNFa and IL1ß, which can be secreted by activated microglia, are fine-tuners synaptic scaling.
However, little is known about surveying microglia impact on neuronal signaling.

We sought to determine the physiological contribution of microglia to the Schaffer collateral pathway properties of the adult mouse hippocampus. Therefore we developed a model of in vivo depletion in adult BL6 mice, in which the Herpes Simplex Virus Thymidine Kinase (HSVTK) has been inserted under the promoter of CD11b microglia marker. This technique allows inducing microglia ablation by in situ application of ganciclovir, a blocker of HSVTK.

Investigation of the Schaffer collateral pathway properties in acute hippocampal slices by extracellular field potential recordings revealed changes in short term synaptic plasticity as well as in the electrical coupling between CA1 stratum radiatum and stratum pyramidale (fEPSP – Spiking coupling).

Our findings provide evidence that surveying microglia attenuate peripheral input propagation to the soma, hence they might contribute to prevent neuronal hyperexcitation in the adult mouse hippocampal circuitry.

# Influence of the complement fragment C5 and Albumin in rat glial cocultures as an in-vitro model of an experimental meningitis

Andreas Schöbel[1], Kathrin Hoppenrath[1], Daniel Hinkerohe[1/ 2], Nora Prochnow[1], Hannes Dambach[1], Christophe Berthold[1], Uwe Schlegel[2], Pedro Faustmann[1]

[1]Department of Neuroanatomy and Molecular Brain Research, Ruhr-University Bochum, Germany[1]

[2]Department of Neurology, Knappschafts-Hospital, Ruhr-University Bochum, Germany

Background: Acute bacterial meningitis induced by gram positive bacteria is accompanied by high liquor concentrations of complement factors and albumin. The aim of this study was to analyse the effect of the complement fragment C5 and albumin on mixed astroglial and microglial cocultures. Astrocytes are of crucial importance for the cerebral homeostasis and are coupled via gap junctions (Connexin43; Cx43) to a functional syncytium. Microglia are the immune competent cells of the brain.

Method: After an incubation (0,5h and 2h) with C5 or human albumin in concentrations of 2,25 or 4,5 µg/50*$10^3$, we investigated the cellular and molecular responses of microglial and astroglial cells. Microglial activation was detected using a monoclonal anti-ED1 antibody. The astroglial membrane resting potential (MRP) and the gap junctional intercellular astroglial coupling (Lucifer yellow uptake) were measured by using the patch clamp recording technique (whole -cell). The presence of Cx43 was analyzed by Western blotting and immunocytochemistry.

Results: Application of C5 and albumin led to a switch from the resting to an activated microglial phenotype. Furthermore, Cx43 protein expression was significantly reduced in C5 and albumin treated cocultures. In both tested conditions this effect was time- and concentration independent. The MRP was reduced and intracellular coupling was not compromised under C5 and albumin in the highest concentration.

Conclusion and discussion: This study demonstrates that C5 and Albumin changed the response of microglia and astroglial cells within 30 minutes. In further studies we want to analyze whether the binding of C5 to its special C5 receptor C5aR, a G-protein-coupled receptor, results only in a direct activation of signaling pathways like MAPK, ERK or have an indirect effect on the release of proinflammatory cytokines like Tumor Growth Factor-ß (TGF-ß). The conductance of Cx43 channels has been shown to depend on phosphorylation through diverse kinases, including protein kinase A (PKA) and protein kinase C (PKC). It is described that albumin activates astrocytes and microglia over the mitogen- activated protein kinase pathway or a TGF-ß receptor. These glial reaction could be the essential initial process in the development of life -threatening complications during bacterial meningitis, leading to cereberal edema and epileptic seizures.

# Is the voltage-dependent anion channel 1 (VDAC-1) involved in H-Ras activity-induced neuroprotection?

Sebastian Neumann[1], Konstantin Kuteykin-Teplyakov[1, 2], Rolf Heumann[1]

[1]Ruhr-University of Bochum, Faculty of Chemistry and Biochemistry, Department of Molecular Neurobiochemistry, Universitätsstraße 150, 44780 Bochum, Germany

[2]present address: Ludwig-Maximilians-University, Center for Neuropathology, Feodor-Lynen-Strasse 23, 81377 München, Germany

The small GTPase Ras is a key regulator of different signal transduction pathways regulating cell division, cell growth, differentiation and survival. For investigating the role of Ras in a neuronal system, Heumann et al. established the synRas mouse model in which constitutive active Val12Ha-Ras is expressed in postmitotic neurons due to the synapsin-promotor (Heumann et al., J Cell Biol 151, 1537-48 2000). One of the phenotypic changes of the synRas mice is protection against lesion -induced neuronal degeneration. Proteome studies of cortex and hippocampus of the synRas mice revealed changes in the expression level of several energy metabolism proteins and especially the voltage -dependent anion channel 1 (VDAC -1) had a 70% decrease. As a result of alternative splicing VDAC -1 is located in the outer mitochondrial membrane (mt -VDAC) and in the plasma membrane (pl-VDAC) in mice. Previously, each VDAC isoform has been claimed to be involved in apoptosis. Furthermore high expression levels of VDAC sensitises the cells for apoptosis whereas decreased expression lead to protection. Here we showed by RT -PCR that selectively the pl -VDAC-1 but not mt -VDAC-1 mRNA was decreased in hippocampus and cortex of adult synRas mice. Interestingly, in a cellular model of Parkinson disease 6-OHDA induced degeneration of PC12 cells was enhanced by overexpression of each VDAC-1 splice-variant and attenuated by co -expression of activated Ras. Using primary cortical cultures derived from synRas mice the selective decrease of pl -VDAC-1 mRNA was confirmed. In line with these data the enzymatic NADH-ferricynaide reductase activity of the pl -VDAC-1 was reduced in synRas cortical cultures. Furthermore, the activated Ras/MAP kinase pathway may influence the alternative splicing of VDAC-1 in primary cortical synRas neurons.

Taken together, constitutive activation of Ras may change the splicing activity towards VDAC-1 leading to a lowered expression of pl-VDAC-1 protein which could contribute to the mechanism of neuroprotection in synRas mice.

# Proinflammatory Cytokines from patients with Crohn's disease affect cultured enteric neurons

Svenja Häuser[1], Cornelia Irene Hagl[1], Sabine Heumüller[1], Elvira Wink[1], Ulrich Böcker[2], Karl-Herbert Schäfer[3]

[1]Klinik of Peddiatric Surgery,AGENS, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, D-68163 Mannheim, Germany

[2]Clinic of Internal Medicine, Medical Faculty Mannheim, University of Heidelberg, Theodor-Kutzer-Ufer 1-3, D-68163 Mannheim

[3]University of Applied Sciences, Life Science, Department of Computer Sciences and Microsystems Technology, Amerikastrasse 1, D-66482 Zweibrücken

Background: The Crohn's Disease (CD) belongs to the group of inflammatory bowel diseases. Its pathogenesis is not yet definitely clarified. According to the most acknowledged theory, it is characterized as a sort of barrier dysfunction of the intestinal gut and subsequently raising of antibodies against physiological parts of the gut and its colonization.
Changes in the serum composition concerning cytokine and trophic factors affects the enteric nervous system, either the glial cells or the neurons or both.

Methods: Myenteric plexus from newborn rat pubs was dissected by enzymatical digestion and dissociated. 10.000 myenteric plexus cells were seeded on coverslips and incubated with either 10µl, 50µl or 100µl control serum (n=10) or serum from patients with Crohns disease (n=10) with varying stages of activity.
Cultures were kept for 48h and fixed in 4% formaldehyde for 30 minutes. Immunostainings for neuronal (PGP 9.5) and glial (S100) markers were performed. Neurite outgrowth of the individual neurons were measured.

Results: In general, the incubation with serum from crohns disease patients led to a reduced neurite length of the regrowing neurons. Depending on the concentration of the Crohn serum the survival of the cells varied. The higher the concentration of the serum, the more cells died.

Conclusion: The serum changes which occur during Crohns disease influence the enteric nervous system and might also lead to extraintestinal manifestations.

# A characteristic establishment of immunoreactive extracellular matrix in the human lateral geniculate body.

David Lendvai[1]

Lendvai D[1], Morawski M[2], Bruckner G[2], Negyessy L[1], Baksa G[1], Glasz T[1], Matthews RT[3], Arendt T[2] and Alpar A[1]

The extracellular matrix surrounds different neuronal compartments in the mature nervous system and may establish typical structures around the somatodendritic part of certain neurons that are termed perineuronal nets. These mesh-like structures were shown in various vertebrates, with different frequency in the various brain fields. The present study reports that chondrotin sulfate proteoglycan-based matrix accumulation is differently established in the human lateral geniculate body. Using various antibodies we show that perisomatic matrix labelling is rather weak or absent whereas dendrites are outlined by small oval-shaped structures termed axonal coats (ACs, BrÃ¼ckner et al. 2008, Neuroscience 151:489- 504). Confocal laser scanning microscopy and electron microscopy showed that these typical structures are associated to synaptic loci on dendrites. At the same time, components recognized by other antibodies seem to relate rather to glial structures as shown by double labelling investigations. The various immunostainings show an evident difference in the intensity of immunoreactivity between magno- and parvocellular layers that confirm previous results in primates that underline the functional segregation between these layers.

# Minocycline attenuates the microglia-assisted glioma expansion and invasion

Katyayni Vinnakota[1], Darko Markovic[1, 2], Rainer Glass[1], Helmut Kettenmann[1]

[1] Cellular Neurosciences group, Max Delbrueck Centre for Molecular Medicine, Campus Buch,Robert Roessle Str.10, 13125 Berlin, Germany

[2]Department of Neurosurgery, Helios Klinikum Berlin-Buch, Schwanebecker Chaussee 50, 13125 Berlin

Minocycline attenuates the microglia-assisted glioma expansion and invasion

Vinnakota K1, Markovic DS1, 2, Glass R1 and Kettenmann H1.
1 Department of Cellular Neurosciences, Max-Delbrück Center for Molecular Medicine, 13125 Berlin, Germany. 2 Department of Neurosurgery, Helios Klinikum Berlin-Buch, Schwanebecker Chaussee 50, 13125 Berlin

Gliomas are most common brain tumors arising in humans. The hallmark of malignant gliomas is invasion into the brain parenchyma, which limits the efficacy of conventional therapies. Invasion is facilitated by the release of metalloproteases from the tumor that degrade the extracellular matrix. These enzymes are released as pro-forms and get activated upon cleavage by membrane -bound metalloproteases. We have previously reported that membrane type 1 metalloprotease (MT1 -MMP) is upregulated in glioma -associated microglia. Soluble factor(s) released from glioma cells trigger the expression and activity of MT1-MMP via Toll-like Receptor (TLR) signaling and activation of p38 MAPK (Markovic, D., et al 2009). The microglial MT1-MMP then activates glioma-derived pro-MMP-2 and promotes glioma invasion. Currently, we are investigating the role of minocycline, a second -generation tetracycline antibiotic, on microglia -assisted invasion of glioma using in vitro and in vivo mouse glioma models. Our data so far indicate that minocycline, a reported blocker of p38 MAPK and microglia cell activation, interferes with MT1- MMP activity and expression at the mRNA and protein levels in primary microglia exposed to glioma conditioned medium. In organotypic brain slices inoculated with glioma cells, minocycline induced a significant decrease in tumor expansion, with no effect in microglia-depleted slices. We also observed a reduction in tumor size in an experimental glioma mouse model upon minocycline administration. Minocycline treated mice also showed a decrease in MT1-MMP expression upon immunostaining. The results from our study indicate that minocycline could serve as a potential adjuvant to existing glioma therapies.

# Interaction of glioma cells with intrinsic brain cells

Min-Chi Ku[1], Rainer Glass[1], Helmut Kettenmann[1]

[1]Max-Delbrück-Centrum für Molekulare Medizin (MDC), cellular Neuroscience, Robert-Rössle-Str. 10, 13125 Berlin, Germany

Gliomas are the most common primary tumors of the central nervous system. Gliomas, like other solid tumors, are not a homogenous cellular mass, but contain many parenchymal cells, which have substantial impact on tumor progression. In the present study, we analyzed which cell types were influenced by soluble factors released from glioma cells. We have demonstrated previously that glioma cells attracted neural progenitor cells and microglial cells surrounded or in the tumor region. We now established a protocol to encapsulate glioma cells into a hollow fiber (HF) which allows the passage of diffusible molecules, but not cells.
With this model we can study the impact of released factors and exclude effects mediated by cell-cell contacts. The cells within these HF survived and expanded for at least 2 weeks after cell encapsulation as studied with metabolic and histological assays. In vivo, HF with glioma cells attract neural progenitor cells, microglial cells but not NG2 cells as compared with controls (HF loaded with fibroblasts in contra lateral hemisphere). Moreover, we observed an increase in GFAP positive astrocytes which either indicates an increased GFAP expression in individual astrocytes or an attraction of astrocytes. A small numbers of T cells were also found surrounding the glioma fiber. In addition, when implanted HF were encapsulated with microglial cells, no microglia attraction nor GFAP increase occurred. Furthermore, human glioma cells can also be entrapped in HF and attracted microglial cells and lead to an increase in GFAP positive astrocytes. Taken together, this approach demonstrated that glioma released factors act on microglia, astrocytes and on endogenous neural progenitor cells.

# Region- and cell-specific expression of matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase genes in the brain during de- and remyelination

Jelena Skuljec[1], Viktoria Gudi[2], Reiner Ulrich[3], Konstantin Frichert[4], Elke Voß[5], Refik Pul[6], Kirsten Wissel[7], Wolfgang Baumgärtner[8], Martin Stangel[9]

[1]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[2]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[3]University of Veterinary Medicine Hannover, Department of Pathology, Bünteweg 2, 30559 Hannover, Germany
[4]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[5]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[6]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[7]Hannover Medical School, Department of Otolaryngology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany
[8]University of Veterinary Medicine Hannover, Department of Pathology, Bünteweg 2, 30559 Hannover, Germany
[9]Hannover Medical School, Department of Neurology, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

Matrix metalloproteinases (MMPs) are a family of endopeptidases which together with tissue inhibitors of MMPs (TIMPs) play an essential role in tissue remodeling. Apart from being involved in the pathogenesis of demyelinating diseases like multiple sclerosis (MS), there is emerging evidence that MMPs also promote remyelination and repair. Since differences between white and gray matter lesions have recently come into the focus of MS research, we investigated region-specific expression pattern of eleven MMPs and four TIMPs in the cuprizone murine model in order to identify factors that regulate regeneration in the central nervous system (CNS).

Demyelination was induced in young adult C57BL/6 mice by feeding with 0.2% cuprizone following normal chow to allow remyelination. At nine different time points mRNA was extracted from microdissected cortex and corpus callosum as well as from isolated microglia from both regions and analyzed using quantitative PCR.
In comparison with the age-matched controls, TIMP-1 and MMP-12 mRNA were significantly upregulated in both areas during de- and remyelination. MMP-12 mRNA was mainly expressed by microglia. Interestingly, only in the white matter, MMP-3, MMP-11, and MMP-14 mRNA were upregulated during remyelination, while MMP-24 mRNA was significantly downregulated during demyelination. Moreover, different mRNA expression patterns of TIMP-3 and TIMP-4 were also observed in cortex and corpus callosum.
These findings suggest that, besides demyelination, MMPs contribute to the regulation of remyelination in the CNS, where MMP-12 production in microglia might account for neuroprotective events. Differences in MMPs and TIMPs expression levels in white and gray matter reveal different molecular mechanisms in these areas.

# TGF-beta in Interleukin-4-mediated alternative activation of microglia

Björn Spittau[1], Xiaolai Zhou[1], Kerstin Krieglstein[1,2]

[1]Institute of Anatomy & Cell Biology, Department of Molecular Embryology, Albert-Ludwigs-Universität Freiburg, Albertstraße 17, 79104 Freiburg, Germany

[2]Freiburg Institute for Advanced Studies (FRIAS), Albert-Ludwigs-Universität Freiburg, Albertstraße 19, 79104 Freiburg

Microglia are the resident immune cells of the central nervous system and are thought to be involved in a variety of neurodegenerative diseases. Stimulation with the bacterial lipopolysaccharide (LPS) is known to induce a microglia phenotype that promotes neurodegeneration of several distinct neuron populations including mDA neurons. This classical activation of microglia is characterised by the upregulation of inducible nitric oxide synthase (iNOS) and neurotoxic factors, such as TNF-alpha. However, recent studies suggest that microglia, like peripheral macrophages, can also be alternatively activated to induce tissue repair and cellular regeneration. Here we report that treatment of primary microglia with interleukin-4 (IL-4) results in upregulation of the alternative activation markers Arginase-1 and YM1. IL-4-mediated induction of this markers is dependent on active TGF-beta signalling. Interestingly, treatment of primary microglia with TGF-beta alone results in downregulation of classical activation markers and upregulation of alternative activation markers. Moreover, treatment of primary microglia cultures with IL-4 increased the secretion of TGF-beta, suggesting an involvement of endogenous TGF-beta in IL-4-mediated activation process. Taken together, our data indicate that alternative activation of microglia involves active TGF-beta signalling and further supports the role of TGF-beta as an anti-inflammatory molecule.

# THE ALTERNATIVE ACTIVATION OF MICROGLIAL CELLS IN ASSOCIATION TO NEURODEGENERATIVE DISEASES

Anke Witting[1], Anne Buttgereit[1], Valentina Reimer[1], Loretta Campanelli[1], Heike Tritschler[1], Patrick Weydt[1]

[1]Ulm University, Experimental Neurology, Helmholtzstrasse 8/1, 89081 Ulm, Germany

Microglia are the resident macrophages in the central nervous system and screen their environment for insults. One activation status of microglia, the alternative activation, is characterized by a resolution of inflammation. We have recently shown that the alternative activation is inhibited by mitochondrial dysfunctions associated with neurodegenerative diseases. We hypothesize that mitochondrial dysfunctions in neurodegenerative diseases are directly linked to the inflammatory activation state of the microglia and that targeting the metabolic status of microglial cells might be a potential therapeutic approach for these diseases. We treated primary mouse microglia cells with agonists and antagonists of PPAR -receptors in combination with LPS and/or the alternative activation inducing cytokine IL -4 and quantified the production of IL -6. PPAR receptors play an essential part in the regulation of metabolism. We found that PPAR-alpha has a stimulatory effect on the alternative activation of microglial cells. In addition we isolated primary mouse microglial cells from the G93A SOD1 mouse model of Amyotrophic Lateral Sclerosis and investigated the alternative activation. We found that the alternative activation was reduced in microglial cells from transgenic mice with the mutated form of the SOD-1 gen in comparison to corresponding controls. Targeting the metabolic system might therefore be a promising therapeutic approach for neurodegenerative diseases.

# The differential impact of the two antiepileptic drugs levetiracetam and valproate on glial properties in an in vitro co-culture model

Hannes Dambach[1], Daniel Hinkerohe[2], Christophe Berthold[1], Andreas Schöbel[1], Uwe Schlegel[2], Pedro Faustmann[1]

[1]Ruhr-Universität Bochum, Abteilung für Neuroanatomie und Molekulare Hirnforschung, Universitätsstraße 150, D-44780 Bochum, Germany

[2]Knappschaftskrankenhaus, Klinik für Neurologie, In der Schornau 23-25, D-44892 Bochum, Germany

Background: There are several studies that epileptic activity is accompanied by inflammatory processes. Astrocytes as well as microglia play an important role in cerebral inflammatory reactions and have also frequently been suggested to be involved in the pathogenesis of epilepsy. Astrocytes build a via gap-junction (connexin 43) coupled, functional syncytium that is necessary for preservation of cerebral homeostasis. Physiological microglia, the main inflammatory effector cells in the CNS, are found in a resting ramified form and comprise 5%–20% of the glial cell population. Typically, the number of microglia cells increases up to 30% in inflammatory processes and they become activated. In the present study we evaluated the effect of the two antiepileptic drugs levetiracetam (LEV) and valproate (VPA) on the astroglial Cx43 expression and microglial activation.

Methods: Primary astrocytic cultures were prepared from brains of postnatal (P0–P2) Wistar rats and co-cultured with a physiological amount of 5% (M5) as well as 30% (M30) microglia, to mimic inflammatory conditions. After LEV and VPA incubation (50 and 100 µg/ml) microglial phenotypes were immunocytochemically classified. Astroglial Cx43 expression was detected by immunofluorescence and Western blotting. With regard to its putative anti-inflammatory potential the TGF-beta1 level was measured after LEV incubation by Sandwich-ELISA.

Results: LEV reduced the number of activated microglia and increased the Cx43 expression in M30 co-cultures. In addition the cytokine concentration of TGF-beta1 was dose-dependent increased after incubation with LEV. VPA inversely increases the total number of activated microglia in inflammatory and control co-cultures and does not clearly alter the Cx43 Expression.

Conclusion: Using our microglia-astroglia co-culture model, we could demonstrate that LEV and VPA differentially affect glial cells. In comparison to VPA, LEV enhances Cx43 Expression in M30 co-cultures to a physiological level, which is important for the intercellular metabolic and trophic support of neurons. In contrast to VPA, LEV also showed an anti-inflammatory potential on microglia cells under imitated inflammatory conditions. This effect could be related among others with the increased expression of the anti-inflammatory cytokine TGF-beta1 under LEV treatment.

# The fate of histone H3 during apoptosis in microglia

Barbara Klein[1], Ursula Lütz-Meindl[1], Hubert H. Kerschbaum[1]

[1]University of Salzburg, Department of Cell Biology, Hellbrunnerstraße 34, A-5020 Salzburg, Austria

Apoptosis is the most popular mode of cell death in multicellular organisms. Chromatin condensation and its nuclear marginalization is one of the most important nuclear apoptotic features. In the present study, we evaluated the cellular distribution of condensed chromatin, focusing on histone H3 in the microglial cell line BV-2 and in murine primary microglia. We used transmission electron microscopy and confocal laser scanning microscopy to localize condensed chromatin as well as histone H3-immunoreactive material. Apoptosis was triggered by UV irradiation.

UV-irradiated microglial cells showed chromatin condensation, but not fragmentation of the nucleus. Interestingly, electron-dense condensed chromatin was localized in the nucleus and in the cytoplasm. Outside of the nucleus, condensed chromatin was identified at the nuclear periphery, within the cytoplasm, and at the plasma membrane. Electron energy loss spectroscopy (EELS) revealed elevated concentrations of phosphorus in electron-dense nuclear and cytoplasmic condensed chromatin, which indicates nucleic acids in this structure. Furthermore, antibodies to histone H3 labeled these electron-dense structures in the nucleus and in the cytoplasm. Anti-histone H3 labeling was not only identified in nucleo- and cytoplasm, but also at the plasma membrane. The ultrastructural findings on the distribution of histone H3 were replicated using confocal microscopy. The cellular distribution of condensed chromatin as well as of histone H3 was similar in BV-2 cells and in primary microglial cells. Blockade of effector caspases with z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]- fluoromethylketone) suppressed translocation of condensed chromatin from the nucleus to the cytoplasm.

In summary, these results show that caspase activation in apoptotic microglia leads to a translocation of histone H3 from the nucleus to the plasma membrane.

# The influence of acute *Escherichia coli* infection on disease course and neurodegeneration of MOG-EAE

Katharina Friebe[1], Rieka Schallhorn[1], Sandra Ebert[1], Doron Merkler[2], Mathias Bähr[1], Katharina Hein[1]

[1]University Hospital Göttingen, Neurology, Robert-Koch-Straße 40, 37075 Göttingen, Germany
[2]University Hospital Göttingen, Neuropathology, Robert-Koch-Straße 40, 37075 Göttingen

**Background:** Neuronal and axonal damage lead to long-term disabilities in multiple sclerosis (MS). There is evidence that viral and bacterial infections can evoke or aggravate autoimmune diseases. However, only a few studies have addressed the question of whether bacterial infection has any impact on neurodegeneration. Lipopolysaccarides (LPS) of *Escherichia coli*, the most common pathogen of bacterial urinary tract infection, are potential modifiers of autoimmune inflammation.
**Objective:** To investigate the influence of acute *E.coli* infection on the clinical course of the disease and neurodegeneration in MOG-EAE, an animal model of autoimmune optic neuritis.
**Methods:** Female BN-rats were immunized with 80 ug $MOG^{Igd}$ to induce MOG-EAE. Rats were scored for clinical symptoms of EAE and weighed daily. One group ("early infection") received an inoculum of $10^6$ CFU/ml *E.coli* intraperitonially at day 7 post immunization. The second group ("late infection") received an inoculum of $10^6$ CFU/ml *E.coli* injected intraperitonially at day 1 of EAE. The corresponding control group received 1ml 0,9% NaCl. 36h after infection blood samples were taken, diluted and plated on agar plates and incubated at 37°C to evaluate the bacterial infection. Then 200mg/kg Cefriaxon was administered every 12h for 3 days to assure the survival of the animals. At the end of experiment (day 8 of EAE) blood and spleen samples were taken, diluted and plated on agar to check for residual infection. Retinas were flat mounted to quantify the RGC density. Histopathological analysis were performed on paraformaldehyde -fixed, paraffin embedded transverse sections of optic nerves by staining with Luxol fast blue, ED1 and Bielschowsky silver impregnation.
**Results:** The early infection with *E.coli* aggravates MOG-EAE ($p=0,024$). Also, the severity of the optic neuritis was more pronounced in the early infection group when compared to the control animals ($p=0,024$ for inflammation and $p<0,001$ for axonal damage). However, the extent of neuronal loss did not show any difference between *E. coli* and control group as observed by RGCs density. Late infection with *E. coli* did not substantially modulate the disease course. Also the severity of the optic neuritis was not influenced by late *E. coli* infection.
**Discussion:** There is evidence that viral and bacterial infections can stimulate autoimmune disease development. In our study, EAE disease course as well as the severity of optic neuritis was unaltered in rats infected with *E.coli* at day 1 of MOG-EAE. However, the early infection with *E.coli* leads to a more severe clinical course of the disease and aggravate the pathological changes of the optic nerve during autoimmune optic neuritis. LPS, a surface component of *E.coli*, is potent factor to boost autoimmune reaction, which may be caused by Toll-like receptor activation and consecutively overexpression of interleukins, when administered at the early stage of the disease.

# The P2 receptor antagonist PPADS supports recovery from experimental stroke in vivo

Ute Krügel[1], Alexander Beck[2], Heike Franke[1], Benjamin Grimmich[1], Thomas Krügel[1], Alexandra Lämmer[2]

[1]University of Leipzig, Institute of Pharmacology and Toxicology , Haetelstrasse 16-18, 04107 Leipzig, Germany
[2]Department of Neurology, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany

After ischemia of the CNS, extracellular adenosine 5´-triphosphate (ATP) can reach high concentrations due to cell damage and subsequent increase of membrane permeability. ATP may cause cellular degeneration and death, mediated by P2X and P2Y receptors. Therefore, the effects of the inhibition of P2 receptors by the antagonist pyridoxalphosphate-6-azophenyl-2',4'-disulphonic acid (PPADS) on electrophysiological, functional and morphological alterations in an ischemia model with permanent middle cerebral artery occlusion (MCAO) were investigated up to day 28. Rats received PPADS or vehicle intracerebroventricularly 15 minutes prior MCAO for up to 7 days. The functional recovery monitored by electroencephalography (quantitative EEG; qEEG) was improved by PPADS indicated by an accelerated recovery of ischemia-induced qEEG changes in the delta and alpha frequency bands along with a faster and sustained recovery of motor impairments. Whereas the functional improvements by PPADS were persistent at day 28, the infarct volume measured by magnetic resonance imaging (MRI) and the amount of TUNEL positive cells were reduced by PPADS only until day 7. The persistent beneficial effect of PPADS on the functional parameters without differences in the late (day 28) infarct size and apoptotic events suggests that the early inhibition of P2 receptors might be favorable for the maintenance or early reconstruction of neuronal connectivity in the periinfarct area after ischemic events.

# The role of the $p75^{NTR}$ in experimental inflammation of the CNS

Tobias Dallenga[1], A Bittner[2], W Jäger[2], P Vollmar[3], WH Oertel[2], N Sommer[2], JC Möller[2], B Hemmer[3], C Stadelmann-Nessler[1], S Nessler[1]

[1]Institute of Neuropathology, University Medical Center, Göttingen, Germany
[2]Neurology, Philipps-University , Marburg, Germany
[3]Neurology, Technical University, München, Germany

Permanent clinical disabilities in multiple sclerosis patients emerge from pathological axonal damage and loss. In this study, we show a major role of the low affinity neurotrophin receptor $p75^{NTR}$ for axonal damage in experimental autoimmune encephalomyelitis (EAE). EAE is induced by active immunization with the $MOG_{35-55}$ peptide and adoptive transfer of an encephalitogenic $MOG_{35-55}$ specific T cell clone in $p75^{NTR}$ deficient and wild type (wt) animals. After adoptive transfer the mice show similar disease incidence, onset and kinetics. Comparable degree and quality of inflammation were found by immunohistochemistry and quantitative RT-PCR in both strains at the peak of disease. However, $p75^{NTR}$ deficient animals suffered from significantly more severe disease in chronic disease stages due to increased axonal damage and loss. In vitro studies with bone marrow derived macrophages, activated lymphocytes and astrocytes from $p75^{NTR}$ deficient and wt animals revealed no difference in the expression of inflammatory related mechanisms previously associated with axonal damage such as NO and ROS production, cytokine patterns or the ability to stimulate T cells. In summary, our results suggest that the $p75^{NTR}$ receptor has axono- and/or neuroprotective effects in inflammatory CNS disease.

# The ß-amyloid precursor protein (APP) is a potent growth factor - Implications for Alzheimer's disease and Cancer

Vivek Venkataramani[1], Christian Rossner[1], Lara Iffland[1], Stefan Schweyer[2], Oliver Wirths[1], Thomas Bayer[1]

[1]University of Goettingen, Division of Molecular Psychiatry, Von-Siebold-Straße 5, 37073 Goettingen, Germany[1]
[2]University of Goettingen,Department of Pathology, Robert-Koch-Str. 40, 37075 Goettingen, Germany

The ß- amyloid precursor protein (APP) represents a type I transmembrane glycoprotein that is ubiquitously expressed. In brain it is a key player in the molecular pathogenesis of Alzheimer's disease. Its physiological function is however less well understood. Previous studies showed that APP is up-regulated in prostate, colon, pancreatic tumor and oral squamous cell carcinoma. In the present study, we show that APP has an essential role in growth control of pancreatic and colon cancer. Abundant APP staining was found in human pancreatic adenocarcinoma and colon cancer tissue. Interestingly, treating pancreatic and colon cancer cells with valproic acid (VPA, 2-propylpentanoic acid), a known histone deacetylase inhibitor (HDACi), leads to up-regulation of GRP78, an ER chaperone immunoglobulin binding protein. GRP78 is involved in APP maturation and inhibition of tumor cell growth by down -regulation of APP and secreted sAPPA. Trichostatin A (TSA), a pan-HDAC inhibitor also lowered APP and increased GRP78 levels. In contrast, treating cells with valpromide, a VPA derivative lacking HDAC inhibitory properties had no effect on APP levels. VPA did not modify the level of epidermal growth factor receptor, another type I transmembrane protein, and APLP2, a member of the APP-family, demonstrating the specificity of the VPA effect on APP. Small interfering RNA (siRNA)-mediated knock-down of APP also resulted in significantly decreased cell growth. Based on these observations, the data suggest that APP down-regulation via HDAC inhibition provides a novel mechanism for pancreatic and colon cancer therapy.

# Valinomycin-induced cell death in microglial cells

Hubert Hannes Kerschbaum[1], Barbara Klein[1], Katrin Wörndl[1], Ursula Lütz-Meindl[1]

[1]University of Salzburg, Department Cell Biology, Hellbrunnerstraße 34, 5020 Salzburg, Austria

Apoptosis has been associated with a collapse of intracellular K+ homeostasis. In the present study we evaluated whether an increase in K+ conductance is sufficient to mimic apoptosis induced by classical activators of apoptosis, like UV irradiation or exposure to staurosporine. Accordingly, we exposed the microglial cell line, BV-2, to the K+ selective ionophore, valinomycin. The Nomenclature Committee on Cell Death, characterized apoptosis by intranuclear accumulation of condensed chromatin, no structural changes in cell organelles, cell blebbing, and decreases in cellular volume (Galluzzi et al., Cell Death Differ. 2007 Jul;14(7):1237-43.). Exposure of BV -2 cells for three hours to valinomycin revealed three phenotypes at the ultrastructural level. The first phenotype was characterized by a minor chromatin condensation, but swollen mitochondria. The second phenotype was characterized by intense chromatin condensation and no swelling of mitochondria. The third phenotype showed condensed chromatin as well as swollen mitochondria. In addition, valinomycin promoted cell blebbing as revealed by scanning electron microscopy. In comparison to control cells, valinomycin-exposed cells showed only a minor increase in cell volume. Furthermore, following three hours of valinomycin exposure, only about 10 % of the cells showed DNA-labeling with propidium iodide. This indicates that the plasma membrane is still a barrier. To oppose a valinomycin-induced decrease in the intracellular K+ concentration, we exposed valinomycin-treated cells in high extracellular K+. This maneuver led to nuclear and mitochondrial swelling. In BV-2 cells, valinomycin caused an increase in intracellular Ca2+. Therefore, we exposed BV -2 cells to the membrane-permeable Ca2+ chelator, BAPTA -AM, before application of valinomycin. BAPTA-AM pretreated valinomycin-exposed cells showed a distribution of heterochromatin similar to control cells, but swollen mitochondria.
Our findings demonstrate that perturbation of intracellular K+ homeostasis is sufficient to disassemble the architecture of cell organelles and promotes cell death. However, valinomycin- exposed cells showed not all characteristics of apoptosis.

# Neuroprotection of Melissa officinalis after hypoxic-ischemic injury both In vitro and In vivo

Gholamreza Hassanzadeh [1], Azami Tameh Abolfazl[2], Bayat Mohammad[1]

[1]Anatomy Department, School of Medicine, Tehran University of Medical Sciences, Poorsina St. P.O.Box 1417613151, Tehran, Iran
[2]Anatomy Department, Faculty of Medicine, Kashan University of Medical Sciences, 5th of Qotb-e Ravandi Blvd. P.O.Box: 87155.111, Kashan, Iran

Brain ischemia initiates several metabolic events, some of which involve in the generation of free radicals. These free radicals mediate a large amount of damage that arises after transient brain ischemia as well as some neurodegenerative disorders. Melissa officinalis is considered as a helpful herbal plant in the prevention of various neurological diseases related with oxidative stress.
Here we examined the effect of Melissa officinalis on oxidant-induced neuronal death in a transient hippocampal ischemia as In Vivo model and a cortical neuronal culture system as In Vitro model.
Global ischemia was produced in anesthetized Wistar rats by 20 min bilateral carotid artery occlusion. Hippocampal injury was assessed 5 days later by morphological changes in neurons as well as caspase3 activity assay. Tunnel positive cells in Melissa treated ischemia group were significantly lesser than control ($p<0.01$). In Vitro stress model in mouse cortical neurons was induced by 5% O2 hypoxia for 24 hours which was indicated by 30% decrease in MTT assay and 120% increase in LDH release compare to control. There was around 40% decrease in PI positive neurons by 10ug plant extract administration ($p<0.01$). Methanolic extract of Melissa officinalis provided a relatively high protection against hypoxia induced oxidative stress both in vivo and vitro.
Our results show that Melissa officinalis could be considered an effective agent in various neurological diseases associated with ischemic brain injury.

# Poster Topic

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# Aberrant temporal brain activity during rest in patients with pain-predominant multisomatoform disorder

Michael Noll-Hussong[1], Alexander Otti[2], Harald Gündel[3]

[1]Klinik und Poliklinik für Psychosomatische Medizin und Psychotherapie des Klinikums rechts der Isar der Technischen Universität München, Langerstr. 3, 81675 München, Germany

[2]Abteilung für Neuroradiologie des Klinikums rechts der Isar der Technischen Universität München, Ismaninger Str. 22, 81675 München, Germany

[3]Klinik und Poliklinik für Psychosomatische Medizin und Psychotherapie des Universitätsklinikums Ulm, Am Hochsträß 8, 89081 Ulm, Germany

Patients with severe and disabling pain which cannot be explained by underlying organic pathology are common in all levels of health care and are typically difficult to treat for physicians as well as for mental health specialists. An important pathophysiological mechanism of this psychosomatic disorder seems to be a disturbed affective regulation which is reflected by functional and structural alterations in the medial prefrontal cortex, the anterior cingulate cortex and the insula. The strong spatial and temporal coherence of the spontaneous neural activity within these regions during a resting state recently led to the assumption of a fronto-insular network dedicated to interoception and personal salience. To investigate putative changes in the temporal activity of this circuit in terms of spontaneous BOLD-fluctuations during a resting state, 21 patients suffering from pain-predominant multisomatoform disorder and 19 age- and gender-matched controls underwent functional 3T-fMRI scanning. Data were analyzed using independent component analysis. Compared with the control-group, BOLD-oscillations within the FIN showed significantly stronger power densities between 0.2 and 0.24 Hz in the patients which might reflect a higher inner arousal and stressed vegetative state. Our results suggest altered computational processes in neural pain processing in chronic pain patients, especially concerning the empathetic route, and may lead to an in-depth neurobiological understanding of the well-known clinical impression of underlying emotional deficits in somatoform pain patients.

# Antidepressant-like features of mice with transgenic activation of Ras in differentiated neurons

Oliver Leske[1], Zoe Bichler[2], Rolf Heumann[1]

[1]Ruhr University Bochum, Faculty for Chemistry and Biochemistry, Biochemistry II -Molecular Neurobiochemistry-, Universitätsstr.150, 44780 Bochum, Germany

[2]Prous Institute for Biomedical Research, Barcelona, Spain

Brain-derived neurotrophic factor (BDNF) is implicated in clinical depression and its treatment. Administrations of antidepressants have been shown to enhance BDNF expression and phosphorylation of its cognate TrkB receptor. In contrast, stress exposure and depression are associated with down regulation of BDNF expression. Furthermore, an up regulation of adult neurogenesis in the hippocampus has been proposed to be correlated with drugs effective in the treatment of depression.

The Ras-mediated extracellular signal-regulated cascade (ERK) pathway is considered as a major BDNF/TrkB intracellular signalling pathway. Here, we test its possible contribution on antidepressant activity by utilizing a synRas transgenic mouse model expressing constitutively activated human Ha-Ras in differentiated neurons [Heumann et al., 2000 J Cell Biol 151: 1537].

SynRas mice showed elevated levels of activated Ras and activating phosphorylation levels of ERK in the cortex and hippocampus. Immunoblotting analysis revealed that chronic fluoxetine administration to wild type mice led to an increased Ras activation followed with subsequent elevation of ERK phosphorylation thus mimicking the synRas phenotype. Consistently, our results obtained in depression-associated animal models showed an antidepressant-like behavior of synRas transgenic mice compared to their wild type littermates. Furthermore, synRas mice exhibited a normal basal HPA-axis activity, but a suppression of corticosterone release in response to acute restraint stress. Interestingly, synRas mice displayed a reduction of the number of new born cells within the dentate gyrus of the hippocampus, indicating that the antidepressive-like behavior is not linked to increased neural progenitor proliferation.

Taken together, an antidepressant-like state was established in a genetic model of enhanced neuronal Ras signalling without correlation to hippocampal precursor cell proliferation. Our data support the idea, that the BDNF/Ras/ERK-pathway is critically involved in mood disorders and antidepressant activity. The results indicate that drugs that activate the Ras/ERK -Pathway should produce an antidepressant response. Furthermore, these results raise the hypothesis that genetic variations in the Ras, MEK or ERK activities, could contribute to the genetic or epigenetic susceptibility for depression.

# Behavioural and electrophysiological signs of the effect of lead on the CNS of rats in various routes of exposure

Zsuzsanna Máté[1], Edina Horváth[1], Leila Sárközi[1], Andrea Szabó[1], Gábor Oszlánczi[1], András Papp[1]

[1]University of Szeged, Department of Public Health, Dóm tér 10., 6720 Szeged, Hungary

Because of its widespread use, lead (Pb) is present everywhere in the environment. At the same time, it is one of the heavy metals notoriously harmful for human health. The nervous system is the main target of Pb, with consequences like occupational neuropathy and delayed mental development of children. From the use of leaded petrol, and in Pb processing and reprocessing industries, airborne particles are emitted, exposing people by inhalation. The size of particles entering the body is crucial in inhalation exposure. Whereas microscopic particles cannot pass the blood-brain barrier, submicroscopic particles (that is, nanoparticles, with dimension 100 nm and below) have high mobility within the organism, so these can have direct access to the CNS. Pb can contaminate various food items as well, so per os intoxication can also occur.

Our experiment was designed to compare the neurophysiological and behavioural effects of Pb in male Wistar-rats after exposure per os, through the airways, and combining the two.

For modelling airborn exposure, Pb oxide nanoparticles (mean diameter ca. 20 nm) were suspended in distilled water and instilled into the trachea of the rats (2 or 4 mg/kg b. w.) five times a week for 6 weeks. In per os treatment, 80 or 320 mg/kg b. w. Pb acetate (dissolved in distilled water) was given to the rats by gavage (also five times a week for 6 weeks). To combine the two routes of exposure, rats were treated per os with Pb acetate for 3 weeks, followed by intratracheal instillation of Pb-oxide nanoparticles for another 3 weeks, with the same timing and doses as above.

After the 6 weeks treatment period, open field activity of the rats was tested. Vertical and horizontal activity of the rats changed differently in the three experimental schemes. Ambulation time decreased significantly after per os and combined treatment, but showed significant increase after 6 weeks of intratracheal instillation. The effect on the time spent with rearing, immobility or local activity was also opposite in intratracheal treatment compared to per os and combined exposure.

Following the open field test the animals underwent electrophysiological investigation in urethane anaesthesia. Spontaneous and stimulus-evoked activity was recorded from the somatosensory, visual and auditory areas of the cortex.

In the spectral distribution of the spontaneous cortical activity, slow waves increased and fast waves decreased after per os treatment, and to a lesser extent also after the combined treatment, but changed hardly after intratracheal instillation of Pb oxide. The cortical sensory evoked potentials showed mostly dose dependently increased latency, which was particularly seen on the auditory and visual evoked activities.

The behavioural and electrophysiological parameters were both sensitive to the effects of Pb but the differences indicated that chemical form and route of exposure may have their own influence on the functional alterations seen in the CNS.

# CANNABINOID RECEPTOR AGONIST WIN55212,2 IMPROVES PREPULSE INHIBITION IN PSYCHOSOCIALLY STRESSED MICE

Ursula Havemann-Reinecke[1], Magdalena M. Brzozka[1], Andre Fischer[2], Peter Falkai[1]

1 University of Goettingen, Dept of Psychiatry and Psychotherapy, von Sieboldstr.5, D 37075 Göttingen, Germany
2 European Neuroscience Institute Göttingen, Griesebachstr.5 D 37077 [1]Germany

## INTRODUCTION

Cannabis, similar to psychosocial stress, is well known to exacerbate psychotic experiences and can precipitate psychotic episodes de novo in vulnerable individuals. Cannabinoid receptors 1 (CB1) are widely expressed in the central nervous system and are particularly important for the effects of cannabis. CB1 are stimulated by WIN55212,2 and inhibited by specific antagonist Rimonabant (SR141716A). Prepulse inhibition (PPI) is the phenomenon in which a weak prepulse stimulus inhibits the response to a startling stimulus. Schizophrenic patients display impairment of PPI. Similarly, chronic psychosocial stress decreases PPI in juvenile mice. The effects of cannabinoids on PPI in experimental animals are contradictory.

## AIM

In the present study, we investigated the synergistic effects of substances modulating the CB1 receptors and chronic psychosocial stress (social defeat) on PPI in mice.

## METHODS

Adult C57Bl/6J mice were exposed for three weeks of psychosocial stress using the resident-intruder paradigm and were subsequently acutely treated with CB1 agonist WIN55212,2 (3 mg/kg, i.p.) or vehicle only. One group of mice was additionally pre-treated with Rimonabant (3 mg/kg, i.p.). After the drug treatment animals were subjected to open field and PPI test.

## RESULTS

Psychosocially stressed mice treated with vehicle only showed decreased locomotor activity and impaired PPI similarly to published data for juvenile mice (Adamcio et al., 2009). Treatment with WIN55212,2 (I) decreased locomotor activity in open field in stressed and control mice and (II) 2 reversed effect of stress by increasing of PPI. The effects of WIN55212,2 on locomotor activity and on PPI were antagonized by Rimonabant suggesting an involvement of CB1 receptors in sensorimotor gating.

## CONCLUSION

Our data showed that an acute CB1 agonist treatment improves PPI in psychosocially stressed mice. Since previous studies showed the opposite effects of WIN55212,2 on PPI in rats (Schneider and Koch, 2002), it may thus be possible, that depending on the doses of cannabinoids/CB1 receptor agonists applied and environmental conditions (e.g. psychosocial stress) contradictory effects can be evoked by CB1 receptors.

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# Contextuals versus Fragmentals *highly gifted versus autistic thinking*

Michiel Goudriaan[1]

[1]St. Mathematisch Instituut ter bevordering van de Geneeskunst, neurologie biochemie , achterom 53, 2611 PM Delft, Netherlands

No abstract available

# Depression-like behavior of mice with induced ablation of both the mineralo- and the glucocorticoid receptor

Miriam Annika Vogt[1], Stefan Berger[2], Natascha Pfeiffer[1], Günther Schütz[2], Peter Gass[1]

[1]Zentralinstitut für seelische Gesundheit, AG Psychiatrische Tiermodelle, J5, 68159 Mannheim, Germany
[2]Deutsches Krebsforschungszentrum dkfz, Molekularbiologie der Zelle I, Im Neuenheimer Feld 280, 69120 Heidelberg, Germany

Current molecular and pathophysiological concepts of depression suggest that glucocorticoid receptors (GRs) play a crucial role in the pathogenesis and therapy of affective disorders. However, so far it is not known where in the brain GRs exert their effects and whether these receptor disturbances are cause or consequence of these diseases in humans.
The impact of forebrain-specific corticosterone receptors, i.e. the mineralocorticoid receptor (MR) and the GR, to mediate depressive-like behavior was analyzed in mutant mice with ablation of MR or GR as well as simultaneous ablation of both receptors in neurons of the forebrain. The ablation was induced at an age of 8-9 weeks using tamoxifen.
Forebrain-specific male MR, GR and MRGR mutants and their respective littermate controls underwent a behavioral test battery for basal and depressive-like behavior. MR and GR mutant mice showed an inconspicuous behavioral phenotype with respect to basal and depressive-like behavior when compared to their littermate controls. In contrast, MRGR double mutants exhibited also normal basal behavior but demonstrated learned helplessness, when exposed to uncontrollable and inescapable footshocks.
In summary, the combined but not single knockout of MR and GR in the forebrain of the adult mouse results in an increased vulnerability to develop a depressive-like state. These findings suggest that not only the GR, but also the malfunction of both MR and GR receptors play a role in the pathophysiology of affective disorders. This has also to be taken into account for therapeutic considerations.

# Effects of early stress on impulsivity and reward learning in Octodon degus: Behavioral studies using a novel animal model for ADHD

Jan Kunzler[1], Katharina Braun[1], Jörg Bock[1]

[1]Otto von Guericke University, Magdeburg, Institut of[l] Biologie, Leipziger Strasse 44 /Haus 91, 39120 Magdeburg, Germany

ADHD (Attention-Deficit Hyperactivity Disorder) is a heterogeneous behavioural disorder, which affects about 5-10 percent of the children and often persists until adolescence. Characteristic symptoms of ADHD are behavioural disturbances such as hyperactivity, attentional deficits and an enhanced impulsivity.
The following study is based on an animal model for ADHD, in which the behavioural symptoms can be induced by early stress experience (1 hour of social separation/day from day 1 to day 21) in the precocious rodent Octodon degus (Degu, trumpet-tailed rat). Early stressed degu pups display explorative hyperactivity and decreased attention towards species -specific vocalizations in a novel environment. These behavioural alterations are paralleled by a hypofunction of several brain areas such as the prefrontal cortex and alterations of neuronal structure in this brain area. The described dysfunctions and deficits can be partly normalized by treatment with methylphenidate.
In the present study we used this animal model to investigate impulsivity and learning performance in a reward learning task. To test this, the animals had to learn a FCN (fixed consecutive number) schedule in a skinner-box with two levers and one flap installed. To get a food-pellet as reward, the animal had to reach a defined minimum number of lever presses on the left lever (FCN-lever), before pressing the right lever (reward-lever). The number of FCN -lever presses needed to activate the reward lever was increased from 1 to 3 to 5 to 8, after reaching a defined criteria (45 pellets in less then 45 minutes on two following days). The number of lever presses and reward can be used as indicators for impulsivity and learning success. We found significant differences between the early stressed and control animals, since the stressed animals needed longer to reach the respective criteria, indicating deficits in reward learning. Interestingly, this deficit could be observed only in a subpopulation of the stressed animals, whereas another subpopulation obviously seemed to be resilient against the early stress experience. Moreover, the described differences between stressed and control animals were only found in males but not in females. Overall, male animals showed a significant better learning performance than the female animals. Our experiments will be extended to further investigate if the observed learning deficits in a subset of the stressed male animals are possibly related to alterations in impulsivity.
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# Chronic social stress modulated gene expression in astroglia in the hippocampus and prefrontal cortex

Carolina Araya Callis[1,2,4], Eberhard Fuchs[1,2,3], Gabriele Flügge[1,2]

[1]Clinical Neurobiology Laboratory, German Primate Center; [2]DFG Research Center of Molecular Physiology of the Brain (CMPB), Göttingen, Germany; [3]Department of Neurology, University of Göttingen, Germany; [4]GGNB Doctoral Program "Molecular Physiology of the Brain"

Glial dysfunction is regarded as a key factor in the pathophysiology of neuropsychiatric disorders including major depressive disorder. Several studies report on reductions in volume and number of glial cells as well as on glia specific changes of gene expression in the hippocampus and prefrontal cortex (PFC) in stress-related neuropsychiatric illnesses. Nevertheless, the exact mechanisms behind these changes remain to be elucidated.
It has been suggested that a shift in the fluid balance between the ventricles and the brain parenchyma could significantly contribute to the volume diminution. One of many proteins involved in maintaining the fluid balance within the brain is aquaporin 4 (AQP4), a water channel expressed in the brain specifically in glial and ependymal cells, but not in neurons. It has also been previously linked to cell proliferation, migration and differentiation and some studies relate it to the pathophysiology of depression.
On the other hand GLT1, a glial specific glutamate transporter, has been shown to be modulated by restraint stress in the hippocampus. Since extracellular glutamate levels may be detrimental to neurons, changes in GLT1 expression could account for some of the effects induced by stress in distinct brain regions. Therefore we investigated the potential changes in AQP4 and GLT1 mRNA expression in a chronic social stress model in male Wistar rats.
We found no changes of AQP4 mRNA expression both in hippocampus and PFC. However, stress upregulated GLT1 mRNA expression in the right PFC but not in the hippocampus.
We conclude that AQP4 channels do not play a role in hippocampal volume changes at least in this model, and further mechanisms responsible for the above stress -induced changes need to be clarified. Moreover, GLT1 upregulation might indicate an activation of glutamatergic neurotransmission, possibly associated with detrimental effects of stress on affect and cognition.

# Enhanced neuronal Ras activity modulates the adverse phenotype in a mouse model of Rett Syndrome

Daniela Damen[1], Oliver Leske[1], Rolf Heumann[1]

[1]Ruhr-University Bochum, Department of molecular Neurobiochemistry, Universitaetsstrasse 150, 44780 Bochum, Germany

Mutations in the gene of Methyl CpG binding Protein 2 (MeCP2) cause the majority of cases of the X-linked neurodevelopment disease known as Rett Syndrome (RTT). Knockout mouse models with loss of MeCP2 function mimic clinical key features of RTT, including developmental regression that leads to motor impairment, irregular breathing and early mortality. Expression of MeCP2 increases during postnatal development with neuronal maturation and this correlates with the onset of the RTT syndrome. However, RTT syndrome models show a deficit in long-term potentiation at neocortical and hippocampal synapses (Moretti et al. 2006; Asaka et al. 2006).
Ras is an universal intracellular membrane-anchored protein cycling from the inactive GDP-bound to the signalling active GTP-bound conformation. During postnatal development Ras expression is increased in the brain cortex and its relative GTP-levels are regulated by neuronal activity (Manns et al. 2005). In order to investigate the possible effects of neuronal Ras activity in RTT syndrome we used transgenic mice expressing activated human Val12 Ha-Ras selectively in neurons resulting in enhanced cortical synaptic long-term potentiation (Heumann et al. 2000; Arendt et al. 2004). The crossbreeding of heterozygous $MeCP2^{+/-}$-females with transgenic synRas-males resulted in a dramatically increased postnatal lethality. Furthermore, in contrast to heterozygous $MeCP^{-/y}$-males double transgenic synRas/$MeCP^{-/y}$-males did not survive beyond postnatal day 28. Female synRas/$MeCP2^{+/-}$-mice had an almost normal lifespan. However, these double transgenic female mice developed an enhanced anxiety-like behaviour in the elevated plus maze comparable to that of the strongly affected $MeCP2^{-/y}$-males.
In summary, neuronal activation of the Ras/MAPkinase pathway accelerated the development of the adverse RTT phenotype and therefore this may lead to new concepts of therapeutical treatment.

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# Exposure of mice to long-light: A new animal model to study depression

Astrid Becker[1], Andras Bilkei-Gorzo[1], Kerstin Michel[1], Andreas Zimmer[1]

[1]University of Bonn, Institute of Molecular Psychiatry, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

Depression is a complex disorder with a lifetime prevalence of about 16%. Unfortunately, two third of the patients do not achieve remission with first-line antidepressants. Thus, there is an urgent medical need for a better and more effective pharmacotherapy, as well as for valid animal models to study depression. Today it is believed that depression is caused by an interaction between genetic and environmental factors, but the underlying molecular and cellular pathomechanisms are still not fully understood. Stressful life events or periods of chronic stress are often preceding the onset of depressive illnesses and thus increased attention has been paid on stress in the context of depressive disorders in the recent years. Therefore, the aim of the present work was to analyze the influence of a chronic stressful environment on laboratory mice using a new animal model, designed and established in this project, to study depression. This new animal model comprises the exposure of nocturnal active, melatonin proficient C3H/HeN mice to an extended amount of artificial daylight, like constant illumination, instead of a circadian 12:12 hour light-dark cycle. Extension of the light phase induced a complex behavioral depression-like syndrome consisting of decreased home cage activity, sucrose anhedonia and hyperactivity in unfamiliar environments like the open field or a novel cage as well as a reduced social affinity for conspecifics. Consistent with behavioral effects, a modulation of stress-axis key components was observed in stress-exposed mice. Changes included increased blood corticosterone levels and modified expression of corticotropin-releasing genes and other stress associated once. Although the design of this new model differs from other rodent models of depression, analogue changes have been observed in prominent animal models of depression such as olfactory bulbectomy, social isolation or conflict models, the chronic mild stress model or in the model of genetically modified animals. Chronic administration of antidepressants had strong impact on the observed behavioral and molecular symptoms. As a result, disruption of the circadian rhythm by extending the daily light period produced a complex depression-like state in mice and represents a useful, reliable and novel animal model to study depression.

# Impact of a deletion of srGAP3 on brain architecture and dendritic spines

Oliver von Bohlen und Halbach[1], Jonathan Bertram[1], Robert Waltereit[2], Dusan Bartsch[2]

[1]University of Greifswald, Institute of Anatomy and Cell Biology, Friedrich Loeffler Str. 23c, 17487 Greifswald, Germany

[2] Abt. Molekularbiologie, Zentrum für seelische Gesundheit (ZI), Mannheim, Germany

Several genes are known to be involved in mental retardation (MR). In 2002, a gene encoding for a protein called srGAP3 (or MEGAP) was discovered, which was disrupted and functionally inactivated in a patient displaying severe mental retardation (MR). Since srGAP3 shares homologies with other RhoGAP-proteins, it is thought that srGAP3 plays crucial roles in neuronal plasticity and higher brain functions. SrGAP3 -deficient mice have recently been generated and we have started to analyze them with respect to putative morphological alterations in the brain. The brains of srGAP3 deficient mice display an increase in total brain size and weight (increase in wet-weight of more than 20%). This enlargement was accompanied by increases in the mean thickness of several white mater tracts within the forebrain. Moreover, the srGAP3 deficient mice display an enlargement of the ventricles. Consistent features of neurons in patients with MR or in several mouse models of MR are abnormal dendritic structures and/or alterations in dendritic spine morphology. By using computer-based reconstructions of Golgi-stained material, we found that spine densities were not altered in hippocampal neurons of srGAP3 deficient mice, but the individual spine length was increased. In summary, these data support the notion that srGAP3 plays specific roles in the brain. Further investigations will not only provide insight in the roles of srGAP3, but may also be helpful getting insight in fundamental mechanisms involved in neuronal plasticity and the development of mental dysfunctions.

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# Impact of the Glucocorticoid Receptor on Maternal Neglect

Natascha Pfeiffer[1], Sabine Chourbaji[1], Christiane Brandwein[1], Miriam Annika Vogt[1], Peter Gass[1]

[1]Zentralinstitut für seelische Gesundheit, AG Psychiatrische Tiermodelle, J5, 68159 Mannheim, Germany

A plethora of epidemiological studies revealed a substantial impact of childhood adversities on the individual risk of stress-related psychiatric disorders in adulthood. Thus, to be able to examine the molecular consequences of a specific low-quality mother-child relationship in the offspring, it is fundamental to design valid animal models reflecting respective maternal behavior.
In an attempt to establish a model for maternal depression, hypothetically indicated by increased maternal neglect, we investigated two commonly used mouse strains, C57BL/6 and Balb/c, which have been reported to exhibit high and low maternal care. To additionally challenge maternal coping, it seemed furthermore interesting to investigate if particular features of care/neglect behavior are emphasized by a heterozygous mutation of the glucocorticoid receptor (GR), which has been shown to be involved in depression-like behavior.
The present study indicates that there is an overall effect of "strain", confirming previous findings of "high-caring" (C57BL/6) and "low-caring" mothers (Balb/c). A decreased GR expression did, however, not generally affect maternal behavior, but led to significantly decreased "licking/grooming" behavior in C57BL/6 dams, which, however, is an essential aspect in the maintenance of the pups' welfare.
These results suggest that the impact of GR on the maternal behavior of C57BL/6 mouse may be secondary compared to the factor "strain", or simply that its effects depend on a rather different stressful challenge than the one given by the "natural" perinatal stress.

# Influence Of Maternal Care On The Adult Social Phenotype: A Cross-fostering Study In MIce

Francesca R D'Amato[1], Valentina Lampis[2], Anna Moles[1], Diego Oddi[1]

[1]CNR, Neuroscience Institute, Behavioral Neuroscience, Via del Fosso di Fiorano 64, 00143 Roma, Italy
[2]Academic Centre for the Study of Behavioural Plasticity, Vita-Salute San Raffaele University, Milan, Italy.

Maternal care has a major impact on pup's development. Cross-fostering litters between strains characterized by different amount of maternal care has demonstrated that variations in this behavior exert profound effects on the offspring that last till adulthood. BALB/c and C57BL/6 strains of mice have been extensively described: relative to BALB/c, C57BL/6 mice display higher levels of maternal care, reduced anxiety and lower response to stress. It is known that the increased levels of maternal care experienced by BALB/c pups raised with C57BL/6 dam are associated with reduced anxiety in adulthood compared to non-fostered same strain mice. Furthermore, fostered mice exhibit lowered response to stress in terms of reduced HPA axis activity and improved performance in learning/memory tasks. By contrast, having a BALB/c dam has been shown to have only minor consequences on the C57BL/6 offspring, apparently more resilient to the reduced levels of maternal care received. Here, we present the results of a cross-fostering study aimed to investigate the impact of such paradigm on sociability in BALB/c and C57BL/6 strains of mice. Infants were tested for ultrasonic vocalizations (USVs) using the maternal separation paradigm (PND 8), and for odour preference in the homing test (PND 10). At weaning (PND 28), we observed the time spent by each subject investigating two social stimuli (one BALB/c and one C57BL/6) in a three-chambered apparatus, and, later on, mice emotionality was tested in an open arena (PND 75). Finally (PND 90), social interaction was evaluated using the resident/intruder paradigm, both in a sexual context (male/female pairs) and in a social investigation context (female/female pairs). Encounters were videotaped and the USVs emitted stored for subsequent spectrographic analysis. Our results are consistent with previous data showing a decrease in anxiety in BALB/c mice reared with C57BL/6 dams, but surprisingly indicate reduced social competence in the cross-fostered C57BL/6 adult offspring.

# Is stereotypic behaviour correlated with cognitive impairment in starlings?

Gesa Feenders[1], Melissa Bateson[1]

[1]Newcastle University, Institute of Neuroscience, Framlington Place, NE2 4HH Newcastle, United Kingdom

Some evidence exists that animals, including humans, that perform high levels of stereotypic behaviour (abnormal repetition of an action) also show higher levels of recurrent perseveration (inappropriate repetition of a response) in cognitive tasks (e.g. Garner et al 2003; Garner 2005). This is due to an impairment in the underlying inhibitory mechanisms within the motor system of the basal ganglia, affecting various kinds of motor output. Thus, if animals with stereotypies are tested in cognitive tasks, the test results may not reflect the natural response of the animals.
We asked whether this correlation between stereotypic behaviour and recurrent perseveration is found in European starlings. We kept birds in cages for ~2 weeks and measured different aspects of stereotypic-like behaviour alongside performance in decision-making tasks (extinction learning, two-choice-guessing). We found that the level of stereotypy was not correlated with perseveration measured during the cognitive tasks. This may be because correlations between stereotypy and perseveration are only found when the stereotypies are fully crystallized and inhibitory motor circuits in the basal ganglia have been permanently altered. This finding is important as it supports validity of research in avian cognition at least under conditions of short-term caged housing in the absence of crystallized stereotypies.

# Juvenile stress - a valid model of depression/anxiety?

Nicole Yee[1], Kerstin Plaßmann[1], Markus Wöhr[2], Gal Richter-Levin[3,4], Eberhard Fuchs[1,5]

[1]Clinical Neurobiology, German Primate Center, Göttingen, Germany
[2]Experimental and Physiological Psychology, Philipps-University of Marburg, Marburg, Germany
[3]Department of Psychology and Neurobiology & Ethology, University of Haifa, Haifa, Israel
[4]The Institute for the Study of Affective Neuroscience, University of Haifa, Haifa, Israel
[5]Department of Neurology, Medical School, University of Göttingen, Göttingen, Germany

Epidemiological studies have strongly implicated early-life stressful experiences as a major risk factor for the development and exacerbation of mood and anxiety disorders. Adolescence, in particular, is a critical developmental stage during which stressful events may predispose individuals to behavioural deficits in adulthood. A model for juvenile stress-induced depression/anxiety has been developed which involves exposing young rats to acute variable stressors over a 3-day period (Avital and Richter-Levin, 2005). We investigated the effects of this juvenile stress paradigm on anxiety related-behaviour, circadian rhythms and responsiveness to stressful stimuli in adult rats. Similar to previous studies, we hypothesized that juvenile stress affects individuals such that they display marked physiological and anxiety-related deficits in adulthood. Furthermore, exposure to traumatic events during juvenility may negatively affect their ability to cope with subsequent stressors encountered as an adult. In separate experiments, the juvenile stress paradigm was applied and outcomes were measured when rats reached adulthood. In the pilot experiment, all rats were implanted with abdominal telemetric devices approximately 2 weeks following juvenile stress to monitor body temperature rhythms. However, given the outcome of this experiment (see results below), surgery was not carried out in subsequent experiments. When rats reached adulthood (age 50+ days), they were tested on the elevated plus maze (EPM) to assess anxiety-like behaviour. Then, a fear-conditioning paradigm was conducted to investigate responsiveness to stress in adulthood. In these tests, ultrasonic vocalizations (USVs) were measured as the behavioural parameter. In the first experiment, no differences were observed between control and juvenile stress groups on the EPM. We hypothesized that this was due to the trauma/stress of the surgical procedure. Therefore, in later experiments, surgery was omitted from the protocol altogether. Nonetheless, some interesting differences were observed between groups in terms of body temperature. It appears that the circadian rhythm of mean body temperature in the juvenile stress group differs from control group (i.e., daily temperature means, changes in temperature between dark/light phases). In subsequent experiments (with no surgery), significant differences in anxiety-related parameters between control and juvenile stress groups were observed on the EPM. A consistent trend was also observed during fear conditioning in terms of fear-related behaviour (USVs). Rats from the juvenile stress group had a shorter latency to begin calling and elicited more 22-kHz USV calls of longer duration. Thus, the juvenile stress protocol was validated in terms of eliciting behavioural and physiological differences in adult rats. However, it is clear that the paradigm is strongly influenced by various factors and still needs to be optimized in terms of reliability and reproducibility.

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Avital A, Richter-Levin G (2005) Exposure to juvenile stress exacerbates the behavioural consequences of exposure to stress in the adult rat. Int J Neuropsychopharmacol 8:163-173.

# Oxytocin and social aggression: what, where and how?

Federica Calcagnoli[1], Christine Stubbendorff [1], Sietse F. de Boer[1], Jaap M. Koolhaas[1], Johan A. den Boer[2]

[1]Department of Behavioral Physiology, University of Groningen, The Netherlands

[2]Department of Psychiatry, University Medical Centre Groningen, The Netherlands

Across many animal species, the neuropeptide oxytocin is widely acknowledged as an important CNS modulator of various social behaviours. In addition to its well established role in enhancing pair bonding, affiliation and anxiolysis, oxytocin has also been suggested to have serenic effects, thus encouraging more positive social interactions. For example, preclinical studies have shown that impaired oxytocin function by knocking out the oxytocin gene or its receptor in mice, results in an enhanced aggressive phenotype. This pro-aggressive effect of oxytocin dysfunction may also extend to humans where a negative correlation was observed between cerebrospinal fluid levels of oxytocin and life history of aggression in patients with personality disorders. It has been also shown that the presence of oxytocin receptor dysfunction impairs social behaviour of autistic patients. Although some preclinical studies where oxytocin was manipulated directly by exogenous peptide administration support the claim that oxytocin decreases aggression, others have found the opposite or no effect. Moreover, it is unknown where and via which mechanisms in the brain the putative anti-aggressive actions of oxytocin are mediated.
In parallel with a clinical study investigating the potential therapeutic value of intranasal oxytocin treatment of patients with autistic and anti- social personality disorders, we are carrying out a preclinical project to study the behavioural effects of central administration of oxytocin on social behaviour in male rats. We hypothesize that oxytocin will reduce the level of aggression particularly in animals that display excessive and maladaptive forms of aggressiveness. To test this hypothesis, we have assessed the effects of single icv administration of various dosages of oxytocin (0.25 – 4 μg) in medium-high aggressive adult male Wild-type Groningen resident rats that were confronted with another male intruder in their home territory. To assess the importance of endogenous oxytocin signaling during these residents, we also evaluated the effects of the oxytocin receptor antagonist L-368,99 (7.5 μg icv). Moreover since serotonin (5-HT) is one of the major neurotransmitters that has been linked to escalated aggression, we tested the hypothesis that the aggression reducing effect of icv oxytocin might be expressed by modulating dorsal raphe 5-HT neuronal activity.
The results show that oxytocin administration significantly reduced offensive behaviour in a dose-dependent manner and without impairment of locomotor or exploratory activities. In addition, treatment with an oxytocin receptor antagonist induced a more pronounced offensive behavioural profile. On the other hand, intermale offensive aggression was not affected by oxytocin (0.1 μg) or oxytocin antagonist (0.75 μg) manipulation of the dorsal raphe. Based on these results, we conclude that enhancing or attenuating brain oxytocin function has anti- or pro- aggressive effects, respectively. Furthermore, the dorsal raphe 5-HT neurons do not seem to be the key neurobiological mechanism for these effects. Overall this study emphasizes the importance of oxytocin as a neuropeptide regulating social behaviour, thus making oxytocin a promising candidate for future therapeutic application in human patient groups suffering from pathological aggression that dramatically impairs their social and empathic skills in social interaction.

# PDE4-Inhibition Facilitates Hippocampal Synaptic Plasticity and Rescues MK801-induced Impairment in LTP in Freely Moving Rats

Valentina Wieschollek[1], Denise Manahan-Vaughan[1,2]

[1]Ruhr University Bochum, Department of Neurophysiology, Universitaetsstrasse 150, 44780 Bochum, Germany

[2]International Graduate School of Neuroscience, Universitaetsstrasse 150, 44780 Bochum, Germany

The acute application of NMDA receptor antagonists, such as MK801 (dizolcilpine), have been shown to induce psychotic symptoms in humans and to give rise to an acute and short-lasting behavioral state in rats, which mirrors many symptoms of schizophrenia. It has therefore been adopted as an animal model of psychosis. We have previously reported that acute MK801-treatment results in a profound impairment of long-term potentiation (LTP) in the dentate gyrus of freely behaving rats (Manahan-Vaughan et al, 2008, Hippocampus 18:125-134; Manahan-Vaughan et al, 2008, Eur J Neurosci 28:1342-1350). PDE4-inhibion specifically increases cAMP-levels, thereby facilitating the cAMP/PKA/MAPK/CREB cascade which is essential for long-lasting LTP (long-term potentiation). Rolipram is a selective PDE4-inhibitor that may have permissive effects on LTP under conditions where plasticity is weak. In this study, we investigated whether intracerebral injection with Rolipram of adult freely behaving control and MK801-treated rats leads to alterations of the ability to express plasticity at the perforant path- dentate gyrus synapses.

Male Wistar rats (7 – 8 weeks old) were implanted chronically with a bipolar stimulation electrode in the medial perforant pathway and a monopolar recording electrode in the dentate gyrus granule cell layer, as well as with a cannula in the ipsilateral cerebral ventrical to enable drug application. In vivo electrophysiological experiments were conducted approx. 10 days after surgery. Short-term potentiation (STP) or LTP were elicited by using high frequency tetanisation (three bursts of 15 pulses at 200 Hz and 10 sec interburst interval or ten bursts of 15 pulses at 200 Hz and 10 sec interburst interval, respectively) in freely moving rats. After a "pretreatment LTP" experiment, rats were injected systemically with either MK801 or saline. 1 week later LTP was assessed again, whereas Rolipram or saline was applied i.c.v. 30min prior to stimulation.

Treatment with Rolipram significantly transformed STP into LTP in control animals. In MK801-treated rats LTP was significantly disrupted one week after injection as compared to pretreatment LTP, exhibiting a smaller induction magnitude and enduring for only ca. 2h. PDE-4 inhibition attenuated this impairment in the induction phase and rescued the maintenance phase of LTP fully. Thus, MK801-induced impairment in the ability to express LTP was rescued by Rolipram.

Under certain pathological conditions, specifically after MK801-induced psychosis, disruption of LTP might be rescued through boosting the cAMP-signalling by Rolipram. This suggests that PDE4-inhibition could be given consideration as a therapeutic strategy in the treatment of psychosis-related diseases.

# Psychophysics and EEG of visual motion processing in children with ADHD

Bettina Lange-Malecki[1], Stefan Treue[1,2], Aribert Rothenberger[3], Björn Albrecht[3]

[1]German Primate Centre, Göttingen, Germany, Cognitive Neuroscience Laboratory, Kellnerweg 4, 37077 Göttingen, Germany
[2]Bernstein Centre for Computational Neuroscience Göttingen, Germany
[3]Child and Adolescent Psychiatry, University of Göttingen, von Sieboldstr. 5, 37075 Göttingen, Germany

Abstract

The aim of our study was to assess perceptual influences to attention-linked task relevant behaviour in children with ADHD and matched controls by a comparison between visual motion and form processing. The well-established Eriksen flanker task was performed incorporating one measurement with coherently moving random dot patterns (RDPs), moving either to the left or right, and one with static triangles, pointing to the left or right. The performance parameters response times and error rates, controlled by a given feedback for speed-accuracy trade-off, as well as N2 -enhancement due to stimulus incongruency as an electrophysiological parameter of cognitive control, were analyzed.

Children with ADHD (combined type) showed significantly prolonged response times and reduced accuracy for both different flanker tasks. Flanker effects are significantly smaller in the motion task compared to the static triangle task in both groups due to unequal prolonged reaction times, more prolonged in congruent than in incongruent moving configuration. These results are reflected in significantly diminished stimulus-locked N2-enhancement. Congruency effects tend to be smaller in ADHD children, and, for both groups, are significantly smaller in RDPs due to larger N2 amplitudes for congruent moving stimuli (see fig.).

Congruent and incongruent flanked moving targets seem to produce a more general flanker effect in the sense of more distraction than attraction by reflexive bottom -up attention with a reduced benefit from congruent configuration as a characteristic. Both the recognition of different stimuli and the allocation of attention by their features seem to be essential requirements for further executive control. As both groups of children exhibited smaller flanker effects in the motion flanker task, children generally seem to have their motion vision not fully developed until early adulthood. The retardation of visual motion development compared to visual static form was more pronounced in the ERP-Data of the ADHD group. Altogether, visual feature dependencies could be proven for executive attention and attention dependent cognitive control in children.

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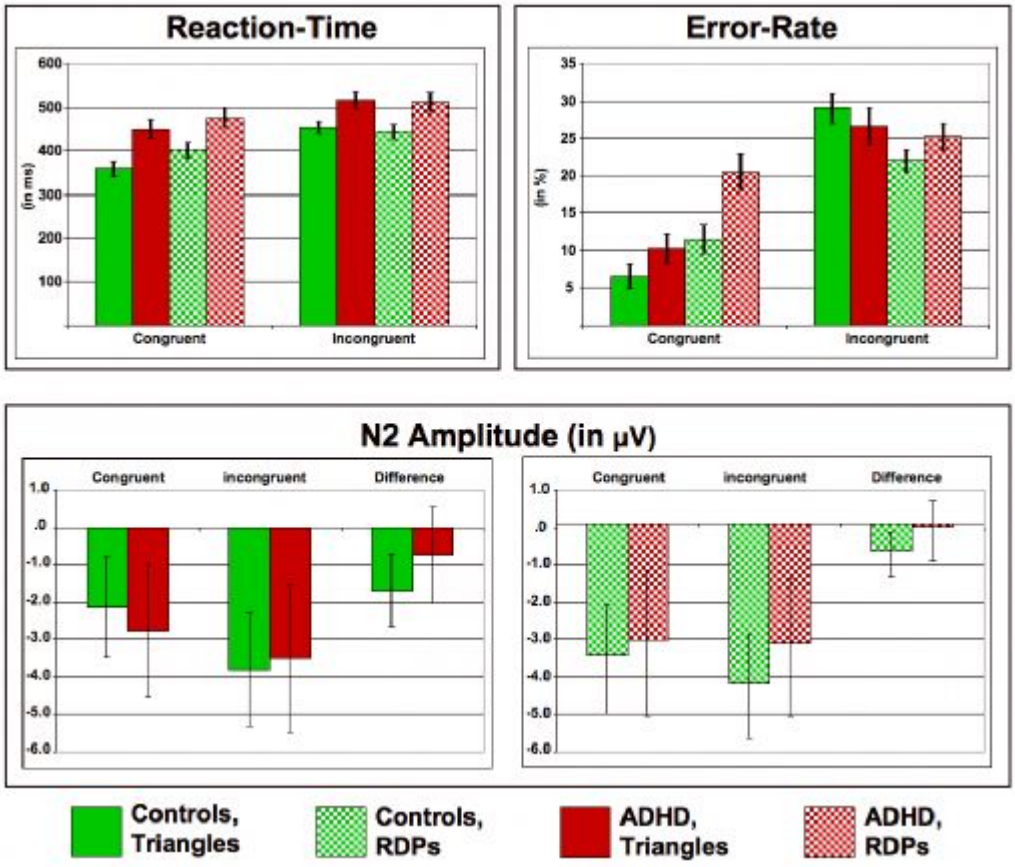
Reaction-Time
(in ms)
600
500
400
300
200
100
Congruent
Incongruent
Error-Rate
(in %)
35
30
25
20
15
10
5
Congruent
Incongruent
N2 Amplitude (in µV)
Congruent
incongruent
Difference
1.0
.0
-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
Congruent
incongruent
Difference
1.0
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-1.0
-2.0
-3.0
-4.0
-5.0
-6.0
Controls, Triangles
Controls, RDPs
ADHD, Triangles
ADHD, RDPs

# Shank1-deficient mice display deficits in developmental milestones, ultrasonic communication, scent marking behavior and social memory – an autism-like phenotype?

Markus Wöhr[1], Florence I. Roullet[2], Albert Y. Hung[3], Morgan Sheng[3], Jacqueline N. Crawley[2]

[1]Philipps-University of Marburg, Experimental and Physiological Psychology, Gutenbergstraße 18, 35032 Marburg, Germany

[2]Laboratory of Behavioral Neuroscience, Intramural Research Program, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892, USA.

[3]The Picower Institute for Learning and Memory, Department of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Autism is a neurodevelopmental disorder characterized by abnormal reciprocal social interactions, qualitative impairments in social communication, and repetitive and stereotyped patterns of behavior with restricted interests. Genome-wide and pathway-based association studies led to the identification of several susceptibility genes for autism, including the SHANK gene family. Mutations in SHANK2 and SHANK3 have been detected in several autistic individuals. SHANK genes code for a family of scaffolding proteins located in the postsynaptic density of excitatory synapses. Recently, we disrupted in mice the Shank1 gene to investigate its function in vivo. Shank1-/- null mutant mice showed altered protein composition of the postsynaptic density and smaller dendritic spines and synapses, which correlated with weakening of excitatory synaptic transmission. Behaviorally, motor deficits were found in Shank1-/- mice in a number of tasks, including open field activity, accelerated rotarod and wire hanging. Shank1-/- mice further displayed impairment of contextual fear conditioning, normal cued fear conditioning, and enhancement of spatial learning in the radial maze. This behavioral phenotype might be reminiscent of the heterogeneous cognitive phenotype seen in people with autism.

In order to test the hypothesis that a mutation in SHANK1 contributes to the symptoms of autism, we evaluated Shank1-/- null mutant mice for behavioral phenotypes with relevance to autism, focusing on social communication. Ultrasonic vocalizations and the deposition of scent marks appear to be two major modes of social communication in mice. Our findings revealed evidence for low levels of ultrasonic vocalizations and scent marks in Shank1- /- mice as compared to wildtype Shank1+/+ littermate controls. Shank1- /- pups emitted fewer ultrasonic vocalizations than Shank+/+ pups when isolated from mother and littermates. Call characteristics and changes herein over time differed between genotypes. Developmental milestone analyses revealed significant delays in several measures of physical development and motor abilities in Shank1-/- pups. In adulthood, scent marking behavior in the presence of female urinary pheromones was affected by genotype. Specifically, adult male Shank1-/- mice deposited fewer scent marks in proximity to female urine than Shank+/+ mice. Number of urine traces in a clean open field did not differ between genotypes. While adult male Shank+/+ and Shank1- /- mice emitted a similar amount of ultrasonic vocalizations when exposed to female urinary pheromones, Shank+/+ mice changed their calling pattern dependent on previous interactions with an adult female, whereas Shank1-/- mice were unaffected by previous exposure to a female, indicating social memory deficits in Shank1-/- mice.

As all members of the SHANK gene family appear to fulfill similar physiological roles, and display considerable neuroanatomical co-expression, the present findings support future investigation of SHANK1 in autism association studies to further test the hypothesis that synaptic defects underlie the symptoms of autism. Our findings of reduced levels of ultrasonic vocalizations and scent marking behaviors in social contexts are consistent with a Shank1-/- phenotype relevant to communication deficits in autism.

# Significant impact of p-glycoprotein on the HPA-system and potential consequences for antidepressant effects.

Yvonne Schönfelder[1], Christoph Hiemke[1], Ulrich Schmitt[1]

[1]University Medical Center of the Johannes Gutenberg University Mainz, Germany, Department of Psychiatry and Psychotherapy, Untere Zahlbacher Str. 8, 55131 Mainz, Germany

P-glycoprotein (P-gp, ABCB1), a member of the multidrug resistance gene family is strongly expressed in the blood-brain-barrier (BBB) and regulates the entrance of xenobiotics into the brain. Multiple antidepressants (ADs) and the glucocorticoid corticosterone are among the substrates of P-gp. One hypothesis for antidepressant effectiveness is based on well balanced brain corticosterone levels, regulated by P-gp. Here we demonstrate in a P-gp knockout mouse model that the regulation of the HPA -axis depends on the P -gp-related homeostasis of corticosterone. Modulations of intracerebral glucocorticoids moreover affect the pharmacological profile of different ADs in behavioral paradigms.

P-gp k.o. mice (abcb1a/1b-/-) were compared to wildtype FVB/N in the forced swim test (FST) and the tail suspension test (TST). Mice were treated by i.p. injection with either saline (1ml, 0,9%), the P-gp substrates venlafaxine (10mg/kg), citalopram (10mg/kg), and duloxetine (10mg/kg) or with fluoxetine (20mg/kg) as a non-substrate of P-gp 30 min. before testing. Immobility of the mice in each test was measured by an automatic computer based system (EthoVision®XT 5.1, Noldus). Brain corticosterone and BDNF concentrations were determined by an enzyme immunoassay and glucocorticoid-receptor (GR) expression by Western-Blot analysis.

Knockout mice showed a clear phenotype displaying altered immobility compared to wildtyp mice in the FST and the TST without treatment, indicating an influence of corticosterone. Furthermore, in vitro analysis showed decreased corticosterone concentrations under basal and stressful conditions and increased GR expression in the brains of P-gp knockout mice. Additionally, we found BDNF brain levels in P-gp deficient mice to be decreased. With respect to AD-treatment wildtype mice showed a clear reduction of immobility in the FST. In contrast, P-gp knockout mice showed no effect independent of the mode of action of the drug or its substrate properties. Immobility was identical to saline treatment.

These results gave strong evidence that entrance of glucocorticoids into the brain regulated by P-gp transport activity has a strong impact on antidepressant-related behavioral changes seen in different ethological paradigms. This supports the hypothesis that antidepressant effects in behavioral tests like the FST are related to corticosterone and are therefore modulated by P-gp and depend finally on an intact BBB.

# Studying the gene-environment interaction in Tcf4 transgenic mice, an animal model of schizophrenia

Dorota Badowska[1], Magdalena M. Brzozka[1], Moritz J. Rossner[1]

[1]Max-Planck-Institut fuer Experimentelle Medizin, Gene Expression, Hermann-Rein-Str. 3, 37075 Goettingen, Germany

TCF4 is a bHLH transcription factor and was recently described as a novel schizophrenia risk gene (Stefansson *et al*., Nature 2009). Its function in the brain, however, still remains largely unclear. A transgenic mouse model overexpressing Tcf4 in the adult forebrain (Tcf4tg) revealed cognitive and sensori-motor disturbances often seen in schizophrenic patients as well (Brzozka *et al*., Biol. Psychiatry 2010).
We present the results of the analysis of cohorts of mice that are either single cage housed (social isolation, SI) or are kept after weaning in an enriched environment (sensory and social stimulation, SS). These groups of mice will be analyzed after 3 and 12 months in a series of tests monitoring standard and schizophrenia related behaviors (explorartory activity, curiousity, learning etc.). The behavioural phenotyping is paralleled by histological and molecular analyses. Immuno-histochemical methods will be applied to analyze GxE dependent synaptic or axonal alterations in different forebrain structures. Molecular studies will include state-of-the-art gene expression and CHIPseq techniques to identify the direct target genes of TCF4.

# The effect of cadmium on behavioral and electrophysiological parameters of rats after subacute exposure in two different forms

Edina Horváth[1], Gábor Oszlánczi[1], Zsuzsanna Máté[1], Andrea Szabó[1], András Papp[1], Gábor Kozma[2], András Sápi[2], Zoltán Kónya[2], László Nagymajtényi[1]

[1]University of Szeged Faculty of Medicine, Department of Public Health, Dóm tér 10., 6720 Szeged, Hungary

[2]University of Szeged Faculty of Science and Informatics, Department of Applied Chemistry, Rerrich Béla tér 1., 6720 Szeged, Hungary

Cadmium (Cd) is a toxic heavy metal which is, and even more was, used in steel and other alloys, pigments, in nickel-cadmium batteries and in electroplating. It is also frequently detected in environmental and food samples. Although the nervous system was not traditionally considered among the main targets of Cd toxicity within the organism, behavioral and neurological consequences of Cd burden have been described. In occupational exposure, reduced visuomotor performance, and difficulties of concentration and postural balance were observed. In exposed children, a straight relationship between elevated hair Cd and altered visual or auditory evoked potential parameters, as well as behavioral problems, was found. High-temperature industrial procedures typically generate metal fumes, containing solid particles of various sizes, including nanoparticles, i.e. airborne solid parts of diameter below 0.1 μm. In this study, Cd was given to rats (in groups of 10) in two different chemical forms and with combining exposure via the airways and the gastrointestinal tract.

One group of adult male Wistar rat had daily oral treatment for 3 weeks with aqueous solution of CdCl2 at 3 mg/kg b.w. dose (group C3). Another group received, after 3 weeks oral exposure, intratracheal application of a suspension of CdO2 nanoparticles for 1 week at 0.04 mg/kg b.w. dose (C31). The third group received the same dose of CdO2 nanosuspension for 3 weeks intratracheally (CIT3). The control groups had identical treatment with the vehicle only (V3, V31, VIT3).

Following the treatment period, spontaneous motility was tested in an open field box. Treated rats showed hypomotility with increased periods of local activity and immobility. Reduction of ambulation distance was significant in the C3, C31 and CIT3 groups vs. the corresponding controls, and the reduction in C31 was also significant vs. C3 and CIT3.

The rats were then prepared for electrophysiological recording in urethane anesthesia. Spontaneous and stimulus-evoked cortical activity was recorded from the primary somatosensory, visual and auditory field, and compound action potential from the tail nerve. The changes of the spontaneous activity were slight. The latency of the sensory evoked potentials typically increased. The increase was more pronounced in C3 vs. V3 and in C31 vs. V31 than in CIT3 vs. VIT 3. Also, the frequency dependence of the latency increase of the somatosensory evoked response was stronger in the treated groups than in the controls. Conduction velocity of the tail nerve decreased, and the fatigability of the nerve, tested by high-frequency stimulation, increased.

The results showed that modelling the nervous system effects of Cd exposure in the described way is possible. It was also seen that the effects were partly dissimilar after various forms of exposure and that certain effects in the combination group were synergistic.

# Ultrasonic communication in rats: Effects of post-weaning social isolation on social approach behavior and brain activity in response to playback of 50-kHz calls in the rat

Dominik Seffer[1], Christoph Renninger[1], Rainer K. W. Schwarting[1], Markus Wöhr[1]

[1]Philipps-University of Marburg, Experimental and Physiological Psychology, Gutenbergstr. 18, 35032 Marburg,

Rats emit distinct types of ultrasonic vocalizations (USVs), which serve as situation-dependent affective signals. Juvenile and adult rats produce 50-kHz USVs in appetitive situations such as rough-and-tumble play, mating or when tickled playfully, whereas 22-kHz USVs occur in aversive situations such as fear-conditioning or social defeat, probably reflecting positive and negative affective states, respectively. In support of a communicative function of USVs, it was demonstrated that 50-kHz USVs and 22-kHz USVs induce call-specific behavioral responses in the receiver. 50-kHz USVs induce social approach behavior, supporting the notion that they serve as social contact calls. In contrast, 22-kHz USVs leads to freezing behavior, indicating an alarming function (Wöhr & Schwarting, PLoS One, 2007). The opposite behavioral responses are paralleled by distinct patterns of brain activation. While 22-kHz USVs induce activation in amygdala and periaqueductal gray, 50-kHz USVs are followed by activation in the nucleus accumbens (Sadananda et al., Neuroscience Letters, 2008). Since rats are social animals and rough-and-tumble play during adolescence has an important role for social development, separation from conspecifics during this phase is known to impair social behavior.

Here, we tested whether post-weaning social isolation impairs social approach behavior in response to playback of 50-kHz USVs. Rat weanlings were housed under one of the following three conditions: group housing, short-term isolation, i.e. 24 hours, or long-term isolation, i.e. 28 days. Then, they were tested for social approach behavior in response to 50-kHz USVs. As acoustic control stimuli served 22-kHz USVs and background noise. We applied c-fos immunocytochemistry to screen for playback-induced neuronal activation in brain areas implicated in the regulation of affect and social behavior.

Confirming previous findings, playback of 50-kHz and 22-kHz USVs elicited opposite behavioral responses and distinct neuronal activation patterns. Post-weaning social isolation specifically affected the behavioral response to 50-kHz USVs. Group housed rats showed a clear preference towards 50-kHz USVs. Socially deprived short-term isolated rats exhibited an even stronger reaction, possibly due to a higher level of social motivation. In contrast, no social approach behavior was observed in long-term isolated rats, highlighting the importance of social experience during adolescence for affiliative behavior.

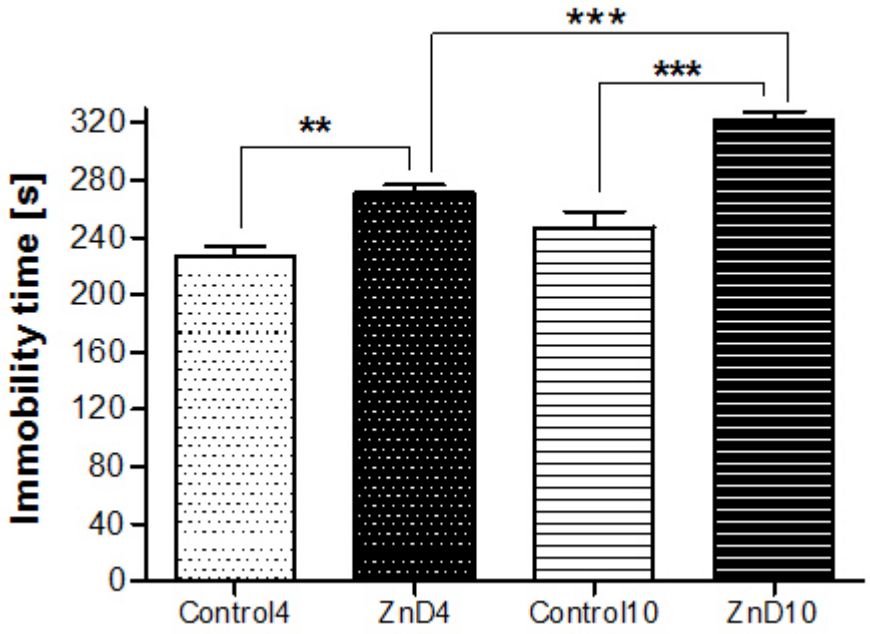
# Zinc deficiency and depression

Katarzyna Mlyniec[1], Boguslawa Budziszewska[2], Witold Reczynski[3], Gabriel Nowak[4]

[1]Jagiellonian University Collegium Medicum, Department of Pharmacobiology, ul. Medyczna 9, 30-688 Krakow, Poland

Zinc is one of the most important trace element in live organisms. Its deficiency retards the growth of humans and animals and also affects brain function. It is believed that the metal element zinc plays an important role in depression, but it is unknown if deprivation of this trace element contribute to development of depressive-like behavior. Some data suggest presence of link between zinc and mood disorder. Zinc shows antidepressant-like properties in tests and models of depression. Moreover, zinc enhances efficacy of antidepressants. Patients suffering from major depression and treatment resistant depression exhibit lower serum zinc, which may be normalized with successful antidepressant treatment. Behavioral abnormalities in zinc deficiency seems to be related to the hyperactivity of HPA axis and glutamatergic system. Zinc is thought to be an inhibitor at the NMDA receptor, which itself may be involved in the psychopathology and therefore a target for the treatment of depression.

The aim of the study was to examine the contribution of zinc to the development of depressive-like behavior, and to correlate this behavior with serum zinc and corticosterone concentration. Mice behavior was assessed following administration of zinc deficient diet for 4 and 10 weeks. To evaluate animal activity, we used modified forced swim test. Zinc-deficient mice show prolonged immobility time in comparison to control in the test (Figure), which means that zinc deficiency contributes to development of depressive-like behavior. This behavior correlates to lower serum zinc and higher serum corticosterone level. Mice were also observed after 4 weeks of zinc deprivation accompanied with acute, intraperitoneally treating with antidepressants. Results show prolonged immobility time in comparison to control group in forced swim test. Lack of zinc impairs antidepressants efficacy which means that zinc deficiency is probably also cause of treatment resistance depression.



# Poster Topic

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is light-dependently phosphorylated
*Olaf Voolstra, Stefan Kaltenbach, Katherina Beck, Claudia Oberegelsbacher, Jens Pfannstiel, Armin Huber*

T14-6C The fine structure of homing behaviour in bees and its consequences for optic flow processing
*Marcel Mertes, Laura Dittmar, Martin Egelhaaf, Norbert Boeddeker*

T14-7C Retracted

T14-8C Visual Motion Detection in Tethered Flying Flies
*Sarah Nicola Jung, Alexander Borst, Juergen Haag*

# A comparison of flight and sight strategies in flies

Bart R.H. Geurten[1], Roland Kern[1,2], Martin Egelhaaf[1,2]

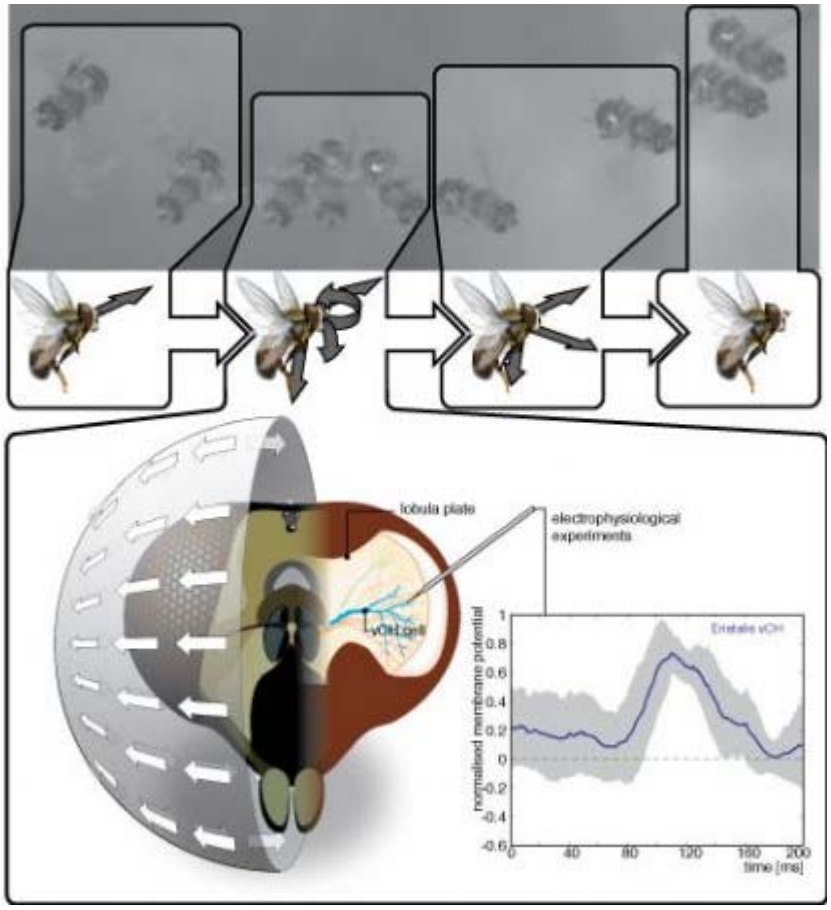
[1]Bielefeld University , Neurobiology, Postbox 10 01 31, 33501 Bielefed, Germany

[2]CITEC Cognitive Interaction Technology - Center of Excellence, Bielefeld University

Visual guided movement is a closed loop situation. The way an animal moves shapes its visual information, which is then analysed and used for movement control. Different movements induce different visual information available to the animal. Flies undertake ultrafast flight manoeuvres with a relative small brain. This makes them ideal to study visually controlled movements. The two fly species *Calliphora vicinia* and *Eristalis tenax* have homologous motion sensitive visual interneurons, but exhibit different flight styles. We compared both species' potential neuronal adaptations to the different motion dynamics resulting from these flight styles. To categorize the complex nature of the flight trajectories we employed clustering algorithms to derive prototypical movements (PMs). These PMs allow us to reduce the complex flight trajectory to a series of discrete events.

As a consequence of the closed-loop nature of flying, each PM leads to characteristic visual input changes. We determined these prototypical optic flow patterns on the retina by reconstructing the flight trajectory and arena and by rendering the correpsonding distinct optic flow patterns. Even though both species exhibit a similar saccadic flight style, we found the visual input to vary greatly between fly species. In contrast to *Calliphora* which mainly flies fast in forward direction, *Eristalis* may also move at slow speeds in nearly every possible direction.

To test whether identified homologous visual interneurons are adapted to the specific optic flow encountered by the two fly species, respectively, we presented the reconstructed behaviourally generated optic flow of both species on a panoramic high-speed LED stimulator (FliMax) to either species. By using the segregation of flight trajectories into sequences of PMs, we characterised the neuronal response patterns to distinct prototypical optic flow patterns. *Eristalis* interneurons seem to have a broader modulation depth than *Calliphora*. This might reflect one of the neuronal adaptations to the specific flight style of *Syrphidae*.


lobula plate
electrophysiological
experiments
vCH1 cell
Excitable vCH
normalised membrane potential
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
0
40
80
120
160
200
time [ms]

# A distinct layer of the medulla integrates polarized light information in the locust brain

Basil el Jundi[1], Uwe Homberg[1]

[1]Philipps-University Marburg, Dept. of Biology, Animal Physiology, D-35032 Marburg

The desert locust *Schistocerca gregaria* perceives polarized light with a specialized dorsal rim area of its compound eye and, like several other insects, most likely uses polarization information for spatial orientation and navigation. In the brain, polarized light signals are passed through the optic lobe and anterior optic tubercle to the central complex which most likely serves as an internal sky compass (Heinze and Homberg, 2007, Science 315:995). Polarized-light sensitive (POL) neurons of the anterior optic tubercle receive additional information about the celestial chromatic contrast and may, thus, distinguish between the solar and antisolar hemisphere of the sky (Pfeiffer and Homberg 2007; Cur Biol 17:1).

To further elucidate the integration of sky polarization and sky chromatic information in the optic lobe, we recorded intracellularly from neurons of the locust medulla. During recording, animals were stimulated with a rotating polarizer from dorsal direction and with an unpolarized green or UV light spot that rotated around the locust head. After recording, neurons were dye injected and analyzed anatomically in three dimensions.

In addition to previously characterized line tangential neurons that transfer polarized light information from the dorsal rim area of the medulla to the anterior optic tubercle (Homberg et al 2003; J Comp Neurol 462:415), our data show that three further classes of medulla neurons are involved in the processing of polarized light information: *(1)* intrinsic neurons with arborizations in a single medulla layer and additional ramifications in the dorsal rim area of the medulla or the accessory medulla, *(2)* tangential neurons with processes into the lamina, and *(3)* intermedulla neurons with ramifications in one layer of the medulla and axonal projections to the contralateral medulla and accessory medulla. All POL-neurons arborize in the same layer (layer 4) of the medulla. In addition to dorsally presented polarized light, all neurons also responded to a rotating green/UV light spot.

Our data suggest that neurons of layer 4 of the medulla are specialized to integrate polarized light information from the blue sky with azimuth-dependent unpolarized light input that might represent information on the chromatic contrast of the sky. Ramifications in the accessory medulla suggest early comparison of sky compass information with time of day. Supported by DFG grant HO 950/16-2.

# Behavioural studies and modelling of gaze stabilization in the blowfly *Calliphora*

Daniel A. Schwyn[1], Francisco J. Hernández Heras[1,2], Gino Bolliger[1,3], Matthew M. Parsons[1], Holger G. Krapp[1], Reiko J. Tanaka[1,4]

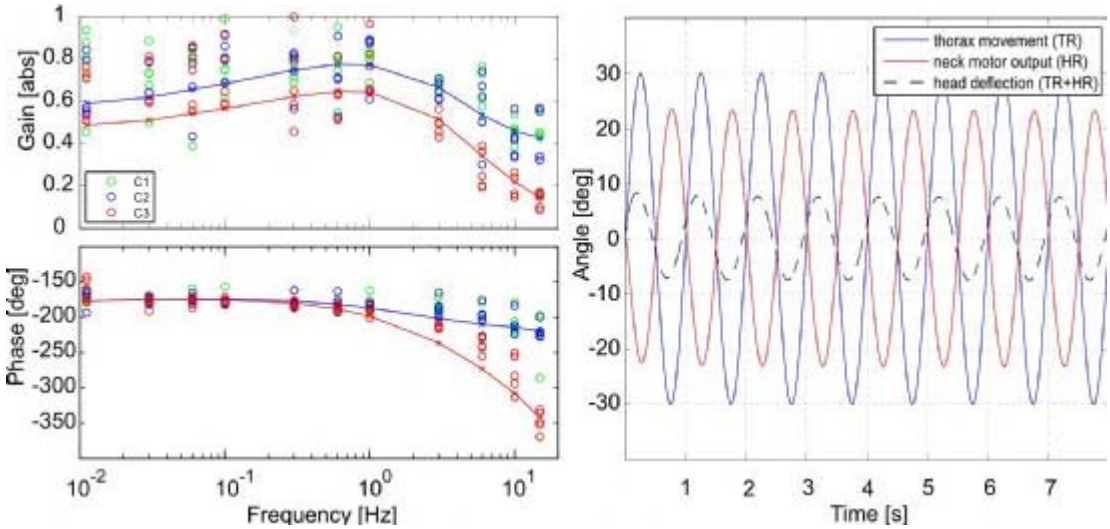
[1]Imperial College London, Department of Bioengineering, South Kensington Campus, SW7 2AZ London, United Kingdom
[2]Université Montpellier 2, Departément de Mathématiques, Place Eugène Bataillon, 34095 Montpellier cedex 5, France
[3]ETH Zurich, Institute for Biomedical Engineering, Gloriastrasse 35, 8092 Zurich, Switzerland
[4]Imperial College London, Institute for Mathematical Sciences, South Kensington Campus, SW7 2PG London, United Kingdom

For self-motion estimation and flight control, flies rely heavily on visual cues. To facilitate visual information processing during acrobatic aerial manoeuvres or when encountering turbulences the animals maintain a default orientation of their head and thus their eyes. This powerful gaze stabilization reflex is driven by input from different sensory modalities. Together, visual and mechanosensory signals enable the fly to keep its eyes level even if the body experiences considerable attitude changes. Here we focus on the contributions of three contributing sensor systems: (i) the compound eyes, providing self motion estimates based on visual wide-field motion, (ii) the ocelli, signaling fast attitude changes by integrating and comparing light levels at three different areas in the dorsal visual hemisphere and (iii) the mechanosensory halteres, measuring body rotations at high angular rates. Our ultimate goal is to establish a closed-loop simulation platform that helps us to analyse the functional properties and performance limits of multisensory control designs in biological systems.

We started with a simple model confined to one degree of freedom that simulates head roll under closed-loop conditions. To specify the model we first performed behavioural experiments to establish the input-output relationships between body rotations and compensatory head movements. Flies were tethered to a step motor that induced rotations around their longitudinal body axes. The animals were presented with an artificial horizon and frontal airflow to encourage extended periods of stable flight. We used high speed videography to monitor the fly's head roll in response to sinusoidal body rotations of +/- 30 degrees at frequencies from 0.01 to 15 Hz. Experiments were carried out in three conditions: (C1) all sensory systems intact, (C2) after occluding the ocelli, and (C3) after removing the halteres. The head roll data was then used for system identification under minimal assumptions regarding subsystems' behaviour.

Fig. A shows the Bode plot of the head roll responses obtained from 5 flies tested under C1 (green), C2 (blue), and C3 (red). The results obtained for C1 and C2 are similar. A noticeable change of the behavioural output was only obtained in flies where both ocelli and haltere function were disabled. Comparatively high gains at low frequencies suggest that other sensory mechanisms involving the prosternal organ and the integration of light levels by the compound eyes may have contributed to the head roll responses. The blue and red lines show the transfer functions derived from the experimental data under C2 and C3, respectively. These transfer functions were used to specify our simulation of the gaze stabilization system. We assumed a linear combination of the signals generated by the sensory systems before they actuate the neck motor system. Fig. B shows the results of a simulation run including 8 stimulus cycles at 1Hz and +/- 30 degrees amplitude (TR) in terms of head roll relative to the thorax (HR) and relative to the vertical (HR+TR).

Although the simulation produces results which are in agreement with independent data sets obtained under comparable stimulus conditions further experimental work is required (a) to derive transfer functions for the contribution of sensory mechanisms which have not been considered explicitly and (b) to test whether the linear combination of signals provided by different modalities is justified.


Gain [abs]
Phase [deg]
Frequency [Hz]
C1
C2
C3
thorax movement (TR)
neck motor output (HR)
head deflection (TR+HR)
Angle [deg]
Time [s]

# Blowfly brain-machine interface: building a closed loop setup between the brain of a blowfly and a mobile robot platform using an implantable micro-recording probe

Kristopher David Peterson[1], Naveed Ejaz[1], Holger G. Krapp[1]

[1]Imperial College London, Bioengineering, South Kensington Campus, Bessemer Building, SW7 2AZ London, United Kingdom

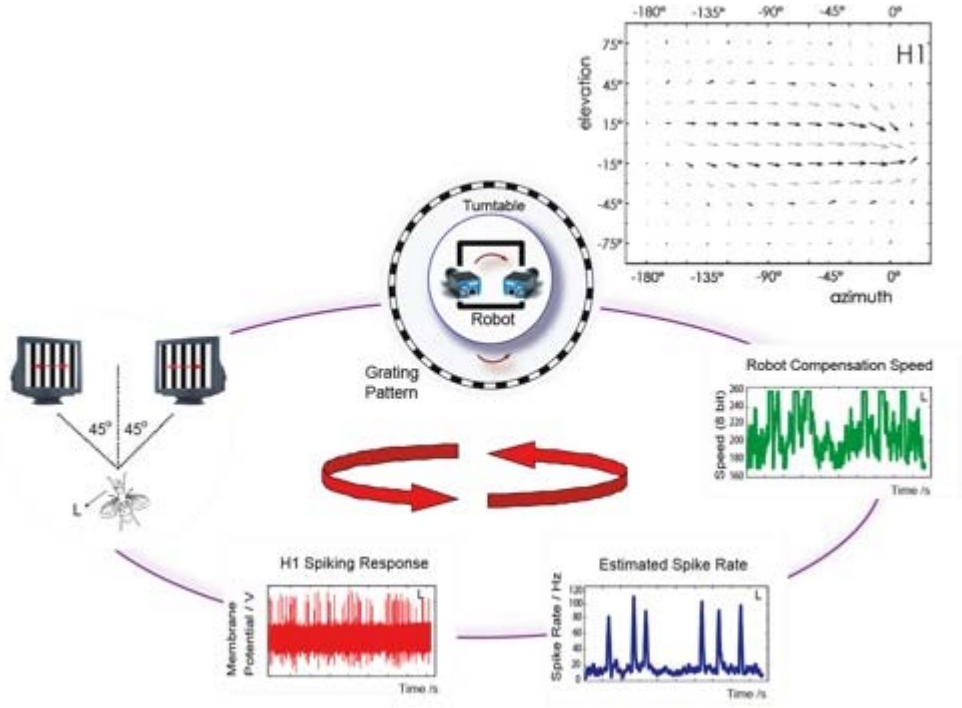
As a model system for understanding general principles of nervous systems, flies and insects offer one several orders of magnitude less complex than mammals. Flies, in particular, are an excellent model system lending themselves for study of biological control. Researches have used behavioural and electrophysiological studies to characterize fly nervous systems. However, currently recording neural activity from moving flies is difficult. We are developing an experimental system where an implantable micro-probe records the brain activity in a minimally restrained fly which is mounted on and controls a mobile robot platform by modulating its brain activity via a closed-loop brain-machine interface.

As a first step, we have developed a closed-loop brain-machine interface between an identified cell, H1, in the blowfly visual system and a mobile robot to demonstrate that neural activity from a single cell is sufficient for a gaze stabilization task. In our setup, an immobilized fly faced two computer monitors as shown in Fig 1. Extracellular spiking activity from the left H1 cell was recorded and filtered to estimate the smooth spike rate. A feedback controller used this spike rate to update the robot speed at an interval dt = 50ms. The robot was placed on a turntable that was surrounded by a vertically oriented grating (contrast ~ 100%, spatial wavelength = 11°) and the turntable was rotated with a sinusoidal speed profile within the horizontal plane. High-speed cameras mounted on the robot captured and transmitted the resultant visual motion to the monitors at 200 fps.

We tested rotation frequencies from f = 0.03–3 Hz and resulting robot speed profiles were recorded for static feedback gain Kp = 0.6, 1.0, 1.4. Bode magnitude plot of a proportional or P-controller shows a low-pass filter trend over the frequencies probed. Bode phase plot shows system is stable for f < 3 Hz and approaches instability at f = 3 Hz. Performance of the P-controller was compared to an adaptive controller, P-controller with adaptive gain Kp and an integration time of 500ms, and was found to be better for the P-controller with a similar phase profile. Performance of the P-controller was also slightly better under naturalistic lab environment than under grating pattern visual stimulus.

To record neural activity from the moving fly, we designed a micro-recording probe containing a high gain, low noise, high linearity, and low power differential amplifier. To achieve small size the probe is an unpackaged die with attached electrodes. Extracellular neuronal signals are typically < 300 μV, last < 2 ms, and have most of their power < 5 kHz. Digitizing such small signals requires > 1000x amplification and very low circuit noise. The amplifier was designed for low power to increase battery life and avoid excessive heat production, which may effect neural function or desiccate and harm the fly. We experimentally assessed the amplifier by recording from a interneuron in the fly visual system. The signals we recorded were similar to those measured by a commercial amplifier, both in the time and in the frequency domain.

The objective of this project is the design and testing of a closed-loop experimental setup that would enable the acquisition of electrophysiological data from moving flies to study the neural activity underlying multisensory motor control and to directly correlate neural processing with behavioural performance.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Robot
Grating
Pattern
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45°
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Robot Compensation Speed
Speed (8 bit)
Time /s
H1 Spiking Response
Membrane
Potential / V
Time /s
Estimated Spike Rate
Spike Rate / Hz
Time /s

# Correlations between head size, eye, brain and optic lobes in male and female blowflies determined by micro-CT imaging

Martina Wicklein[1], Daniel A Schwyn[1], Richard L Abel[2], Thomas J Simonsen[3], Holger G Krapp[1]

[1]Imperial College London, Department of Bioengineering, London, UK
[2]The Natural History Museum, Department of Mineralogy, London, UK
[3]The Natural History Museum, Department of Entomology, London, UK

Virtual 3D models are powerful tools for the study of the functional organization of complex anatomical structures. The creation of such models usually requires sophisticated procedures including the dissection, fixation, and sectioning of the preparation, followed by microscopic inspection. Such techniques are rather time consuming, and may lead to tissue distortions. In addition, it is virtually impossible to recombine different body structures into a complete model of the original specimen.
X-ray micro computed tomography (micro-CT) is a 3D imaging technique that can be used to quickly analyse invertebrate preparations, and thus overcome some of the limitations of microscopic techniques. It is possible to differentiate between cuticular structures and different types of soft tissues due to differences in radio-opacity, as we demonstrated earlier [1].
In this study, we utilize micro-CT to determine sexual dimorphisms in brains of blowflies. Differences in volume of specific brain areas are thought to be due to an interaction of the species-specific behavioural repertoire, metabolic constraints and evolutionary processes.
Female blowflies (Calliphora vicina) that were reared under the same conditions as their male siblings are generally larger and tend to have larger heads. It is unclear, however, whether this difference in head size is reflected in differences regarding the size of the brain.
Male and female blowflies are easily distinguished based on the size and the shape of their eyes in the fronto-dorsal area of the head. In this area male blowflies have an enlarged and specialised region called the 'love spot'. This sexual dimorphism helps male flies chase and catch females on the wing and is caused by a bigger number and diameter of ommatidia in the love spot area. Is this anatomical dimorphism reflected by any volume differences between the male and female visual neuropils?
For both questions to be answered it is essential to obtain volumetric measurements of the intact head and eyes in the same individual in which the volumetric measurements of the relevant brain areas are observed. Because micro-CT allows us to create virtual 3D models of the specimen in question without the need of prior dissection of the specimen both of these requirements are fulfilled.
We obtained 3D virtual reconstructions and volumetric measurements of male and female fly brains including the neuropils of the optic lobes, the compound eyes and the head capsule. We fixed whole male and female blowflies in ethanol and elemental iodine and scanned the specimens using a micro-CT scanner at the Natural History Museum, London. The specimens were reconstructed using 3D rendering software. The optic lobes, head capsule and the compound eyes were manually segmented and subjected to volumetric measurements. Then, brain volume was correlated with head size and the volumes of the optic lobes were correlated to the size of their input, measured by the number of ommatidia in the eyes.
The fast processing time and the possibility to simultaneously distinguish between a wide range of different tissues and structures in situ allows for a comprehensive 3D model of the animals' functional anatomy and its analysis. This makes micro-CT scanning well-suited for studies of anatomical features and to reveal their natural variability.

[1]Schwyn et al (2010) Imaging the fly brain using microCT. Proceedings for the 9th International Congress of Neuroethology, Salamanca Spain

# Extracellular long-term recordings from polarization-sensitive interneurons of the locust brain

Miklós Bech[1], Uwe Homberg[1]

[1]Philipps-University Marburg, Department of Biology, Animal Physiology, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

The desert locust Schistocerca gregaria perceives polarized light with a specialized dorsal rim area of the compound eye. For maintaining constant flight directions during long-range migration, it potentially uses the polarization pattern of the blue sky as a compass cue. In the brain, polarization information is passed through the optic lobe and the anterior optic tubercle to the central complex which most likely serves as an internal sky compass (Heinze and Homberg, 2007, Science 315:995). Because the electric field vectors (E-vectors) of the blue sky are arranged in concentric circles around the sun, the sky polarization pattern changes during the day like the position of the sun. Thus, for long range navigation, the processing of polarized light information has to be time-compensated.

We performed extracellular long-term recordings from polarization-sensitive neurons of the locust central complex using copper wire electrodes. Electrodes were inserted into the brain while the locust was fixed vertically in the experimental setup. The animal was stimulated with polarized blue light (465 nm), obtained by an LED. The blue light passed a motor-driven rotating polarizer, which was set on a perimeter apparatus. It allowed stimulation with a rotating E-vector from different elevations in the visual field. The receptive field of the recorded neuron was tested every hour by stimulation through a clockwise and counterclockwise rotating polarizer (rotating speed of 30°/s). The recordings lasted for up to 24 hours. Single units were identified through template matching combined with a cluster analysis.

The units showed polarization-opponency and had wide receptive fields over a range up to 180° along the left-right meridian. Within the visual field, E-vector tuning was not constant but changed considerably along the right-left meridian. In addition, changes in response amplitude and E-vector tuning depending on the time of day were observed. Several recorded units showed strong excitation or inhibition when the light source was shifted along the right-left meridian in the visual field of the locust suggesting directionally-specific motion sensitivity. Comparison of the physiological characteristics of the recorded units with data from previous intracellular recordings allowed for an assessment of the recorded cell types. Supported by DFG grant HO 950/21-1.

# How does conflicting compass information affect desert ants' orientation?

Fleur Lebhardt[1], Bernhard Ronacher[1]

[1]HU Berlin, Biologie / Verhaltensphysiologie, Invalidenstr. 43, 10115 Berlin, Germany

In this study we investigated the influence of the polarization pattern on the orientation of desert ants (*Cataglyphis fortis*). Desert ants navigate in the featureless environment of their desert homeland by means of path integration. During their foraging course they continually ascertain the direction and the distance of their path segments and combine these to a home vector. While distance information is derived by an egocentric odometer, directional information is visually determined using the sky's polarization pattern and the sun's position (Wehner and Müller 2008, Proc.Natl.Acad.Sci. USA 103(33):12575). If no sky compass information is accessible, ants do not incorporate the distance travelled into their path calculation (Ronacher *et al*. 2006, J.Exp.Biol. 209:3301). However, a constant polarization pattern over a certain distance, such as a uniform linearly polarized light created by a polarization filter, will be accepted as sufficient optic input.
We aimed at elucidating how ants actually combine different directional information derived by the polarization pattern from the sky, the sun's position, and idiothetic cues. Different experiments were performed in which the ants were confronted with contradictory information about their movement direction. Ants were trained to visit a feeder (~ 7m distance from the nest) through an aluminium channel covered with a linear polarizing filter in vertical, horizontal and diagonal orientation relative to the ant's walking direction. After the arrival at the feeder individual ants were transferred to a test field where their homing direction could be observed and documented.
In a first series of experiments the straight channel was shaded from direct view of the sun, and a bend was simulated by a 90° turn of the polarization filter, while the propioceptive direction information signalled a straight path. As a reciprocal experiment the ants travelled in a channel with a 90° turn, while a straight ahead movement was visually pretended by a constant polarization pattern. In a second series, the sun was visible in the channel covered with a polarization filter, thus providing conflicting directional information via the two sky compass cues. The polarization cue turned out to be dominant over idiothetic cues and over the sun compass information.

# Identification of novel genes required for the internalization of Drosophila TRPL

Alexander Cerny[1], Nina Meyer[1], Thomas Dürr[1], Claudia Oberegelsbacher[1], Armin Huber[1]

[1]University of Hohenheim, Biosensorics, Garbenstr. 30, 70599 Stuttgart, Germany

In Drosophila, phototransduction occurs by a G-protein mediated signalling cascade. Upon absorption of a photon, the G-protein coupled receptor rhodopsin is converted to its activated form metarhodopsin. As a consequence the alpha subunit dissociates from the G-protein and activates phospholipase Cß which hydrolyses PIP2 into IP3 and DAG. By a yet unknown mechanism the activity of the phospholipase Cß initiates the opening of two cation channels TRP and TRPL. While TRP is constitutively anchored in the rhabdomeric membrane by the inaD complex, TRPL translocates between the rhabdomeric membrane after dark adaptation and an intracellular compartment after several hours of illumination thereby changing the biophysical properties of the rhabdomeric membrane. Studies of Drosophila phototransduction mutants revealed that the phototransduction cascade and calcium influx through TRP is required and sufficient for TRPL internalization.

In order to identify novel genes required for the internalization of Drosophila TRPL, we conducted a FRT/FLP mutagenesis screen of the chromosome arms 2R, 2L and 3R giving rise to 14 mutants defective in TRPL translocation. Of the mutants on chromosome arm 3R two mutants could be identified as novel alleles of TRP and Rhodopsin1, respectively, demonstrating the feasibility of the screen. Four mutants on chromosome arm 2R (ttd7, ttd11, ttd13 and ttd14) turned out to not affect any known phototransduction genes and were analyzed in greater detail. At first, the mutants were mapped by a complementation analysis with a set of overlapping deletions, covering 98% of the genes located on chromosome arm 2R. Among the four mutants a candidate region could be identified for three mutants (ttd11, ttd13 and ttd14). Then, candidate genes were identified in the respective region for every mutant and, if available, mutant alleles of candidate genes were analyzed with our mutants for complementation. For ttd11, no mutant of a candidate gene displayed a non-complementation. Therefore, it is likely that one of the five genes not tested due to a lack of mutant alleles is mutated. The mutant ttd12 did not complement with two mutant alleles of alicorn (alicornAd2 and alicornDP00037) the Drosophila ortholog of the ß-subunit of the AMP activated protein kinase (AMPK). However, the molecular lesion of the alicorn gene in ttd12 is still elusive. The mutant ttd13 did not complement with two mutant alleles of the CG30118 gene (CG30118EY01823 and CG30118KG03769) and on the molecular level, a substitution of a conserved Prolin75 residue to Leucin could be identified in ttd13 mutant flies. Bioinformatic analysis of CG30118 identified a CYTH-like domain which is supposed to be a metal ion dependent hydrolase of triphosphate containing substrates. However, functional data about the CG30118 are not yet available.

Taken together, the results of our mutagenesis screen suggest that beyond the phototransduction cascade which serves as a trigger also other genes are involved in the translocation of TRPL. The identification and molecular characterization of these genes might give new insights into the cellular mechanisms driving the translocation of TRPL.

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# Influence of ventral optic flow on distance estimation in desert ants (*Cataglyphis fortis*)

Kathrin Judith Schwannauer[1], Siegfried Bolek[1], Harald Wolf[1]

[1]Ulm University, Institute for Neurobiology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

The desert ant *Cataglyphis fortis* is a central place forager living in the salt pans of North Africa. This species proved one of the most suitable objects in navigation research (Wehner 2003, J Comp Physiol A 189, 579) in the past decades. Foragers of *C. fortis* search the featureless surroundings of their nest for food items, mostly dead insects, for distances of up to several hundred meters. After finding a food item, the ants return to the nest on a direct path, instead of following the meandering outbound path of the search. This form of navigation is independent of external landmarks and similar orientation cues (though these may be used if present), and it is termed path integration, or vector navigation.

Vector navigation requires information about angles steered and distances covered. Angular information is provided by a sky compass that is similar to the sky compass of honey bees (Wehner & Müller 2006, PNAS 103, 12575). Distance estimation is accomplished by the combination of two systems, a ventral optic flow integrator (Ronacher & Wehner 1995, J Comp Physiol A 177, 21) and a stride integrator (Wittlinger et al 2006, Science 312, 1965). Here we examine the characteristics of the ventral optic flow integrator and how it contributes to overall distance estimation in different test situations.

In earlier experiments, we had observed that ants which were not manipulated during their outbound journey (training), but had their ventral eye halves covered during the homebound journey (test), underestimated their homing distances significantly. In continuation of these experiments, individually marked ants were trained to shuttle between nest and feeder through an aluminium channel. After sufficient training, ants were caught at the feeder and prepared for the test. In one group, the ants had only parts of the ventral halves of both of their eyes covered, instead of the complete ventral eye halves. A second group had the ventral halve of only either the left or right eye covered before being tested. A final group had their frontal ventral eye halves covered during training, but for the test this eye cover was removed and replaced by a cover on the caudal ventral eye halves of both eyes. A control group had the ventral halves of both eyes covered during training as well as during testing, another control group remained completely unmanipulated.

After manipulation the ants were transferred to a test channel where they performed their homebound runs. All groups of ants which had parts of their ventral eyes covered consistently showed reductions in homing distance that were roughly half as pronounced as in the original experiment with complete ventral eye halve covers. The two control groups showed normal homing distances. These results indicate that different portions of the ventral eye, and the ventral halves of both eyes, contribute about equally to distance estimation.

# Internalization of the *Drosophila* TRPL ion channel is mediated by Rab5 and RabX4

Claudia Oberegelsbacher[1], Carina Schneidler[1], Armin Huber[1]

[1]University of Hohenheim, Biosensorics, Garbenstrasse 30, 70599 Stuttgart, Germany

Signaling at the plasma membrane can be modulated by up- and down-regulation of signaling proteins. A prominent example for this type of regulation is the *Drosophila* TRPL (TRP-like) ion channel which changes its spatial distribution within the photoreceptor cell depending on the light condition (BÃ¤hner et al., 2002 Neuron 34:83-93). In dark-raised flies TRPL is localized in the rhabdomeral photoreceptor membrane and translocates to the cell body upon illumination. Analysis of phototransduction mutants revealed that the internalization of TRPL depends on the activation of the phototransduction cascade and requires $Ca^{2+}$ influx through light-activated TRP channels. Although complete internalization of TRPL requires more than six hours of constant illumination, first changes in the rhabdomeral localization of TRPL can already be observed after five minutes of light exposure. As reported previously (Cronin et al.; 2006 J. Cell Sci. 119:2935-2944), the internalization of the TRPL channel is a multistep process. In stage 1, TRPL is transported to the base of the microvilli and to the adjacent stalk membrane. The subsequent transfer of TRPL to the storage compartment (stage 2) is accomplished by vesicular transport, also used for the internalization of rhodopsin. This conclusion is based on the observation that - in immunocytochemical studies - most of TRPL-containing vesicles are also labeled with antibodies against Rh1 rhodopsin, indicating a co-transport of these two proteins. In the case of rhodopsin, the internalization is mediated by the small GTPase Rab5 (Han et al., 2007 EMBO J. 104:17730-177734), which leads to the assumption that Rab proteins might also be involved in TRPL trafficking. To investigate this possibility, we screened 29 dominant negative Rab-mutants by deep pseudopupil analysis. Flies defective in TRPL translocation were further examined by immunocytochemistry. Our screen resulted in the identification of two dominant negative Rab-mutants Rab5DN and RabX4DN, which show defects in light-induced TRPL internalization. Cross sections of dominant-negative Rab-mutants show that only the first stage of TRPL transport can be accomplished in these mutants, but not the step depending on vesicular transport. These observations indicate that Rab5 and RabX4 mediate TRPL internalization.

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# Localisation of chimeric TRP/TRPL ion channels in Drosophila photoreceptors

Tina Oberacker[1], David Richter[1], Alexander Cerny[1], Armin Huber[1]

[1]Universität Hohenheim, Fachgebiet Biosensorik, Garbenstr. 30, 70599 Stuttgart, Germany

TRP cation channels serve as sensors and transducers of environmental stimuli. The Drosophila TRP (transient receptor potential) channel, and its closest relative, TRPL (TRP-like), are essential components of the phototransduction cascade of the compound eye. TRPL but not TRP translocates between the light harvesting membrane and an intracellular compartment. In dark-adapted flies TRPL is localised in the rhabdomeral photoreceptor membrane and translocates into the cell body upon illumination. We addressed the question which portions of the TRPL protein are required for triggering the light-dependent translocation of the TRPL ion channel. Using eGFP-tagged chimeric TRP/TRPL channels we tested if it is possible to "transplant" the property of light-dependent translocation observed for the TRPL channel onto the TRP channel. The chimeric constructs were expressed in the photoreceptor cells of living transgenic flies. In these constructs the N-terminal region, the C-terminal region, both parts or the transmembrane regions of TRP were replaced by the corresponding regions of TRPL. Their subcellular loaclisation was analyzed by detecting the eGFP fluorescence on immunocytochemical cross sections through the eyes. Chimeras containing the N-, the C-terminal region or only the transmembrane regions of TRPL showed little change in subcellular localisation in response to light. They were predominantly localized in the cell body irrespective of the light condition. The chimera containing the N- and C-terminus of TRPL (chimera 3-eGFP), however, showed a light-dependent translocation behavior almost identical to TRPL. To estimate the relative amount of chimera 3-eGFP in the rhabdomers, we quantified the relative amount of eGFP-fluorescence in the rhabdomers in comparison to flies expressing TRPL-eGFP on water immersion images of intact eyes. Of chimera 3-eGFP more protein was present in rhabdomeres in the dark than in the light, indicating that it was translocated to the cell body upon illumination with orange light. This translocation was reversible since subsequent dark-adaptation resulted in redistribution of the chimeric protein to the rhabdomeres. We also studied the subcellular distribution of chimera 3-eGFP in the trpl302-nullmutant to exclude that endogenous TRPL affects the localisation of chimera 3-eGFP. In this mutant background the localisation of chimera 3-eGFP is similar as in wild-type background, except that in dark-adapted flies some labelling is also observed outside the rabdomeres. In summary, we conclude that the N-terminal and C-terminal region of TRPL must cooperate to allow for the specific light-dependent translocation that is typical of the TRPL ion channel, indicating that this process is not mediated by a simple single internalization motif.

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# Modeling wavelength discrimination in *Drosophila*: Evidence for a contribution of Rhodopsin 1 to color vision

Christian Garbers[1], Carol O'Brien[2], Christopher Schnaitmann[3], Hiromu Tanimoto[3], Thomas Wachtler[1]

[1]Ludwig-Maximilians-Universität München, Department Biology II, Planegg-Martinsried , Germany
[2]Department of Anatomy, University College Cork, Ireland
[3]Max-Planck-Institut für Neurobiologie, Martinsried, Germany

In each ommatitdium of the compound eye of *Drosophila*, the six outer photoreceptors (R1-R6) express rhodopsin 1 (Rh1) as their light sensing pigment. The inner photoreceptors R7 and R8 express Rh3 or Rh5, and Rh4 or Rh6, respectively, opsins with different spectral sensitivities. The neural mechanisms of color vision in *Drosophila* are unknown, but it is commonly assumed that in dipterans, spectral discrimination is mediated by signals from the inner photoreceptors [1]. The outer photoreceptors, and thus Rh1 are usually not considered to play a role in *Drosophila* color vision. We tested these assumptions by analyzing predictions of models of color discrimination derived from the spectral absorption properties of *Drosophila* opsins. Based on a previous approach to predict color discrimination thresholds [2] we developed a computational model for wavelength discrimination in *Drosophila*. The model combines the different photoreceptor spectral sensitivities into opponent mechanisms. Relative wavelength discrimination is calculated as the distance in the space of opponent signals. We considered various model variants with different combinations of *Drosophila* rhodopsins. Models were fit to published data on wavelength discrimination in *Drosophila* [3] by adjusting the relative weights of the opponent mechanisms. Most models yielded poor fits to the data. Only those models with Rh1 in at least one of their opponent mechanisms were able to fit the data well. This finding is corroborated by comparisons between the experimental discrimination curves and the spectral sensitivity curves of the different opsins. Our results indicate that wavelength discrimination in *Drosophila* involves opponent channels that include rhodopsin 1.

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# Multimodal Sensory Integration in a Fly Motoneuron

Juergen Haag[1], Adrian Wertz[1], Alexander Borst[1]

[1]Max-Planck-Institute for Neurobiology, Dept. Systems and Computational Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

Integration of information from different sensory modalities into a unified percept is one of the most important roles of the central nervous system. However, at what level of the nervous system this integration occurs and according to which rules are still open questions. Here, we investigate multi-modal sensory integration in a fly motoneuron, the so-called VCNM-cell (Ventral Cervical Nerve Motoneuron), controlling head movement of the animal [1]. We show that this neuron receives input from two identified wide-field motion-sensitive neurons, the HSN and the HSE-cell [2,3], from the wind-sensitive Johnston organ on the antenna, from the campaniform sensilla of the halteres and from a central neuron signaling flight activity. We find that visual motion alone leads to only subthreshold responses. When combined with wind stimuli or flight activity, VCNM responds to visual motion by modulating its spike activity in a directionally selective way. This nonlinear enhancement of visual responsiveness in VCNM by other sensory modalities is reflected at the behavioral level, when compensatory head movements are measured in response to visual motion: while head movements of flies have only a small amplitude when flies are at rest, the response amplitude is increased by a factor of 30-40 during flight.

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# Optic Flow Processing in a Fly Neck Motor Neuron

Adrian Wertz[1], Juergen Haag[1], Alexander Borst[1]

[1]Max Planck Institute of Neurobiology, Department of Systems and Computational Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

In order to control complex flight maneuvers, chasing or landing behavior, flies rely heavily on the computation of optic flow. The processing of optic flow is performed in the third visual neuropil by a set of about 60 lobula plate tangential cells. These motion sensitive tangential cells are well described with respect to their visual response properties [1] and the connectivity amongst them [2]. They have large and complex receptive fields with different preferred directions in different part of their receptive fields matching the optic flow that occurs during various flight maneuvers. However, little is known about the specific connectivity of tangential cells to postsynaptic neurons descending to neck muscles or to the motor circuits in the thoracic ganglion. Here we describe the physiology and the connectivity of a neck motor -neurons called CNMN6 [3]. Measuring locally the motion sensitivity of the cell, we found that CNMN6 has a receptive field tailor – suited to detect a nose-up pitch of the fly. Injection of neurobiotin into the cell reveals a dye coupling to a subset of ipsilateral VS cells and the contralateral HSS cell. The receptive field of CNMN6 can be explained by the coupling to VS cells whereas the input from HSS cell is not observable in the local motion sensitivity. However measuring the global motion sensitivity either with both eye's intact or with the contralateral eye occluded reveals a binocular integration of optic flow in CNMN6. In addition, we found that CNMN6 responds to wind stimuli delivered to the antennae.

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# Receptive field properties and pattern-dependent response modulations in motion-sensitive visual interneurons – a model study

Hanno Gerd Meyer[1], Jens Peter Lindemann[1], Martin Egelhaaf[1]

[1]Bielefeld University, Neurobiology & CITEC, P.O. Box 10 01 31, D-33501 Bielefeld, Germany

Even if a stimulus pattern moves at a constant velocity across the receptive field of motion sensitive neurons, such as lobula plate tangential cells (LPTCs) of flies, the response amplitude is usually not constant but may modulate over time in a pattern-dependent fashion. Hence, it is not easily possible to infer the time course of pattern velocity from such neural signals. The response properties of motion-sensitive LPTCs in the brain of the blowfly give evidence, that motion detection in Diptera is incorporated via integration of several spatially separated correlation-type elementary motion detectors (EMDs). The receptive fields and spatial sensitivity distributions of LPTCs are determined by the location and extension of their dendritic fields and the dendritic density distributions within these fields. LPTC responses depend on the contrast of the spatial frequency components of the visual stimulus. The contrast of time-dependent brightness patterns of the moving retinal image is related to the amplitude of the output signal, resulting in pattern-dependent modulations in LPTC response. Pattern-dependent response modulations have been interpreted as '*pattern-noise*', because they deteriorate the neuron's ability to provide unambiguous velocity information [1]. On the other hand, these modulations could also provide the system with valuable information about the textural properties of the environment. It may be assumed, that due to the sensitivity of LPTCs to contrast and spatial structure of local features within moving scenes, LPTC response might additionally provide information about the spatial localization of pattern motion in the visual field.
In the current project the influence of the size and shape of receptive fields on the amplitude of pattern-dependent response modulations was analyzed. This was done by model simulations of arrays of EMDs which have previously been employed to explain LPTC responses. Four recently proposed model versions were used which differ in their input organization. High dynamic range (HDR) natural panoramic images moving at constant velocity served as input datasets. We quantified the effects of receptive field size and shape on pattern dependent amplitude modulations by their standard deviation over time from the mean response. The receptive field size influences modulation amplitude to a great extent. An increasing number of EMDs included in the receptive field of LPTC models leads to a decrease of the pattern-dependent modulations, since spatial changes within the visual field are smoothed out by the summation over many spatially displaced input signals. This effect - depending on the model version used – is coupled to the geometry of the receptive field. However, large receptive fields deteriorate the ability of the system to localize movements in the visual field, hinting at a trade-off between the quality of velocity signals and their locatability. Hence, the size and geometry of receptive fields should be adjusted according to the task of the motion sensitive neuron: they should be large, if good velocity signals are required, but should be relatively small, if motion dependent pattern information is required that can be localized in the visual field.

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# Responses of central-complex neurons to unpolarized light stimuli in the desert locust

Ronny Rosner[1], Uwe Homberg[1]

[1]Philipps-Universität Marburg, Tierphysiologie, Karl-von-Frisch-Straße 8, D-35032 Marburg Marburg, Germany

Many insects rely on visual information when navigating through their environment. A key role in computing the direction of locomotion is ascribed to neurons of the central complex, a group of neuropils in the center of the insect brain consisting of the central body and the protocerebral bridge (Homberg 2008, Arthropod Struct Devel 37:347). Behavioral studies on mutant fruit flies showed that particular substructures of the central complex are required for recognition of visual landmarks, visual targeting in climbing motor activity, and spatial visual orientation memory (Strauss 2002, Curr Opin Neurobiol 12:633). Evidence from desert locusts suggest that the compass for celestial navigation by means of the polarized light pattern of the blue sky resides in the central complex (Heinze und Homberg 2007, Science 315:995).

To further analyze visual signal processing in the central complex we perform intracellular recordings from neurons in this brain region while the animals experience diverse stationary and moving stimuli presented via flat-screen monitors. Distinct types of central complex neurons are sensitive to e.g. brightness changes, moving gratings, small moving boxes or looming stimuli. Usually the neurons are highly adaptive, responding in a pronounced way only during the first presentation or if long time intervals are allowed in -between the presentations.

Interestingly the neuronal responses to unpolarized light stimuli are not restricted to neurons with ramifications in the upper division of the central body. Instead neurons so far considered to be involved solely in the computation of polarized light information, such as tangential neurons of the protocerebral bridge or columnar neurons with ramifications in the lower division of the central body also responded to complex unpolarized light stimuli, e.g. expanding discs. This suggests that the integration of unpolarized und polarized visual information in the central complex is more widespread and complex than previously thought.

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# Sensitivity for motion and orientation in the blowfly visual system

Christian Spalthoff[1], Rafael Kurtz[1]

[1]Bielefeld University, Department of Neurobiology, Postfach 100131 , 33501 Bielefeld, Germany

The detection and the neural processing of motion is an essential task of the visual system. Although the properties of visual motion-sensitive neurons in vertebrate and invertebrate brains have been studied to some detail, the cellular mechanisms leading to direction-selective responses to motion remained largely unresolved, because the input circuitry of these neurons is often difficult to access with electrophysiological techniques.
Much of our knowledge on motion computation stems from recordings of the axonal output signals of lobula-plate tangential cells (LPTCs) in the fly brain. These neurons have large receptive fields and some of them show a pronounced specificity for distinct patterns of optic flow, i.e. retinal image shifts during self-motion. However, at this time it is not precisely known how these neurons obtain their sensitivity for motion in distinct directions, because the small size of their retinotopically arranged local input elements renders electrophysiological recording an inefficient approach. We circumvented this problem by monitoring localized neuronal activity in two types of calcium imaging experiments:
1) By measuring the spatial patterns of calcium concentration changes across the large dendrites of LPTCs we visualized regional activity elicited by local presynaptic neurons providing retinotopic input to LPTCs.
2) We stained small groups of neurons in the medulla, the input neuropile to the lobula plate, with calcium-sensitive dyes via local electroporation.

We found that calcium signals at LPTC dendrites are highly direction selective, with characteristic spatial variations of the local preferred direction across the dendrite. The recordings in the medulla do not show a similarly distinct directional selectivity but suggest that orientation selectivity might already be expressed at this stage of the visual pathway.

# Stochastic and mutually exclusive expression of *Drosophila rhodopsin* genes

Jens Rister[1], Claude Desplan[1]

[1]New York University, Department of Biology, 1009 Silver Center, 100 Washington Square East, 10003-6688 New York, USA

The compound eye of *Drosophila melanogaster* is a powerful model system to study the development of a retinal mosaic and underlying cell-fate decisions. In the fly compound eye, each unit eye (ommatidium) contains eight photoreceptors, six "outer" (R1-6) and two "inner" (R7/8) ones. They express different Rhodopsin (Rh) photopigments that allow wavelength discrimination (1). R7 and R8 Rhs are tightly coupled in two ommatidial subtypes which are stochastically distributed over the retina (1, 2): 30% are of the pale (p) *rh3/rh5* subtype while the remaining 70% belong to the yellow (y) *rh4/rh6* subtype. This remarkable cell-type specificity is mainly achieved at the level of transcription: Short "minimal" promoters of less than 300 base pairs are sufficient to recapitulate these complex patterns (3).
To gain insights into the underlying regulatory logic, we performed an extensive promoter structure-function analysis. We have identified cis-regulatory elements that are required for subtype specific expression of Rhodopsins *rh5* and *rh6*. Our data suggest that activation mediated by Orthodenticle and Senseless (3, 5) and subtype specific repression play a crucial role in the stochastic expression of *rhs*. To test the sufficiency of the identified motifs and to reconstruct this regulation, we are currently using artificial binding sites to reverse-engineer a synthetic *rhodopsin* promoter.

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# Synaptic plasticity in visual and olfactory brain centers and behavioral effects in *Cataglyphis fortis* after unilateral sensory deprivation

Anna Hellwig[1], Sara Mae Stieb[1], Rüdiger Wehner[2], Wolfgang Rössler[1]

[1]University of Würzburg, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany

[2]Brain Research Institute, University of Zürich, Switzerland

The desert ant *Cataglyphis fortis* is native to salty areas or even salt pans of the North African desert. Because of the volatility of trail pheromones in this harsh area with its high temperatures, the ants predominantly rely on vision to find their way back home after foraging. The ants use a polarization compass in combination with a proprioceptive odometer for path integration in addition to landmark-dependent orientation (Wehner, J Comp Physiol A, 2003) and olfactory cues (Wolf et al., J Comp Physiol A, 2005). *C. fortis* undergoes an age-related polyethism which subdivides the colony into dark-adapted interior workers and short-lived outdoor foragers. It was shown that light exposure – both under natural conditions as well as precociously - leads to structural synaptic changes in the mushroom body (MB) calyces (Stieb et al., Dev Neurobiol, 2010) – sensory integration centers involved in learning and memory. In this study we addressed the question whether light exposure is not only affecting synaptic changes in visual brain centers, but also in the ants` behavior. A previous study revealed that despite unilateral occlusion of one eye, the ants were still capable to navigate using the pattern of polarized light (Wehner and Mueller, Nature Vol 315, 1985) indicating that *Cataglyphis fortis* may exhibit an inter-occular transfer.

To study the different effects of light treatments on the ants` behavior we exposed callows (1-day old ants) for specific time periods to light. The activity of light-exposed and a dark-control group was recorded during light pulses as well as during dark phases. First results indicate that light exposure induces a boost in the ants` activity. Visual and olfactory deprivation experiments were performed to explore structural plasticity of synaptic complexes – so-called microglomeruli (MG) – in the visual and olfactory compartments of the MB calyces. Deprivation of one of the compound eyes and its three ocelli and amputation of one antenna was done to study multimodal effects of light and olfactory cues on synaptic microcircuits in the MBs. We analyzed on the number of MG in the visual (collar) and olfactory (lip) input regions of the MB calyx as well as on overall volume changes. Fluorescent labeling of pre-and postsynaptic sides, laser-scanning confocal microscopy and image analyses tools were used to visualize and quantify potential changes in MG synapses.

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# Targeting of middle- and hind legs of the stick insect *Carausius morosus* on the slippery surface

Anne Wosnitza[1], Viviane Fischer[1], Ansgar Büschges[1], Matthias Gruhn[1]

[1]Universität zu Köln , Zoologisches Institut , Zülpicher Straße 47 b , 50674 Köln , Germany

In their natural habitat, stick insects (*Carausius morosus*) climb on branches. Previous work (e.g. Cruse 1979) suggested that stick insects perform targeting movements with their legs to find support for middle- and hind legs more easily. Based on behavioral experiments, it has been assumed that they use position information from the posterior extreme positions of front- and middle legs to control the anterior extreme position of the ipsilateral middle- and hind legs, respectively. Here we address the question whether this targeting of the middle- and hind legs is also present when influences through mechanical coupling through the ground are removed. If targeting is present under these conditions, that would emphasize the role of underlying neuronal mechanisms. We used a slippery surface setup (Graham & Cruse, 1981; Gruhn et al. 2006) to provide a walking situation with removed mechanical influences between the legs.

First, we looked for evidence of leg targeting in animals walking continuously on the slippery surface. No targeting of posterior legs could be observed, neither for hindlegs, nor for middle legs. We found no correlation between the extreme positions in the direction parallel (x) or perpendicular (y) to the body axis. The distances between the pairs of extreme positions did not show a correlation either. Secondly, we studied whether targeting occurred in hind- or middle legs during walking on the slippery surface, when the rostral neighboring leg, i.e. either middle- or front leg, was positioned at a defined position relative to the body (cf. Cruse, 1979). Targeting precision during the first step of a sequence of steps of the ipsilateral posterior leg was analyzed with respect to dependency on that position. Under these conditions, the extreme positions of front- and middle legs show a tendency to correlate parallel to the body axis. However, no targeting was observed for leg positions perpendicular to the body axis. These results indicate that a neural mechanism exists for controlling the anterior extreme position of a stepping leg along the axis of the body during the first step of a walking sequence. Results from experiments evaluating the first step during free walking on the slippery surface are pending.

# The *Drosophila* Transient Receptor Potential Ion Channel is light-dependently phosphorylated

Olaf Voolstra[1], Stefan Kaltenbach[1], Katherina Beck[1,3], Claudia Oberegelsbacher[1], Jens Pfannstiel[1,2], Armin Huber[1,2]

[1]University of Hohenheim, Biosensorics, Garbenstrasse 30, 70599 Stuttgart, Germany

[2]University of Hohenheim, Life Science Center, Garbenstrasse 30, 70599 Stuttgart, Germany

[3]present adress: University of Würzburg, Institute of Medical Radiation and Cell Research, Versbacher Strasse 7, 97078 Wuerzburg, Germany

The activity of many ion channels is controlled by phosphorylation. For example, in the major delayed rectifier K+ channel Kv2.1 that is expressed in most central neurons, reversible phosphorylation contributes to graded modulation of voltage-dependent gating (Park et al., 2006 Science 313:976-979). The *Drosophila* phototransduction cascade terminates in the opening of an ion channel, designated transient receptor potential (TRP). TRP has been shown to become phosphorylated in vitro, suggesting regulation of this channel through post-translational modification. Here, we identified 21 phosphorylation sites of the *Drosophila* TRP ion channel in a mass spectrometry approach. 20 of these sites are located in the C-terminal portion of the channel and one phosphorylation site resides near the N-terminus. At least 7 of these phosphorylation sites were phosphorylated predominantly in the light, whereas one site, serine 936, was predominantly phosphorylated in the dark. Since serine 936 was the only site that was found to be phosphorylated predominantly in the dark, we generated a phosphospecific antibody against this site. In head extracts of dark-adapted wild type flies, large amounts of TRP phosphorylated at serine 936 were detected whereas no phosphorylation of serine 936 could be observed in extracts from light-adapted flies. Visually impaired mutants exhibited strong phosphorylation of serine 936 regardless of the light condition. In a mutant expressing a constitutively active TRP channel, no phosphorylation of TRP at serine 936 was observed. These results indicate that the dephosphorylation of serine 936 depends on the activation of the phototransduction cascade in vivo. However, the last step of the cascade, i.e. the opening of the TRP channel, is sufficient for triggering dephosphorylation of S936. We have now generated transgenic flies that express modified TRP channels in which specific phosphorylation sites were changed to alanine by site directed mutagenesis. A preliminary analysis of these flies will be presented. In our future work we hope to dissect the roles of single phosphorylation sites in the functioning of the TRP ion channel.

# The fine structure of homing behaviour in bees and its consequences for optic flow processing

Marcel Mertes[1], Laura Dittmar[1], Martin Egelhaaf[1], Norbert Boeddeker[1]

[1]Bielefeld University, Center of Excellence 'Cognitive Interaction Technology' (CITEC), Department of Neurobiology, Postfach 100131, 33501 Bielefeld, Germany

Bumblebees, Honeybees and ground-nesting wasps can memorize the spatial location of important places using a visual representation of the goal environment. To understand how they solve this complex task it is necessary to reconstruct the visual input they receive while acquiring a representation of the goal environment.

In this study we aim to understand what aspects of natural optic flow are being sensed by the animals and how optic flow may be used for spatial learning. To identify and critically assess the underlying mechanisms we first analyse the bee's behaviour in great temporal and spatial detail. This is important because the insects actively organize the visual information they receive through series of structured movements. On departure from a goal they perform learning flights that generate motion parallax information, which presumably helps them to identify close landmarks.

We used two high-speed cameras to record bees (honeybees and bumblebees) approaching and departing from a food source that was located between three landmarks in an indoor flight-arena. Subsequently we measured the 3D-flight trajectory and the bee’s body and head orientation off-line. The analysis revealed a fine structure of the coordinated head and body movements: fast, stereotyped head turns and, thus, gaze changes precede saccadic body turns.

Using a computer 3D-model of the flight arena we reconstructed what the bees saw during such learning and return flights. These reconstructions of original flight trajectories are used as visual stimuli to analyse under what conditions motion sensitive neurons in the bee brain reliably detect landmarks.

# The neuronal pathways through the anterior optic tubercle in the *Papilio* butterfly

Michiyo Kinoshita[1]

[1]Sokendai ( The Graduate University for Advanced Studies), Laboratory of Neuroethology, Shonan village, Hayama, 240-0193 kanagawa, Japan

Foraging behavior of the Japanese yellow swallowtail butterfly, *Papilio xuthus*, strongly depends on visual information. I have demonstrated its sophisticated color vision by behavioral experiments. Although the spectral organization of the compound eye retina of *Papilio* is well known, processing of the visual information in the central brain is poorly understood. Here I focused on the anterior optic tubercle (AOTu), a region in the protocerebrum receiving inputs mainly from the optic lobe. In order to identify the input as well as the output pathways through the AOTu, we injected tracer (dextran-conjugated biotin) into various regions of the optic lobe and also into the AOTu.

The AOTu receives inputs both from the medulla and lobula in the optic lobe via the anterior optic tract. When we injected the tracer into the ventral region of the medulla, medial region of the AOTu was strongly stained. Injection into the very dorsal region of the medulla stained the lateral region of the AOTu and three globular structures immediately ventral to the AOTu. Thse globular structures probably correspond to the lower unit of the AOTu in the locust, which is known to function in the polarization -based navigation. In preparations injected into the AOTu, stained neurons in the optic lobe are 1) fine columnar fibers throughout the medulla and the lobula with cell bodies located between the lamina and the medulla, 2) cells with branches in the layer 4 and 5 of the medulla and 3) cells with branches in the layer 2 of the lobula. Those neurons most likely construct a regional organization in the AOTu corresponding to the compound eye regionalization.

The injection into the AOTu stained at least three regions in the protocerebrum; the AOTu in the contralateral hemisphere, the lateral accessory lobes (LALs) in both hemispheres and the posterior medium protocerebrum. The connection between bilateral AOTus indicates integration of visual information from the both eyes. Considering the fact that the LAL receives olfactory information and contains descending neurons to the thorax in *Bombix mori*, the *Papilio* neurons from the AOTu to the LAL may contribute to integrate visual and olfactory information for multimodal sensation.

# Visual Motion Detection in Tethered Flying Flies

Sarah Nicola Jung[1], Alexander Borst[1], Juergen Haag[1]

[1]MPI of Neurobiology, Systems and Computational Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

Sensory neurons are mostly studied in fixed animals, but do they respond the same when the animal is free to move? Interestingly, recent studies found differences between responses of sensory neurons in resting versus moving insects ([1-3]). Since, in real life, the dynamic range of visual motion input in flies changes dramatically from the situation at rest over walking towards flying, it is interesting to investigate whether the visual system adjusts to these changes. Lobula plate tangential cells of flies lend themselves well to study this question because they are known to code for ego -motion based on optic- flow [4]. We compared the responses of the lobula plate tangential cell H1 to a visual pattern moving at different speeds under three different conditions: fixed flies before and after application of the octopamine agonist chlordimeform (CDM) and tethered flying flies. CDM has been previously shown to induce arousal in flies ([5]). We found that flying as well as the application of CDM significantly shifts and broadens the tuning of H1 towards higher speeds.

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T15-16C Identification of connexins in photoreceptors of 129/Sv mice
*Petra Bolte, Regina Herrling, Ulrike Janssen-Bienhold , Reto Weiler*

# Chromatic Bipolar Cell Pathways in the Mouse Retina

Tobias Breuninger[1], Christian Puller[3], Silke Haverkamp[3], Thomas Euler[1,2]

[1]MPI for Medical Research, Dept. Biomed. Optics, Heidelberg; [2]Centre for Integrative Neuroscience (CIN) / Institute for Ophthalmic Research, University of Tübingen; [3]MPI for Brain Research, Dept. Neuroanatomy, Frankfurt/M.

Like most mammals, mice feature dichromatic color vision based on short (S) and medium (M) wavelength-sensitive cone types. It is thought that mammals share a common retinal circuit that compares S- and M-cone output (in trichromats S- and M+L-cone) to generate blue/green (blue/yellow) opponent signals, with distinct bipolar cells providing separate chromatic channels. While S-cone selective ON-bipolar cells (in mouse "type 9") have been anatomically identified, little is known about other cone selective channels, for instance M-cone selective OFF-bipolar cells.

We characterized cone connectivity of selected mouse bipolar cell types using immunohistochemical methods. In addition we recorded the electrical responses to photopic dichromatic light stimulation and compared the chromatic tuning of selected bipolar cell types. To target and record specific bipolar cells in retinal slices we used transgenic mice expressing fluorescent proteins in subsets of retinal neurons and visualized individual cells with two-photon microscopy.

We first confirmed that type -9 bipolar cells display blue$^{ON}$ light responses. We then studied other types of cone bipolar cells. Our anatomical data indicate that four of the five mouse OFF-bipolar cell types (types 2, 3a/b and 4) as well as type 7 (as an example for ON-bipolar cells) indiscriminately contact both cones, whereas type-1 OFF-bipolar cells avoid S-cones. Electrical recordings of four types of cone bipolar cells (type 1, 2, 7 and 9) revealed that the chromatic tuning strongly depends on their position along the dorso-ventral axis – due to the dorso-ventral gradient in S-opsin co-expression in mouse M-cones. In *dorsal* retina, where co-expression is low, most type-2 cells were green-biased, with a fraction of cells (˜ 14 %) displaying strongly blue-biased responses, likely reflecting S-cone input. Type-1 cells were also green-biased but did not include blue-biased "outliers", consistent with type-1 cells avoiding S-cone contacts. Our hypothesis that "outliers" represent S-cone contacting bipolar cells is consistent with a simple statistical model of cone-bipolar cell connectivity. Our model was able to reproduce the measured distribution of chromatic tuning in the recorded OFF-bipolar cells. Therefore we suggest that type 1 represents the green$^{OFF}$ pathway in mouse. In *ventral* retina, all bipolar cell types studied displayed similar blue-biased responses, suggesting that color vision may be only supported in the dorsal mouse retina.

In conclusion, our data supports an antagonistically organized blue/green circuit with bipolar cells functioning as chromatically defined channels, which form the common basis for mammalian dichromatic color vision.

# Chromatic processing in mouse retinal ganglion cells

Le Chang[1], Tobias Breuninger[1], Thomas Euler[1]

[1]Centre for Integrative Neuroscience (CIN) / Institute for Ophthalmic Research, University of Tübingen; [2]Institute of Biophysics, Chinese Academy of Sciences, Beijing, China, AG Euler, Röntgenweg 11, 72076 Tübingen, Germany

Color processing has been intensively studied in ganglion cells of the primate retina, but in mouse correspondent knowledge is still incomplete. Extracellular recordings and behavioral studies revealed that mice feature color vision, but the underlying retina circuit, in particular the relevant morphological ganglion cell type(s), have not yet been identified or physiologically characterized.

We used dichromatic light stimulation (in the photopic range) of the short (S) and the medium (M) wavelength-sensitive mouse cone photoreceptors by two LED light sources adjusted carefully to evoke equal photo-isomerization for the corresponding opsins. The relative contribution of S- and M-opsin to ganglion cell input was estimated by modulating the intensity of the two chromatic stimulus components sinusoidally or in steps. Using 2-photon imaging, we targeted GFP -positive ganglion cells in transgenic mice in whole -mounted retina for extracellular recordings. The mouse line we used (GFP-O; Feng et al, 2000) features sparse labeling of various morphological types of retinal ganglion cells. The morphology of the targeted cell was classified using image stacks taken after the recording.

Our preliminary data show that ON alpha ganglion cells (soma diameter > 20 µm, dendritic field size > 200 µm) received strong M-opsin but weak S-opsin input in dorsal retina, whereas in the ventral retina they received significantly stronger S-opsin input. This is consistent with the presence of a gradient in S-opsin co-expression in M-cones along dorsal-ventral axis in mouse retina. Interestingly, we found some evidence for differential blue/green processing in the responses of ventral but not dorsal ON alpha ganglion cells, suggesting that chromatic processing circuit might be more complex than previously thought. In addition, using population 2-photon calcium imaging, we have started to characterize different types of retinal ganglion cells with respect to their chromatic tuning to identify cells that are involved in chromatic discrimination or processing (e.g. color opponent cells).

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# Closed-loop experiments for measuring spatial contrast integration in the retina

Daniel Bölinger[1], Tim Gollisch[2]

[1]MPI of Neurobiology, Visual Coding Group, Am Klopferspitz 18, 82152 Martinsried, Germany

[2]University Medical Center Goettingen, Dept. of Ophthalmology, Waldweg 33, 37073 Goettingen, Germany

The spatial receptive field of a retinal ganglion cell is the region over which it is sensitive to light stimulation. It is often implicitly assumed that a given cell integrates the stimulus linearly over this region, e.g. in the popular linear-nonlinear (LN) model. However, many ganglion cells show signs of nonlinear spatial integration, Y cells in the cat retina being just one example. Assessing the characteristics of these nonlinearities in a quantitative fashion has been difficult and mostly approached indirectly by comparing the performance of different hypothetical models.

Here, we aim at directly measuring potential nonlinearities in the spatial receptive fields of ganglion cells in the amphibian retina. Our approach is to subdivide the receptive field center into different parts and present different contrast steps in each part. For these stimuli, we measure *iso-response curves*, which are defined as those contrast combinations that lead to the same pre-specified response. Iso-response curves can be determined both for given firing rates (iso-rate curves) and first-spike latencies (iso-latency curves). The shapes of these iso-response curves depend on potential nonlinearities of the receptive field. The advantage of the iso-response approach lies in its independence of the cell's output nonlinearity, for example its threshold and saturation.

We record light responses from isolated, whole-mounted retinas with an extracellular multi-electrode array. For an efficient measurement of iso-response curves, we perform *closed-loop experiments*. This allows us to sample mainly the interesting region of stimulus space: The stimulus-evoked action potentials are analyzed on-the-fly. The stimulus is then adjusted according to the measured response characteristics and again presented to the retina.

The obtained iso-response curves reveal substantial nonlinearities in ganglion cells of both axolotl and frog. Iso-latency curves consistently suggest nonlinear stimulus integration that is dominated by half-wave rectification and sublinear summation. Nonlinearities deduced from iso-rate curves, on the other hand, display much more variability. These findings lead us towards exploring a model with nonlinearly integrated subfields and local gain control.

# Complexins in the murine retina: Cellular and synaptic distribution

Johanna Mühlhans[1], Kerstin Reim[2], Andreas Gießl[1], Johann Helmut Brandstätter[1]

[1]University of Erlangen-Nuremberg, Department of Biology, Animal Physiology, 91058 Erlangen, Germany
[2]Max Planck Institute for Experimental Medicine, Department of Molecular Neurobiology, 37075 Göttingen, Germany

The regulated release of neurotransmitter at chemical synapses comprises enormous complexity. Differences in the organization of the presynaptic molecular machinery are responsible for the different modes of neurotransmitter release. This is most obvious when comparing the structure and function of conventional and ribbon synapses in the retina.
The Complexins (Cplxs) are a family of small soluble proteins involved in the regulated release of neurotransmitter at chemical synapses in the central nervous system, including the retina. In the mouse genome four Cplx isoforms encoded by different genes exist: Cplx 1, 2, 3 and 4. While Cplxs 1, 2 and 3 are expressed in retina and brain, Cplx 4 is restricted to the retina. Cplx 1 and 2 – like Cplx 3 and 4 – are highly homologous to each other, whereas limited homology exists to Cplx 3 and 4. Furthermore, Cplx 3 and 4 – but not Cplx 1 and 2 – contain a CAAX box motif, which is important for farnesylation and membrane targeting. To find out more about the role of Cplxs at retinal synapses, we examined their expression, distribution and synaptic localization in wild-type and knockout mouse retinae using various methods, e.g. *in situ* hybridization and immunocytochemistry at the light and electron microscopical level.
Cplx 1 and 2 are distributed in three immunoreactive strata in the inner plexiform layer (IPL). In addition, somata of amacrine and ganglion cells in the inner nuclear layer (INL) and the ganglion cell layer (GCL) are labeled. Double labeling experiments demonstrate the localization of Cplx 1 and 2 at GABAergic and glycinergic synapses. There are no antibodies available to discriminate between Cplx 1 and 2, but *in situ* hybridization experiments show that Cplx 1 is restricted to somata in the GCL, whereas Cplx 2 is expressed in somata in the GCL and the INL.
Cplx 3 and 4 are the isoforms present at retinal ribbon synapses. In the outer plexiform layer (OPL), Cplx 3 is found mainly in cone and, to a lesser extent, in rod synaptic endings; for Cplx 4 it is vice versa. In the IPL, Cplx 3 is found in rod bipolar terminals and in glycinergic and some GABAergic amacrine cells. In contrast, Cplx 4 is found only in a subpopulation of cone bipolar cells. Surprisingly, at the ultrastructural level Cplx 3 and 4 are not concentrated at the ribbon synaptic site where transmitter release happens, but are homogeneously distributed throughout the photoreceptor terminals.
During postnatal development, all four Cplxs are present in the mouse retina already at the day of birth (P0). In accordance with synaptogenesis in the murine retina – conventional synapses develop ahead of ribbon synapses – Cplx 1 and 2 reach their typical adult distribution pattern between P10 to P14, followed by Cplx 3 and 4 at around P14 and P21, respectively.
The differential and synapse specific localization of the four Cplx isoforms most likely reflects differences in the molecular organization of the presynaptic machinery, responsible for the different modes of neurotransmitter release at conventional and ribbon synapses in the retina.

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# Cone bipolar cells in a bat retina.

Brigitte Müller[1]

[111]Max Planck Institute for Brain Research, Neuroanatomy, Deutschordenstrasse 46, 60528 Frankfurt/Main, Germany

Microchiropteran bats are strongly nocturnal, with small eyes and rod -dominated retinae. However, they also possess a significant cone population comprising two spectral types and hence the basis for daylight and color vision (Müller et al., PLoS ONE 4(7): e6390, 2009). Here, at least four types of cone bipolar cells (BPs) were discovered in the retina of *Carollia perspicillata*, a microbat species of the family Phyllostomoidae, by immunocytochemical markers and intracellular injections of DiI.

Neurons of the inner nuclear layer (INL) were assessed using antisera against hyperpolarization-activated and cyclic nucleotide-gated channel 4 (HCN4), carbohydrate epitope CD15, Recoverin, Protein Kinase C alpha (PKCa), vesicular glutamate transporter 1 (vGluT1), nitric oxide synthase (NOS) and cholin acetyl transferase (ChAT). For DiI injection, 150 µm retinal slices were cut with a vibratome and fixed in paraformaldehyde for 5-30 minutes. Additionally, slices were prelabeled by CD15- or Recoverin-antiserum.

Anatomical division of the inner plexiform layer (IPL) into five strata (S1-S5) was determined by the antibodies against ChAT and NOS. Immunostaining of the Gγ13, a marker for ON-BPs, confirmed the extension of the ON lamina of the IPL. Immunolabeling of vGluT1, a marker for all cone BPs, nicely demontrates the numerous occurance of cone BPs and the extension of the IPL.

Labelings with the antisera against CD15, HCN4 and Recoverin turned out to label different cone BPs in the bat retina since no colocalization with PKCa, the well established marker for mammlian rod BPs, was observed. HCN4 antiserum labels somata and processes of numerous cone BPs and some amacrine cells (ACs) in the INL. In the IPL the two HCN4 -positive strata show some colocalization with the two ChAT bands but clearly extend further into the ON-sublayer (S4). The CD15 immunostaining co-localizes to a great extent with the HCN4 label of BP somata in the INL and in S1 of the IPL. Individual axon terminals stratifying in S1 can be identified in vertical sections, representing an OFF cone BP type. The inner CD15-positive stratum exceeds the HCN4-positive one reaching from S3 -S5. It consists mainly of CD15 -IR AC dendrites. Intracellular injection of DiI into CD15-prelabeled BPs confirmed the existence of CD15-positive ON-cone BPs. Quantitative analysis of immunopositive CD15 BPs in retinal wholemounts revealed that the majority of CD15-IR BPs are OFF-cone BPs. Immunostainig with recoverin revealed two more cone BP types in *C. perspicillata* stratifying in S1 and S5 respectively. Since double immunostaining with PKCa shows no colocalization it can be concluded, that recoverin labels an ON- and an OFF cone BP -type. Recoverin and CD15 are not colocalized. Future intracellular injections of prelabled recoverin-positive cone BPs with DiI will reveal their entire morphology. To analyse cone contacts of the immunostained BPs the axon terminals of cone photoreceptors were labelled by peanut agglutinin (PNA) or S-opsin antiserum, the latter labeling the short wave sensitive cones selectively. So far, none of the investigated cone BP types here was found to receive input from one cone type only.

Despite the fact that microbat retinae are considered extremely adapted to nocturnal vision, the existence of various cone BP types gives good evidence, that the microbat retina conforms to the wiring diagram present in many other mammalian retinae.

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# Contrast adaptation in the retina: Global or local mechanisms?

Mona Garvert[1], Tim Gollisch[1,2]

[1]Max-Planck-Institute of Neurobiology, Martinsried, Germany
[2]Dept. of Ophthalmology, University Medical Center Göttingen, Germany

Retinal neurons need to adapt to statistical properties of the environment in order to efficiently encode the visual world. One important statistical property is stimulus contrast. If contrast increases, retinal ganglion cells adapt by accelerating response kinetics and decreasing sensitivity. As a neural basis for this phenomenon, an intrinsic mechanism in ganglion cells was proposed. A competing hypothesis suggests a presynaptic mechanism in bipolar cells, which typically have smaller receptive fields than ganglion cells and therefore could provide local adaptation within a ganglion cell receptive field.

We addressed the question of whether contrast adaptation occurs globally at a ganglion cell level or locally at the level of bipolar cell receptive fields. Ganglion cell spike trains were recorded with multi-electrode arrays from isolated retinas of frog and salamander and analyzed with a standard Linear-Nonlinear (LN) model approach. We designed a stimulus that underwent changes in contrast in locally restricted regions corresponding to the size of bipolar cell receptive fields. Thereby, we aimed at exposing some bipolar cells to changing contrast while exposing others to constant contrast. Bipolar cells processing either of the two stimulus parts were sampled by the same ganglion cell.

As expected, we observed changes in the kinetics of stimulus integration and in the sensitivity at those locations where stimulus contrast changed. However, we also observed comparable changes in kinetics and sensitivity at locations where contrast did not change. This indicates that contrast adaption is not locally restricted to the area of contrast change, but acts globally over the whole receptive field of a ganglion cell. These findings suggest that the site of the effects of contrast adaptation may be within the ganglion cell itself and not at the level of presynaptic bipolar cells.

# Different Pericentrin-isoforms identified in developing and adult mammalian photoreceptor cells

Andreas Gießl[1], Johanna Mühlhans [1], Johann Helmut Brandstätter[1]

[1] University of Erlangen-Nuremberg, Department Biology, Division of Animal Physiology, Staudtstr. 5, 91058 Erlangen, Germany

Pericentrin (Pcnt, also named Kendrin), a conserved protein of the pericentriolar material (PCM), plays an important role in microtubule organization serving as a multifunctional scaffold for numerous proteins and protein complexes. Recent studies indicate that Pcnt mutations are associated with a range of diseases, e.g. Majewski/microcephalic osteodysplastic primordial dwarfism type II (MOPDII) and Seckel syndrome, two rare human autosomal recessive genetic disorders. Moreover, further studies suggest a role for Pcnt in human cancer, mental disorders and ciliopathies.

Until today, three Pcnt splice variants from orthologous genes in mice and humans – Pcnt A, S (250) and B (360) (human 380) – are known. The proteins are characterized by coiled -coil domains throughout most of their structure; two splice variants also contain a PCM targeting motif called the PACT domain. Pcnt localizes to the base of primary and motile cilia and is involved in ciliary development and function in mammalian cells.

In the vertebrate retina, photoreceptor cells are morphologically and functionally arranged in several compartments. The light sensitive photoreceptor outer segment is linked with an inner segment, which contains the typical energy producing and protein synthesizing components of an eukaryotic cell, via a modified, non-motile cilium, termed the connecting cilium. Using various methods, e.g. laser microdissection in combination with RT-PCR, immunocytochemistry, western blotting and real-time PCR, we find two Pcnt-isoforms expressed in photoreceptor cells. Pcnt and several centrosomal interaction partners are localized at the basal body and the centriole of the connecting cilium. Here, Pcnt co-localizes with the whole machinery, which is involved in protein transport from the inner to the outer segment. Moreover, we find in western blotting experiments with different mouse tissues, that the Pcnt-isoforms are expressed in varying intensities between the different tissues and between the isoforms. Recent studies revealed also different intensities of the expression patterns in mouse retina on the mRNA and protein level. In summary, these findings suggest distinct functional roles of the different Pcnt splice variants in photoreceptor cells.

The presence of Pcnt at the connecting cilium, the site of transport regulation and interaction with transport molecules like IFTs, suggests a role of Pcnt in ciliary transport in photoreceptor cells. Studying the role of the different Pcnt isoforms will provide novel insights into human disorders related to defects in ciliary function.

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# Does disruption of photoreceptor coupling affect cone degeneration in a retinitis pigmentosa mouse model?

Katharina Schmidt[1], Ulrike Janssen-Bienhold[1], Karin Dedek[1], Reto Weiler[1]

[1]University of Oldenburg, Department of Neurobiology, Carl-von-Ossietzky-Strasse 9-11, D-26111 Oldenburg, Germany

Retinitis pigmentosa is a form of retinal degeneration in which the rods die first, followed by the cones. The genetic defects which cause retinitis pigmentosa are expressed solely in rods and are based on mutations in rod-specific genes. The mutations result in rod photoreceptor cell death, followed invariably by the death of the genetically normal cone photoreceptors. In this study, we test the bystander hypothesis, which claims that electrical coupling between the two photoreceptor subtypes plays a key role in the disease spread from rods to cones. Propagation of degeneration is mediated by molecules (e.g., death signals or toxic molecules) flowing through gap junctional channels from dying rods to cones. We investigate whether disruption of rod-cone coupling can prevent cone photoreceptor death and reduce the spread of degeneration.

We used C57Bl6/N wild-type mice and two transgenic mouse lines: the rho-/- mouse, which is a model for retinal degeneration, and the rho-/-/Cx36-/- mouse, in which coupling between rods and cones is also disrupted. We used confocal microscopy to compare the retinal structures of these mice at different ages. Cone degeneration was assessed by measuring cone cell density in m- and s-opsin-labeled retinal whole mounts. Detailed effects on cone degeneration and morphological retinal changes were analyzed in retinal cryosections using antibodies specific for different retinal cell types and for dying cells (for example anti-PAR antibodies, anti-caspase3 antibodies, TUNEL staining).

Our preliminary results did not reveal any distinct differences in cone survival between rho-/- and rho-/-/Cx36-/- mice. However, cone degeneration in rho-/-/Cx36-/- retinae progressed more slowly than in rho-/- retinae, likely a result of disruption of rod-cone coupling. We detected the active form of caspase-3 in the degenerating retina, indicating apoptotic mechanisms, as shown in other retinal degeneration mouse models.

The gap junctional coupling between rods and cones is not involved in the major pathway of cone degeneration in rho-/- mice, as predicted by the bystander hypothesis, but the gap junctions may promote the progress of cone degeneration. Disruption of rod-cone gap junctional coupling leads to deceleration of cone photoreceptor loss. Apoptotic pathways are involved in photoreceptor degeneration in the rho-/- mouse model.

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# Does transorbital alternating current stimulation enhance the survival of retinal ganglion cells?

Petra Henrich-Noack[1], Nadine Voigt[2], Sylvia Prilloff[3], Bernhard A. Sabel[4]

[1]Otto-von-Guericke University, Institute of Medical Psychology, Leipziger Str. 44, 39120 Magdeburg, Germany

We have recently used repetitive transorbital alternating current stimulation (rtACS) to treat patients visually impaired after brain lesions and found that it enhances visual functions. The underlying mechanisms of this effect are, however, not yet clear. It has been proposed that rtACS enhances cell survival (Morimoto et al. 2005). Therefore, we now investigated the cellular consequences of repetitive rtACS in rats after traumatic lesion of the optic nerve.

A fluorescent dye (Mini emerald) was stereotactically injected into the superior colliculus for retrograde labelling of Retinal Ganglion Cells. All experiments were performed under Ketanest/Rompun narcosis. A baseline measurement with in vivo confocal imaging (ICON) of the retina was performed 1 week later, counting the number of labelled neurons in a defined area of interest. The rats were randomly assigned to a treatment group or sham-procedures and 5 days after the baseline ICON measurements an optic nerve crush was performed. Eleven rats were treated with rtACS according to the Morimoto protocol. Briefly, rats received directly after the injury a treatment with biphasic square-wave pulses (duration: 1 ms; 100 µA and 20 Hz) for one hour. Control animals (n=10) underwent the same procedures except that they did not receive rtACS. ICON was performed on day 3, 7 and 15 post lesions, allowing us to quantify repeatedly the number of surviving Retinal Ganglion Cells (RGCs). On day 11, a second electrical stimulation was applied with the same parameters as on day 3.

Compared to baseline, the number of RGCs was reduced by 15.5%, 31% and 60.8% in the untreated control group on post-traumatic day 3, 7 and 15, respectively. In the stimulated animals the neuronal loss was 9%, 28.2% and 55.1%. Although slightly below the control group, RGCs death in the stimulated group was not significantly different from un-stimulated animals.

These results are not in agreement with those reported in the literature showing that such a stimulation protocol leads to significant neuroprotection. However, most recent data indicate that published results represent partially delay in neuronal death (Morimoto et al., 2010). As our method of partial optic nerve trauma was different from the published one using total transection , a different timing of the cascades leading to neuronal death may explain why an effect of rtACS was not detectable in the current experiment. Further studies are now underway to systematically evaluate the effects of rtACS after partial optic nerve damage.

# Electrophysiological correlates of retinal ganglion cell degeneration following optic nerve lesion

Christian Leibig[1], Jacob Menzler[2], Günther Zeck[1]

[1]Natural and Medical Sciences Institute at the University of Tübingen, Reutlingen, Germany
[2]Systems- and Computational Neuroscience, Max Planck Institute of Neurobiology, Martinsried, Germany

The optic nerve represents an ideal model to study the degeneration and regeneration of CNS axons [1]. Dysfunction of optic nerve axons has clinical relevance for the pathogenesis of glaucoma or trauma. Morphological changes following optic nerve (ON) injury are well studied; however, electrophysiological correlates are largely missing.

Here we search for electrophysiological alterations at the level of single retinal ganglion cells after either optic nerve crush or optic nerve section. We recorded the ganglion cell spiking activity in the isolated whole-mount retina interfaced to a multi-transistor array ('Neurochip') in rabbit (New Zealand White) and rat (Wistar) retinas. Retinal function in lesioned animals was evaluated at four postoperative time intervals (P4, P7, P11 and P28) and compared to untreated retinas.
RGCs exhibit lower spontaneous activity following ON lesion in both species. Furthermore, the spike patterns of spontaneous activity change following ON lesion. In the healthy rabbit and rat retinas RGCs exhibit prominent spike bursts (3 – 6 spikes with interspike intervals range of 2-20 ms) followed by periods of silence longer 50 ms [2]. The intra-burst-intervals and the percentage of tonic spikes increase after ON lesion. A significant change occurs in RGCs of both, the rabbit and the rat four days after ON lesion.
Intraretinal axonal signals could be 'imaged' in the thin rabbit retina only. Propagation velocities decreased significantly from 1.4 m/sec to 1.1 m/sec for RGC axons when the ON lesion occurred at least seven days before the measurement.
Full field light-flashes were presented to the retina. Average response latencies were calculated for the occurrence of the first spikes of each individual RGC. For populations comprising 40 - 60 ON cells recorded in the same retinal portion the median delay was ~ 55 msec. This delay did not change significantly for ON cells after optic nerve crush. Further protocols are currently investigated to test if temporal RGC response properties change after ON lesion.
Our results demonstrate that 'electrical imaging' at high spatial resolution may be used to detect electrophysiological changes of RGCs after ON lesions.

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# Electrophysiology of the snake retina

Tobias Kohl[1], Bruce A. Young[2]

[1]University of Bonn, Department of Neurobiology, Poppelsdorfer Schloss, 53115 Bonn, Germany
[2]University of Massachusetts Lowell, Department of Physical Therapy, Lowell, USA

Few previous studies have explored the snake's eye from a functional perspective. We recorded electroretinograms (ERGs) from a number of snake species chosen following considerations of phylogeny, habitat, and temporal activity patterns. For each species we determined the flicker-fusion frequency, a contrast sensitivity profile, and an intensity response curve. The flicker-fusion frequencies spanned from 15 – 60 Hz and closely correlated with activity patterns; diurnal species which appeared to be visually-dominant had the highest flicker-fusion frequencies. The normalized contrast sensitivity profiles had an interspecific range of nearly 40% at every contrast level, with the more visually-dominant taxa on the lower end of the normalized response. Nearly half of the species had a continuous increase in response with increasing stimulus contrast; the other half had response peaks below maximal contrast. The normalized intensity response curves had the most interspecific variation. Most of the species displayed a bimodal intensity response, in some cases with more than a 50% difference in response between the high and low intensity response. We suspect that the interspecific variation we document in retinal electrophysiology is a reflection of, among other factors, differences in photoreceptor compositions among the retinas of the different species.

# Encoding of Saccadic Scene Changes in the Mouse Retina

Vidhyasankar Krishnamoorthy[1], Tim Gollisch[1, 2]

[1]Max Planck Institute of Neurobiology, Visual Coding Group, Am Klopferspitz 18, 82152 Martinsried, Germany

[2]University Medical Center Göttingen, Dept. of Ophthalmology, Waldweg 33, 37073 Göttingen

Fast, sudden eye movements ("saccades") form an essential feature of visual behavior. The visual signals on the retina are thus segmented into brief image fixations separated by global motion signals. It has been shown that the activity of retinal ganglion cells is strongly affected by saccade-like image shifts. This suggests that there may be a specific representation of saccades by the retina, either by way of short bursts of spikes or by suppression of spiking activity (e.g., Noda & Adey 1974, Roska & Werblin 2003). Little is known, however, about how the responses to the motion signal interact with the encoding of the image content at the subsequent fixation. Here, we address this question by analyzing ganglion cell responses in the isolated mouse retina to simulated saccades. For about half of the recorded cells, we found strong spiking activity during the saccade. This supports the idea that the retina actively encodes the saccade and may signal the abrupt scene change to downstream centers. Furthermore, we characterized the responses to the newly fixated image. While there appears to be only little influence of the motion signal itself on these responses, the responses depended strongly on the history of the stimulus before the saccade. This suggests that retinal signals under saccadic vision may code for image transitions rather than the currently fixated image.

# Encoding of stimulus direction in archer fish ganglion cells

Viola Kretschmer[1], Malte T. Ahlers[1], Josef Ammermüller[1]

[1]Universität Oldenburg, Neurobiologie, Carl-von-Ossietzky-Str. 9-11, 26129 Oldenburg, Germany

Archer fish have the ability to down aerial prey with shots of water matched to size and distance of the prey. On the basis of estimated prey weight, size and motion, each member of a swarm can calculate the point of impact on the water, turn towards it and match its swimming speed to the distance in order to be the first reaching that point. This very complex computational task is accomplished in a very short time (down to 40ms) and therefore has to be achieved by a very compact neural circuitry.

Fast turning and acceleration is probably triggered by the archerfish's C-start escape network which is known for fast responses to visual information for life saving purpose. The short latencies until the precisely aimed predictive start require high speed processing and transferring of visual information in all processing stages.

In this study we examined the underlying neural mechanisms by extracellular recording ganglion cell activity in the isolated archer fish retina with a 10x10 multielectrode array, while applying visual stimuli mimicking the natural situation of falling prey.

Using white, full-field light flashes ganglion cells showed very short latencies down to 19ms. With an intensity of 4,48µW/cm² mean latencies of 20ms can be observed in archer fish ganglion cells compared to 30ms in other bony fish (carp, gold fish).

To analyze how information about stimulus direction could be encoded by retinal ganglion cells we compared the responses of all recorded ganglion cells to moving edges, taking their position on the array, the receptive field dimensions and the stimulus timing into account. A strong direction sensitivity with different spike rates for different directions can be observed. Simultaneously recorded cells "seeing" the same stimulus also show different time courses in response to different directions. Differences in the rank order, in which cells respond to opposite directions, could be observed. Just with the varying time course of three cells the brain would be able to classify the direction in which the stimulus is moving.

# Experience-dependent plasticity and vision restoration in rats after optic nerve crush

Bernhard A. Sabel[1], Sylvia Prilloff[1], Petra Henrich-Noack[1], Siegfried Kropf[2]

[1]Otto-von-Guericke University Magdeburg, Institute of Medical Psychology, Leipziger Str. 44, 39120 Magdeburg, Germany[2]Otto-von-Guericke University Magdeburg,Institute for Biometry and Medical Informatics, Leipziger Str. 44, 39120 Magdeburg

Within the first weeks after brain damage the visual system can spontaneously recover, but it is not known if visual experience plays a role in this process. Therefore, we studied the role of visual experience during recovery by exposing rats to normal, enriched and impoverished visual conditions.
Adult rats, which had learned a 6-choice brightness discrimination task, received bilateral partial optic nerve crush (ONC) and were then exposed daily for 34 days to either (i) total darkness, (ii) standard 12-h light: 12-h dark cycle, or (iii) a 2 h selective visual enrichment. The percentage of correct choices and the maximal performance levels reached were used as recovery endpoints. Retinal ganglion cell (RGC) morphology was evaluated following retrograde transport of fluorescent tracer with in vivo confocal neuroimaging (ICON) before and after ONC.
Whereas rats kept under normal daylight conditions after ONC recovered their visual functions rather well, rats housed in complete darkness did not recover at all. However, only 2 hrs of daily exposure to visual enrichment induced a significantly recovery which was even faster than in rats housed under normal daylight conditions. RGC soma size changes were observed after ONC, but they did not correlate with any measures of behavioural recovery.
We conclude that visual experience, even if provided for short daily periods, is a critical factor determining the dynamics of early phases of recovery.

# Expression and subcellular localization of Usher syndrome proteins in the human photoreceptor cells

Uwe Wolfrum[1], Tobias Goldmann[1], Nora Overlack[1], Christina Mueller[2], Jan M. Vetter[2], Kerstin Nagel-Wolfrum[1]

[1]Institute of Zoology, Cell and Matrix Biology, Johannes Gutenberg University of Mainz, Germany
[2]Dept. of Ophtalmology, University Med Center Mainz, Germany

The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. It is assigned to three clinical types and 12 genetically heterogeneous subtypes. USH is characterized by profound inner ear defects and Retinitis pigmentosa. In contrast to USH patients, USH rodent models undergo, if at all, a very mild retinal degeneration. So far, there is no explanation for this difference in the retinal phenotype. One possible explanation is that primates and rodent photoreceptor cells differ in structure and in the subcellular distribution of individual USH proteins. Here, we tested the latter hypothesis by analyzing the subcellular localization of USH1/2 molecules in human retinas.
We assessed the expression of USH1/2 proteins by Western blots and by immunohistochemistry on cryosections through human retinas. Furthermore, subcellular localization was analyzed using a combination of high resolution immunofluorescence and immunoelectron microscopy. All USH1/2 proteins are expressed in photoreceptor cells of human retinas. As shown in rodents, USH1/2 proteins are associated with the ciliary region or localize to the outer segment of human photoreceptors. However, staining intensities of USH proteins significantly differed between rods and cones. In addition, a subset of USH proteins were identified in calycal processes which are prominent features of human photoreceptors. In conclusion, there are significant differences between rodents and humans in the expression of USH1/2 proteins in retinal photoreceptors which can explain the lack of retinal USH phenotype in mice. In human defects in USH molecules may lead to disorganization of the USH protein network in calycal processes and thereby to the destabilization of photoreceptor outer segments.

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# Expression of connexin30.2 in amacrine cells and intrinsically photosensitive ganglion cells of the mouse retina

Arndt Meyer[1], Maria M. Kreuzberg[2], Klaus Willecke[2], Reto Weiler[1], Karin Dedek[1]

[1]Department of Neurobiology, University of Oldenburg, Oldenburg, Germany
[2]Institute for Genetics, University of Bonn, Bonn, Germany

Many retinal neurons are electrically and metabolically coupled via gap junctions, complex protein channels made up of connexins (Cx). Recently, we showed that Cx30.2 is expressed by amacrine and ganglion cells in mouse retina. We characterized 6 morphologically distinct types of Cx30.2-expressing ganglion cells, including RGA1 cells (Pérez de Sevilla Müller et al. 2010). Since RGA1 cells have recently been shown to belong to the intrinsically photosensitive ganglion cells, we tested here whether Cx30.2-expressing cells are indeed melanopsin-positive. Also, we aimed to morphologically characterize the Cx30.2-expressing amacrine cells.
To analyze Cx30.2 expression, we used a Cx30.2-LacZ mouse, which expressed the LacZ reporter DNA instead of the Cx30.2 coding region (Kreuzberg et al. 2006). Cx30.2 -expressing neurons were characterized by co-immunolabeling with antibodies against the LacZ-coded ß-galactosidase (ßGal) and markers for various calcium-binding proteins, neurotransmitters and melanopsin. For targeted intracellular dye injections in retinal flatmounts or vibratome sections, LacZ -positive cells were labeled with fluorescein -di-beta-D-galactopyranoside, a fluorogenic substrate for ßGal.
Cx30.2/LacZ-positive cells are present in the ganglion cell layer (GCL) and in the proximal inner nuclear layer (INL) of the mouse retina. They make up about 20% of all cells in the GCL and about 8% of all amacrine cells in the INL. Using dye injections, we identified a novel Cx30.2-expressing ganglion cell type: a small ON ganglion cell stratifying in stratum 3 of the inner plexiform layer (IPL).
Since Cx30.2-expressing RGA1 cells have been reported to be identical to one type of melanopsin-containing ganglion cells, we double stained retinas for Cx30.2/ßGal and melanopsin. Indeed, melanopsin-immunoreactive OFF and ON ganglion cells (M1 and M2 cells, respectively) were ßGal-positive. This was also the case for displaced M1 cells. Thus, it seems that most, if not all types of melanopsin-expressing intrinsically photosensitive ganglion cells express Cx30.2.
To characterize Cx30.2-expressing amacrine cells, we stained retina sections for different calcium-binding proteins and the neurotransmitter GABA. Only occasionally, we found amacrine cells in the INL positive for ßGal and parvalbumin, calretinin and calbindin, respectively. In rare cases we found GABA-immunoreactive displaced amacrine cells which were ßGal-positive. However, most ßGal-positive amacrine cells in the INL did not show GABA immunoreactivity and thus are most likely glycinergic. We dye-injected ßGal-expressing amacrine cells in the INL and found these cells to resemble type 7 glycinergic cells of the rat retina (Menger et al. 1998). Type 7 cells are narrow-field multistratified cells whose dendrites span the entire IPL.

Taken together, our results show that at least one type of glycinergic amacrine cells and many intrinsically photosensitive ganglion cells express Cx30.2. Since melanopsin-containing ganglion cells are coupled to amacrine cells and not to ganglion cells, it appears likely that Cx30.2 plays a role in the "image-forming" function of intrinsically photosensitive ganglion cells.

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# Expression of the Voltage-gated Calcium Channel Subunit $a_2$d-3 in the Mouse Retina is Highly Specific

Hartwig Seitter[1], Antonella Pirone[2], Marlies Knipper[2], Jutta Engel[3], Thomas Münch[1]

[1]University of Tübingen, Centre for Integrative Neuroscience, Paul-Ehrlich-Str. 15, 72076 Tübingen, Germany
[2]University of Tübingen, Tübingen Hearing Research Centre, Elfriede-Aulhorn-Strasse 5, 72076 Tübingen, Germany
[3]Saarland University, Department of Biophysics, Building 76, 66421 Homburg, Germany

The $a_2$d proteins were originally pulled down as part of voltage-gated calcium channels (VGCC). Further investigations revealed their function in trafficking VGCC complexes to the plasma membrane, thus increasing total calcium current magnitude. Recently it was shown, that in addition, $a_2$d subunits can affect synaptogenesis independent of their trafficking function.
We report here the expression pattern of $a_2$d-3 in the mouse retina. In a knockout model of $a_2$d-3 the impact on synaptic morphology and cellular physiology is proposed to give new insights into the function of the $a_2$d-3 subunit.

In this mouse model, $a_2$d-3 is knocked out by insertion of LacZ. Through a combination of immunohistochemistry and LacZ staining procedures, the retinal cell type that expresses LacZ under the $a_2$d-3 promotor was determined to be almost exclusively horizontal cells. The question of whether $a_2$d-3 might have a function in synapse formation in the developing retina was addressed in a time series, with total RNA extracted from P3, P6, P9, P12, P16 and adult wildtype mouse retina. RT-PCR showed the presence of $a_2$d-3 gene expression in all developmental stages and in the adult.
Analysis of retinal wholemounts for horizontal cell numbers and distribution revealed no significant differences between wildtype and knockout mice. Overall retinal morphology also appeared to be unchanged.
The subcellular localization of $a_2$d-3 and the effect of the knockout on synaptogenesis remain to be determined.

The unique expression of $a_2$d-3 in one retinal cell type points to a highly specific function of this VGCC subunit. Its presence all throughout development does not give a clear indication of the importance of $a_2$d-3 for synaptogenesis in the mouse retina. The ongoing expression in adult horizontal cells hints at a function that is not concerned with the formation of synapses. Investigations into the mechanics of this mutation could reveal not only novel properties of the accessory $a_2$d subunits but also give further insights into horizontal cell physiology.

# fMRI of superior colliculi and oculomotor brainstem nuclei in humans

Walter Linzenbold[1], Marc Himmelbach[1]

[1]Hertie Institute for Clinical Brain Research, Section Neuropsychology, Hoppe-Seyler-Str. 3, 72076 Tübingen, Germany

Although the oculomotor brainstem system is well described in non-human primates, functional measurements of this system remain a challenging task due to technical constraints and anatomical characteristics. The vast majority of oculomotor fMRI studies investigating reflexive saccades found no reliable signal changes in brainstem nuclei. In rare cases of positive findings, these are typically confined to the superior colliculus. Despite of the fundamental role of the brainstem nuclei for oculomotor control, examinations of their functional status in humans represent a neglected issue in human neuroimaging. Using high-resolution fMRI we searched for BOLD signal increases in nuclei of the oculomotor system using a reflexive saccade task in a group of healthy subjects. Our measurements were conducted with a conventional 3T Trio Siemens Scanner using a 12-channel head coil. EPI volumes were acquired with a slice thickness of 2 mm and an in-plane resolution of 1.5 x 1.5 $mm^2$. Our group analysis revealed task-related BOLD increases in the superior colliculus, the oculomotor nucleus, the abducens nucleus, and in the paramedian pontine reticular formation. An additional visual stimulation paradigm led to increased signal levels in the superior colliculus consistent with its visual properties but no corresponding signal changes in other brainstem nuclei. In light of the eminent difficulties of brainstem functional neuroimaging in humans, our data demonstrate the feasibility of functional examinations of the oculomotor brainstem system in humans. In combination with refined structural imaging that allows for unequivocal localization of brainstem nuclei, such functional examinations could contribute to conclusive descriptions of neurological disorders affecting the oculomotor system.

# Functional characterization of the oscillatory activity in the rd-1 mouse retina

Wadood Haq[1], Timm Schubert[2], Thomas Ladewig[1], Eberhart Zrenner[1,2], Thomas Euler[1,2]

[1]Institute for Ophthalmic Research, University of Tübingen, Germany
[2] Centre for Integrative Neuroscience (CIN), University of Tübingen, Germany

In the retina of the rd-1 mouse model (Bowes et al., 1990), photoreceptors degenerate within the first 28 postnatal days. However, despite the loss of their outer segments and therefore a loss of function, some photoreceptor cell bodies survive in the outer nuclear layer (ONL). In addition to morphological changes also functional changes have been observed in the remnant retinal network. For example, strong light -independent activity has been reported in rd-1 retinal ganglion cells (Stasheff, 2008, Margolis et al., 2008; Ye et al., 2007). It has been proposed that synaptic input from amacrine cells is, at least in part, responsible for the generation of this ganglion cell activity. Such spontaneous activity in degenerating retina may interfere with artificial stimulation using retinal implants (Zrenner, 2002) and, therefore, it is important to understand the underlying mechanisms. This study aims at identifying the cellular source(s) of light-independent activity in rd-1 mouse retina.

Retinal whole mounts were obtained from rd-1 mice (P28-45) and placed in the recording chamber of a microscope with the ONL side up. The retina tissue was loaded with the $Ca^{2+}$ indicator dye Fura-2 via incubation with the AM-ester form. Calcium signals were measured using the 340/380 nm wavelength ratio with a Till Photonics CCD camera system (acquisition rate: 10 Hz). The recorded $Ca^{2+}$ events were analyzed off-line using custom-written Matlab (Mathworks) routines. In addition, we combined the imaging experiments with immunohistochemistry to identify the active cells. After the $Ca^{2+}$ imaging, the retinal tissue was double-labeled with combinations of antibodies against Recoverin (a photoreceptor and cone bipolar cell marker), Calbindin (a horizontal cell marker), and PKC (a rod bipolar cell marker). Furthermore, 4,6-diamidino-2-phenylindole (DAPI) was applied to label all cell nuclei. This anatomical data was then overlaid with the $Ca^{2+}$ imaging data.

We found that light -independent oscillatory activity – as measured by $Ca^{2+}$ imaging – is present in a substantial fraction of cells (49%, n = 230 cells) in the degenerated and reduced ONL of rd-1 mouse retina. Preliminary immunohistochemical data suggest that active cells included photoreceptors, horizontal cells and bipolar cells. The observed $Ca^{2+}$ activity pattern in these cell classes varied in signal shape, amplitude and frequency. In photoreceptors and bipolar cells, we measured slow and rhythmic transient $Ca^{2+}$ signals up to 1.5 Hz as well as bursts of faster events (with up to 3.5 Hz). Horizontal cells showed more sustained signals with durations of several seconds.

In conclusion, our preliminary data revealed a surprisingly wide-spread and diverse activity in the degenerated and reduced outer retina of the rd-1 mouse. This activity may be responsible for oscillations in the remnant retinal network and, at least in part, for the spontaneous ganglion cell spiking observed earlier.

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# Generation and functional characterization of a transgenic mouse expressing a $Ca^{2+}$ biosensor in cone photoreceptors

Tao Wei[1], Katja Koeppen[2], Thomas Ott[3], Naoyuki Tanimoto[4], Norman Rieger[2], Britta Baumann[2], Oliver Griesbeck[5], Thomas Ladewig[1], Thomas Euler[1], Bernd Wissinger[2]

[1]Centre for Integrative Neuroscience (CIN) / Institute for Ophthalmic Research, university of Tuebingen
[2]Molecular Genetics Laboratory, Centre for Ophthalmology, University Clinics Tuebingen
[3]Transgenic Animals Core Facility, University Medical School, Tuebingen
[4]Division of Ocular Neurodegeneration, Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tuebingen
[5]MPI for Neurobiology, Martinsried, Germany

Calcium plays a crucial role in modulating the photo-response as well as in glutamate release at the photoreceptor synapse. In addition, there is growing evidence that $Ca^{2+}$ is involved in photoreceptor apoptosis in hereditary retinal dystrophies. To monitor [$Ca^{2+}$] fluctuations underlying those processes in mammalian cone photoreceptors we generated a transgenic mouse line that expressed a $Ca^{2+}$ biosensor specifically in cones.

We generated a plasmid construct containing the sequence of the $Ca^{2+}$ biosensor TN-XL under the control of the human long wavelength-sensitive (LW) opsin promoter. The linearized construct was injected into the pronuclei of fertilized oocytes, which were then implanted into pseudo-pregnant mice. One of 10 founder mice lines showed stable expression of TN-XL in the retina. This line with a transgene copy number of about 12-15 (as determined by qPCR) was bred to homozygosity for the transgene insertion. Retinal expression of the biosensor was studied using immunohistochemistry; biosensor function was evaluated using $Ca^{2+}$ imaging in retinal slice preparations. The effect of biosensor expression on retinal function was assessed by electroretinography.

Immunostaining with antibodies against L/M opsin confirmed that the biosensor was exclusively expressed in cones. The transgenic mice possess normal scotopic and photopic ERG responses (tested at one and three months of age). Application of drugs that activate $Ca^{2+}$ channels or $Ca^{2+}$ stores present in cone photoreceptors evoked the expected changes in intracellular [$Ca^{2+}$], as measured by confocal $Ca^{2+}$ imaging. Two photon $Ca^{2+}$ imaging revealed light-stimulus evoked $Ca^{2+}$ transients in the cone pedicles, suggesting that the presence of the biosensor did not detectably affect cone function.

In conclusion, we generated a transgenic mouse line that selectively expressed the $Ca^{2+}$ biosensor TN-XL in cones. Our $Ca^{2+}$ imaging confirmed the functionality of the $Ca^{2+}$ biosensor in cones, allowing us now to study cone photoreceptor $Ca^{2+}$ dynamics under physiological conditions. Important questions, such as the role of internal [$Ca^{2+}$] changes in cone degeneration of mouse disease models can now be approached by crossbreeding the disease models with our transgenic mouse line.

# Identification and characterization of a novel connexin (zfCx53.4) expressed in horizontal cells of the zebrafish retina

Helena Greb[1], Reto Weiler[1], Ulrike Janssen-Bienhold[1]

[1]University Oldenburg, Neurobilogie, 2503, 26111 Oldenburg,Germany

Horizontal cells (HCs) are retinal interneurons which play a major role in a feedback pathway to cones. Within the teleost retina up to four types of horizontal cells have been identified. Horizontal cells of the same subtype form autonomous networks, and we suppose that gap junctions involved in connecting these independent circuits are composed of different connexins. Until today four zebrafish connexins, termed Cx52.6, Cx55.5, Cx52.7, and Cx52.9, all of which are expressed in HCs of the zebrafish retina, have been identified (Zoidl et al., 2004; Shields et al., 2007; Klaasen et al. 2010, submitted). In addition, we have recently cloned a splice variant (designated cpCx53.3) of cpCx52.6 from carp retinal cDNA, which is the ortholog of zfCx52.6 and expressed in all subtypes of HCs in the carp retina. In the present study we wanted to find out, whether a similar splice variant of zfCx52.6 is also expressed in the zebrafish retina and to analyze its cellular distribution pattern.
We used RT-PCR and zebrafish retinal cDNA to clone and identify a new connexin designated as zfCx53.4. Alignments of amino acid sequences of zfCx53.4 and zfCx52.6 revealed 99 % identity. Both connexins differ only in the very C-terminal region (final 12 amino acids), suggesting that zfCx53.4 represents a splice form of zfCx52.6. By performing splice site prediction we found, that the final C-terminal 4 amino acids of the zfCx52.6 sequence were replaced after splicing with 12 different amino acids coded further downstream. Alignments of the zfCx53.4 aa sequence with the recently identified cpCx53.3, and mmCx57, characterized it as an ortholog of cpCx53.3 and a homolog of mmCx57, with identities of 87 % and 48 % with these connexins, respectively. Furthermore, zfCx53.4 shows 100 % identity with cpCx53.3 at the very C-terminal region (final 18 amino acids).
The cellular expression pattern of the newly identified connexin zfCx53.4 in the zebrafish retina was analyzed by using an antibody generated against the C-terminal region of cpCx53.3 (final 15 amino acids), the specificity of which has been proven in the carp retina. Gap junction-like immunoreactive puncta were exclusively found around cells in the distal part of the inner nuclear layer, right at the border of the outer plexiform layer, where horizontal cells are located. In dissociated cells of the zebrafish retina anti-cpCx53.3 exclusively labeled the dendrites of all three subtypes of horizontal cells, and their axon terminals. No other retinal cells were labeled by this antibody.
In conclusion, our data provide the first evidence for the exclusive expression of a newly identified splice variant of zfCx52.6, termed zfCx53.4, in horizontal cells of the zebrafish retina.

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# Increased resistance to retinal degeneration in PARP1 gene knock-out animals

Ayse Sahaboglu Tekgöz[1], Naoyuki Tanimoto[2], Jasvir Kaur[1], Javier Sancho-Pelluz [3], Gesine Huber[2], Edda Fahl[2], Blanca Arango-Gonzalez[1], Eberhart Zrenner[1], Per Ekström[4], Hubert Löwenheim[5], Mathias Seeliger[2], François Paquet-Durand [1]

[1]Institute for Ophthalmic Research, Division of Experimental Ophthalmology, Roentgenweg 11, 72076 Tübingen, Germany

[2]Ocular Neurodegeneration Research Group, Centre for Ophthalmology, Institute for Ophthalmic Research, University of Tübingen, Schleichstr. 4/3, 72076 Tübingen, GERMANY.

[3]Present address: Department of Ophthalmology, Columbia University, 160 Fort Washington Ave, Edward S. Harkness Eye Institute Research Annex, Room 513, New York, NY 10032, USA.

[4]University of Lund, Clinical Sciences, Lund, Dept. of Ophthalmology, BMC-B13, Klinikgatan 26, 22184 Lund, Sweden.

[5]Otolaryngology Department, University of Tübingen, 72076 Tübingen, Germany

Purpose: Retinitis pigmentosa (RP) is a group of inherited neurodegenerative diseases affecting photoreceptors and causing blindness in humans. The retinal degeneration 1 (rd1 or rd) human homologous mouse model for RP is characterized by a loss-of-function mutation in the gene encoding for the ß-subunit of rod photoreceptor cGMP phosphodiesterase 6 (PDE6). PDE6 dysfunction causes accumulation of cGMP which has been shown to be associated with excessive poly (ADP-ribose) polymerase (PARP) activation and oxidative stress in the rd1 mouse. To investigate the role of PARP for photoreceptor cell death and survival, we studied retinal morphology, function and response to pharmacologically induced retinal degeneration in PARP knockout (KO) mice.

Methods: PARP1 knockout (KO), rd1 and corresponding wild-type (wt) animals at post-natal day (P) 11 and P30 were used for in vivo analysis. Haematoxylin/Eosin staining, optic coherence tomography (OCT) and electroretinography (ERG) analysis were performed for PARP1 KO retinal morphology and function. PARP1 KO and wt mouse retinal explants were cultured with/without Zaprinast, a selective inhibitor of cyclic GMP-specific phosphodiesterase, to emulate in vitro a situation comparable to the rd1 mouse model. Immunofluorescent detection of cGMP was used to confirm Zaprinast effects. TUNEL staining, PAR immunohistochemistry and PARP activity were performed for analysis of dying cells, PAR accumulation and PARP activation, respectively.

Results: Retinal histology and OCT revealed no major alterations of retinal phenotype when compared to wild-type (wt). ERG was normal in PARP1 KO animals. PDE6 inhibition in wt retina caused massive photoreceptor degeneration comparable to the rd1 situation. By comparison, in the PARP1 KO situation, Zaprinast induced cell death was markedly reduced.

Conclusions: These findings demonstrate that PARP1 activity is in principle dispensable for normal retinal function, but is of major importance for photoreceptor degeneration under pathological conditions. Moreover, our results suggest that PARP dependent cell death may play a major role in retinal degeneration and highlight the possibility to use specific PARP inhibitors for the treatment of RP.

# Inhibitory neurotransmitter receptors in the retinal circuitry which processes direction of motion

Olivia Nicola Auferkorte[1], Sanjeev Kumar Kaushalya[2], Suneel Reddy[3], Thomas Euler[4], Silke Haverkamp[1]

[1]Max-Planck-Institute for Brain Research, Neuroanatomy, Germany

Far from being a simple sensor, the retina actively participates in processing visual signals. One of the best understood aspects of this processing is the detection of motion direction. The responsible "direction selective" (DS) circuit of the retina includes several subtypes of ganglion cells and a certain type of inhibitory amacrine cells, dubbed "starburst" amacrine cells (SACs). Inhibition from SACs onto DS ganglion cells is key in generating retinal DS signals. Here we are investigating the nature of the GABA receptors mediating this inhibition.
Rabbit retinae were dye -injected to label individual ON -OFF direction -selective ganglion cells (DSGCs) and starburst ACs, then stained with antibodies against GABAA receptor subunits alpha 1 (GABA-R a1) and alpha 2 (GABA-R a2). Retinae of transgenic mice expressing EGFP-tagged gephyrin protein in random ganglion cells (Thy1-gephyrin-EGFP) were also stained with these antibodies, and putative ON-OFF DSGCs were analyzed for postsynaptically anchored receptors.
GABA-R a2 immunoreactive puncta are aggregated in two bands of the inner plexiform layer (IPL, the neuropil containing the dendrites of DSGCs and SACs), which overlap with staining against ChAT (choline acetyltransferase), a marker for SACs. Indeed, GABA-R a2 labeling follows the pattern of the starburst plexus and shows a meaningful association with the output structures of SACs. Varicosities along the distal dendrites of individually labeled ON SACs were found to virtually always be associated with GABA-R a2 puncta (96% of all varicosities analyzed, n = 76 in 2 cells). GABA-R a2 could also be found on DSGCs dendrites (31 puncta/100 µm, n = 202 in 1 cell) and many gephyrin puncta along DSGCs dendrites colocalized with GABA-R a2, both in the ON (36%, n = 143 in 1 cell) and OFF sublayers (35%, n = 165 in 1 cell.) All counts were found to be higher than chance, as assessed by quantifying the random overlap of signals in micrographs where one of the channels was manipulated spatially. Preliminary data on GABA-R a1, the only inhibitory receptor previously reported in the DS literature, suggests a lower incidence of this subunit on elements of the DS circuitry.
We postulate a critical role for the GABA-R a2 subunit in mediating DS inhibition between SACs and ON-OFF DSGCs. Ultrastructural imaging and experiments using GABA-R a2 knock-out mice have been started to test our hypothesis.

# Inhibitory synaptic inputs onto melanopsin expressing retinal ganglion cells

Sonja Neumann[1], Silke Haverkamp[1], Olivia Nicola Auferkorte[1]

[1]Max-Planck-Institut für Hirnforschung, Neuroanatomie, Deutschordenstraße 46, 60528 Frankfurt am Main, Germany

Melanopsin expressing retinal ganglion cells (mRGCs) are recently described photoreceptors, which are involved in circadian photoentrainment and the pupillary reflex. Additionally to their intrinsic photosensitivity, based on melanopsin phototransduction, the mRGCs are also activated by synaptic inputs from the rod and cone pathways. Although many studies focused on the excitatory drive through this extrinsic pathway, almost nothing is known about inhibitory synaptic inputs onto mRGCs. In this study we screened for neurotransmitter receptors possibly involved in the inhibitory modulation of mRGCs.

We took an anatomical approach, in which we used immunofluorescent labeling of macaque retinae with antibodies against melanopsin (as a marker for mRGCs) and different glycine - and GABA-receptor subunits. In confocal micrographs, we analyzed their distribution on the dendrites on the two main types of mRGCs: the M1 and the M2 cells, which stratify in distinct sublaminae of the inner plexiform layer.

Both M1 and M2 mRGCs appear to express glycine receptor alpha subunits 2 and 3 (GlyRa2 and GlyRa3). M1 cells seem to preferentially express the slow GlyRa2 and the GlyRa3 subunit versus the faster GlyRa1 subunit. Overall, we found less GlyR immunoreactive puncta on the innerstratifying M2 cells compared with M1 cells.

Undergoing experiments on GABA receptors will complete this survey and offer valuable indication of the different types of inhibitory amacrine cells modulating the light responses of the mRGCs.

We report here for the first time that the melanopsin-containing ganglion photoreceptors express inhibitory neurotransmitter receptors, confirming a previous physiological finding (in the rodent retina) that the mRGCs receive modulatory extrinsic inhibitory inputs.

# INTRAFLAGELLAR TRANSPORT PROTEINS IN CILIOGENESIS OF PHOTORECEPTOR CELLS

Tina Sedmak[1], Uwe Wolfrum[1]

1 Department of Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg University Mainz, Muellerweg 6, 55099 Mainz, Germany.

2 Department of Biology, Animal Physiology Department Biology, University of Erlangen-Nuremberg, Staudtstrasse 5, 91058 Erlangen, Germany.

Assembly and maintenance of cilia dependent on the intraflagellar transport (IFT) mediated by molecular motors and their interaction with specific IFT proteins. Here the participation of IFT in the ciliogenesis of mammalian photoreceptor cilia was investigated.
Reinvestigation of the ciliogenesis of murine photoreceptor cells by electron microscopy revealed that in contrast to previous reports the photoreceptor cilium is formed in an intracellular pathway. Based on our observations we divided the ciliogenesis in mouse photoreceptor cells in six distinct district stages (S1-S6). While the first stages (S1-S2) are characterized by electron dense centriolar satellites, ciliary vesicle enclosing the elongating ciliary shaft of evolving primary cilium, which at S2 already possesses accessorial appendages. Furthermore, in stage S2 the proximal ciliary membrane has beaded appearance as characteristic feature of the transition zone in the mature cilium. In later ciliogenesis, when growing cilium protrudes into the extracellular space the proximal ciliary shaft remodels into the connecting cilium, whereas the distal forms the light sensitive outer segment.
During photoreceptor cell differentiation IFT proteins are expressed and associated with the ciliary apparatus in all defined stages of ciliogenesis. Higher resolution microscopy analysis revealed that in addition to the centriole and basal body IFT proteins are localized in the photoreceptor cytoplasm. There IFT proteins are present in the centriolar satellites, at post-Golgi vesicles and at the surface of the ciliary vesicle. Later in ciliogenesis (S4-S6), with the exception of IFT20 the localization pattern of IFT52, IFT57, IFT88 and IFT140 correspond in principle to their distribution in mature photoreceptor cilium.
Our data provide evidence for the participation of IFT proteins in photoreceptor cells ciliogenesis, namely in the formation of the ciliary vesicle and the elongation of the primary cilium. In advanced stages of ciliogenesis (S3-S6) the ciliary localization of IFT proteins indicate their participation in IFT as seen in mature cilia. The prominent accumulation of IFT proteins in the periciliary cytoplasm at the base of the cilia in these stages most probably resembles a pool of IFT molecules waiting in the wings for the delivery into the growing ciliary shaft for IFT. Nevertheless, the cytoplasmic localization IFT proteins in the absence of a ciliary shaft in early stages of ciliogenesis indicates roles of IFT proteins beyond its well established function for IFT in mature cilia and flagella.

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# Layer dependent visual receptive field properties in ferret superior colliculus

Iain Maurice Stitt[1], Florian Pieper[1], Gerhard Engler[1], Edgar Galindo-Leon[1], Andreas K. Engel[1]

Institute for Neurophysiology and Pathophysiology, University Medical Center, Hamburg-Eppendorf, Martinistraße 52 (N43), D-20246 Hamburg, Germany

The mammalian superior colliculus (SC) is a highly conserved midbrain structure that responds to novel external events and initiates orienting movements. Interestingly, all layers of the SC, regardless of modality, are organised into juxtaposed, overlaid maps of space. For example, visual responsive neurons in the superficial and deep layers at corresponding dorsoventral locations within the SC respond to stimuli from the same position in the contralateral visual field. Recent anatomical studies have provided evidence for a direct superficial-to-deep projection in the SC. However, it remains unclear how much information is conveyed by this connectivity.

Here, we recorded single and multi neuron activity, along with local field potentials from both superficial and deep layers of the SC with a silicon multichannel probe. Spatial receptive fields of the neurons were determined using small (~3°) drifting gratings presented at 20x20 different locations on a rear projection screen. The location of spatial receptive fields recorded within individual SC penetrations were very similar, as predicted by anatomical connectivity. The smallest spatial receptive fields (~6°) were located most superficially, while deeper neurons (up to 1mm) had larger receptive fields. To assess spatiotemporal tuning characteristics and directional/orientation selectivity across SC layers, full field drifting gratings were presented with 8 directions, 5 spatial frequencies and 4 temporal frequencies.

Spatial and temporal tuning characteristics were variable, however in general, most neurons responded strongest when low spatial frequencies (0.025 cycles/degree) were presented at higher temporal frequencies (8 cycles/s), in accordance with previous studies in the cat. Our analysis revealed both directional and orientation selectivity from SC neurons in all visual responsive layers. Preliminary results suggest that neurons from neighbouring recording sites show the greatest similarity in feature selectivity. Further analysis will address the temporal dynamics of interlaminar interactions in the SC.

# Localization and functional role of Pannexin1 in the mouse retina

Birthe Dorgau[1], Katharina Schmidt[1], Ulrike Janssen-Bienhold[1], Karin Dedek[1], Petra Bolte[1], Regina Herrling[1], Hannah Monyer[2], Silvia Penuela[3], Dale Laird[3], Reto Weiler[1]

[1]Department of Neurobiology, University of Oldenburg, D-26111 Oldenburg, Germany
[2]Clinical Neurobiology, University Hospital, Heidelberg, D-69120 Heidelberg, Germany
[3]Department of Anatomy & Cell Biology, University of Western Ontario, London, Ontario, N6A5C1 Canada

Pannexin, a protein family of vertebrates, is composed of three members (Panx1, Panx2 and Panx3). The functional and physiological role of pannexin proteins is still ambiguous. Due to structural similarities of pannexins with gap junction proteins, pannexins have been hypothesized to form connexin-like plasma membrane channels. Recently, it has been postulated that Panx1 forms single membrane channels, connecting the cytoplasm with the extracellular space. In addition, Panx1 shows different channel properties compared to connexins, e.g. glycosylation sites at extracellular domains. These differences lead to the assumption that pannexins do not participate in gap junction formation but are involved in cellular transmembrane transport. As part of this function, Panx1 has been implicated in several cellular pathways, like ATP-dependent cell death or inflammatory processes. Panx1 is expressed in various neuronal tissues including the retina (Dvoriantchikova et al., 2006). In this study, the distribution and the functional role of Panx1 in the mouse retina has been investigated.
RT PCR analysis was used to identify Panx1 expression in the mouse retina. Anti-Panx1 antibodies were applied for the detection of Panx1 proteins in the mouse retina and in dissociated retinal mouse neurons. Specificity of the antibodies on retinal tissue was demonstrated by Western blot and immunohistochemistry in transfected N2A cells and on transgenic Panx1 knock-out mice.
Consistent with previous studies, we identified Panx1 expression in the mouse retina. Anti-Panx1 antibodies detected Panx1 proteins in cells of the inner nuclear layer. Using intracellular dye injections and immunohistochemistry, we found that Panx1 is exclusively expressed in type 3a bipolar cells in the mouse retina, in particular in their membrane and dendrites. To characterize the putative function of Panx1 channels, we performed ERG recordings on Panx1 knock-out mice in comparison to Panx1-expressing mice. Preliminary ERG data showed abnormalities in scotopic ERG. The ERG of Panx1 knock-out mice showed significantly increased b-waves compared to wild-type littermates, whereas the a-wave was not affected. These data indicate that Panx1 deficiency influences the signal processing in the rod pathway. In summary, we suggest Panx1 to form single membrane channels in the plasma membrane of type 3a bipolar cells which play an important role in the rod pathway.

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# Magi2 is a new interaction partner of the USH1G protein SANS

Katharina Bauß[1], Tina Maerker[1], Erwin van Wijk[2], Ferry Kersten[2], Ronald Roepman[3], Hannie Kremer[2], Uwe Wolfrum[1]

[1]Institute of Zoology, AG Wolfrum, Muellerweg 6, 55099 Mainz, Germany
[2]University Med. Center Nijmegen, Dept. of Otorhinolaryngology, Netherlands
[3]University Med. Center Nijmegen, Dept. of Human Genetics, Netherlands

The human Usher syndrome (USH) is the most common form of inherited combined deaf-blindness in men. It is genetically heterogenous and can be devided into three clinical subtypes (USH1-3). Mutations in USH genes lead to profound inner ear developmental defects and retinal degeneration. The so far identified USH genes encode molecules of diverse protein families. Previous studies revealed that all known USH1 and USH2 proteins are integrated in protein networks, the so called USH interactome. In our studies we investigated the role of the USH1G protein SANS (scaffold protein containig ankyrin repeats and SAM domain) in vertebrate photoreceptor cells. Photoreceptors are specialized, bipolar neurons with well defined structural and functional compartments. For their maintenance and function directed transport mechanisms are essential. Here, we identified the MAGUK protein Magi2 (membrane associated guanylate kinase inverted-2) as a protein newly related to USH as a feasible component for transport processes along photoreceptor cells.
In a yeast-2-hybrid screen with the C-terminal SAM-PBM domain (sterile alpha motif, PDZ-binding motif) of SANS as bait we identified three PDZ-domain containing proteins, including Magi2 as putative interactors of SANS. GST-pull down analyses confirmed direct interaction between PDZ5 domain of Magi2 and the C-terminal SAM domain of SANS. In addition, co-transfection assays in eukaryotic cells result in recruitment of both interaction partners *in vivo*. Co-immunoprecipitations out of retina extract indicated an interaction of SANS and Magi2 in the retina. Indirect immunofluorescence shows partial co-localization of SANS and Magi2 in murine retina especially in inner segment and synaptic region of the photoreceptor cells. Immunoelectron microscopy of murine retina confirms subcellular co-localization of both proteins and additionally revealed the association of the Magi2-SANS complex with transport vesicles in photoreceptor cells. Further analysis of the complex indicated an association with the endocytosis machinery in cultured cells.
The direct binding of SANS to vesicle-associated Magi2 and the subcellular distribution of SANS-associated complexes strengthen our hypothesis that SANS participates in transport processes in photoreceptor cells. Furthermore, binding of both proteins is an evidence for a role of this SANS-Magi2 interaction in endocytotic/exocytotic processes in photoreceptor cells which take place in the subcellular compartments of the apical inner segment and at retinal synapses.
Defects of complex partners may lead to dysfunction of the transport network causing photoreceptor degeneration as seen in USH patients.

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# Measuring spectral intensity thresholds by inducing optokinetic reflex in fresh water turtles

Friedrich Kretschmer[1], Malte T. Ahlers[2], Janina Ahrens[1], Josef Ammermüller[2], Jutta Kretzberg[1]

[1]Universität Oldenburg, Computational Neuroscience, Carl-von-Ossietzky-Strasse 9-11, 26129 Oldenburg, Germany
[2]Universität Oldenburg, Neurobiology, Carl-von-Ossietzky-Strasse 9-11, 26129 Oldenburg, Germany

A common problem in vision research is the characterization of animal visual systems. Since animals are unable to verbally communicate their perception, objective behavioural criteria are needed to determine visual thresholds. One method is to induce a visual reflex in the observed animal by controlled visual stimulation. For this purpose, the optokinetic reflex (OKR) was established to analyze contrast thresholds and spatial frequency thresholds in animals.
The most common setup used for OKR measurements makes use of four LCD monitors for the presentation of the visual stimulus (Prusky et al., 2004), which are positioned around the animal and simulate a virtual cylinder with a constant grating around the animal. Behaviour is then observed and usually classified by a human observer.
In this study, we present an alternative experimental setup to present a well defined stimulus to induce OKR in small animals while using an automated headtracking system that yields to an objective criterion avoiding the human-observer approach. The stimulus is presented in the form of a moveable slide that is projected by a high-intensity LED through a 360° mirror onto the inside of a metal cylinder of 1m in diameter. Using wavelength filters allows to precisely determine the wavelength dependence of OKR thresholds, which is almost impossible with LCD monitors because of their broad and undefined spectrum. Head-movements are recorded with a high-speed camera and the angle of the head is evaluated and stored on-line for further analysis.
We used this experimental approach to determine spectral intensity thresholds in fresh water turtles, where we could show notable differences in the induction of the optokinetic reflex depending on the wavelength of the stimulus and in analogy to the absorption spectra of the photoreceptors. Furthermore, we will discuss how this approach could be used for several different experimental applications to characterize the visual system in small animals.

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# Neuronal Coding in the Retina and Fixational Eye Movements

Christian Bernhard Mendl[1], Tim Gollisch[1,2]

[1]MPI Neurobiology, Munich, Junior Research Group Visual Coding, Am Klopferspitz 18, 82152 Martinsried, Germany

[2]Department of Opthalmology, School of Medicine, Waldweg 33, 37073 Göttingen

Retinal ganglion cells have to transmit visual information accurately to higher brain areas. In terms of neuronal coding, the timing of the first spike after a stimulus change has emerged as a prominent candidate for information transfer. Interestingly, this so-called latency coding might be actively employed by microsaccades, which are believed to enhance vision acuity and counteract perceptional fading.

Here, we analyze spike patterns from retinal ganglion cells under simulated miniature eye movements and evaluate potential coding strategies. We mimic these movements by projecting a rapidly shifting grating onto the isolated, flat-mounted amphibian retina. Ganglion cell activity is recorded with extracellular multi-electrode arrays. The task we set out to solve is to discriminate between different spatial offsets of the presented grating based on the ganglion cells' responses only.

We observe that many cells respond specifically at a short time after a rapid image shift. The corresponding spike patterns show a strong temporal structure; in particular, the latencies of spike bursts are tuned to the spatial location of the presented grating. A more detailed examination points towards a simple encoding model, in which the latency depends largely on the stimulus content within a cell's receptive field after the rapid shift.

# Optometric and MR-Image Analysis of the Visual System in NF-κB Knockout Mice

Ronny Haenold[1], Alexandra Kretz[2], Karl-F. Schmidt[3], Karl-Heinz Herrmann[4], Marc Riemann[1], Jürgen R. Reichenbach[4], Siegrid Löwel[3,5], Otto W. Witte[2], Falk Weih[1]

[1]Leibniz-Institute for Age Research - Fritz-Lipmann-Institute (FLI), Immunology, Beutenbergstr. 11, 07745 Jena, Germany

[2]Friedrich-Schiller-University of Jena Medical School, Department of Neurology, Erlanger Allee 101, 07747 Jena, Germany

[3]Friedrich-Schiller-University of Jena, Institute of General Zoology and Animal Physiology, Erbertstr. 1, 07743 Jena, Germany

[4]Friedrich-Schiller-University of Jena Medical School, Institute of Diagnostic and Interventional Radiology, Medical Physics Group, Philosophenweg 3, 07743 Jena, Germany

[5]present address: Bernstein Focus Neurotechnology (BFNT) and Johann-Friedrich-Blumenbach Institute for Zoology und Anthropology, Georg-August-University, Berliner Str. 28, 37073 Göttingen, Germany

A multitude of neurological pathologies and organismic aging itself are associated with an impairment of sensory system functions such as the deterioration of visual capacity. This is thought to be due to defects in neuron-intrinsic determinants as well as noxious immune responses. Recent experimental evidence suggests an involvement of the transcription factor NF-κB, commonly known for its anti-apoptotic action in the immune system and in neuronal cell survival including the aging and injured retina [1,2].

Here, we investigated the role of NF-κB in the visual system by functional analysis and *in vivo* MR-imaging of mice deficient for the NF-κB subunits RelA/p65, p50, RelB, and p52. Visual function was analyzed by optometric measurement of visual acuity and contrast sensitivity. Disruption of classical and alternative NF-κB activation resulted in subunit-specific modulation of the visual functions. Lack of classical RelA/p65 revealed no obvious phenotype for visual acuity (0.36 ±0.007 cyc/deg; $P = 0.2$) and contrast sensitivity (86.2 ±9.7% at a frequency of 0.064 cyc/deg; $P = 0.3$) in 1-year-old knockout mice. In contrast, both visual acuity (0.34 ±0.004 cyc/deg) and contrast sensitivity (47.2 ±1.8%) were reduced in p50 deficient mice already at an age of 2 months compared to controls ($P < 0.001$). At 1 year of age, contrast sensitivity progressively declined down to 21.5 ±0.4% ($P < 0.001$). We also found that components of the alternative NF-κB pathway were involved in proper visual function. Deletion of either RelB (27.2 ±0.9%; $P < 0.01$) or p52 (25.0 ±0.6%; $P < 0.01$) caused a significant impairment particularly in contrast sensitivity in 1-year-old mice.

Manganese-enhanced magnetic resonance imaging (MEMRI) of the retino-tectal projection was used to detect structural alterations in the lateral geniculate nucleus (LGN) of NF-κB p50 and p52 deficient mice *in vivo*. MEMRI was performed on a human 3T scanner and T1-weighted images were analyzed following bilateral injection of a manganese solution into the vitreous body. We observed a significant reduction in contrast-to-noise ratio in the LGN of p50 (14.7 ±1.3; $P < 0.01$) and p52 (16.5 ±0.6; $P < 0.01$) knockout animals compared to wild-type mice (19.7 ±0.9) at the age of 1 year. Interestingly, longitudinal analysis of 15-months-old p50 deficient mice revealed a progressive reduction of the LGN signal intensity (p50 knockout 11.7 ±1.3 vs. wild-type 18.1 ±1.1; $P < 0.01$).

Our observations suggest a pivotal role for NF-κB in the visual system and indicate subunit-specific functions for structural and/or functional maintenance of the retino-tectal projection. Ongoing investigations are designed to clarify specific neuronal function such as retinal ganglion cell survival, and to potentially modulate developmental and aging processes within the visual system.

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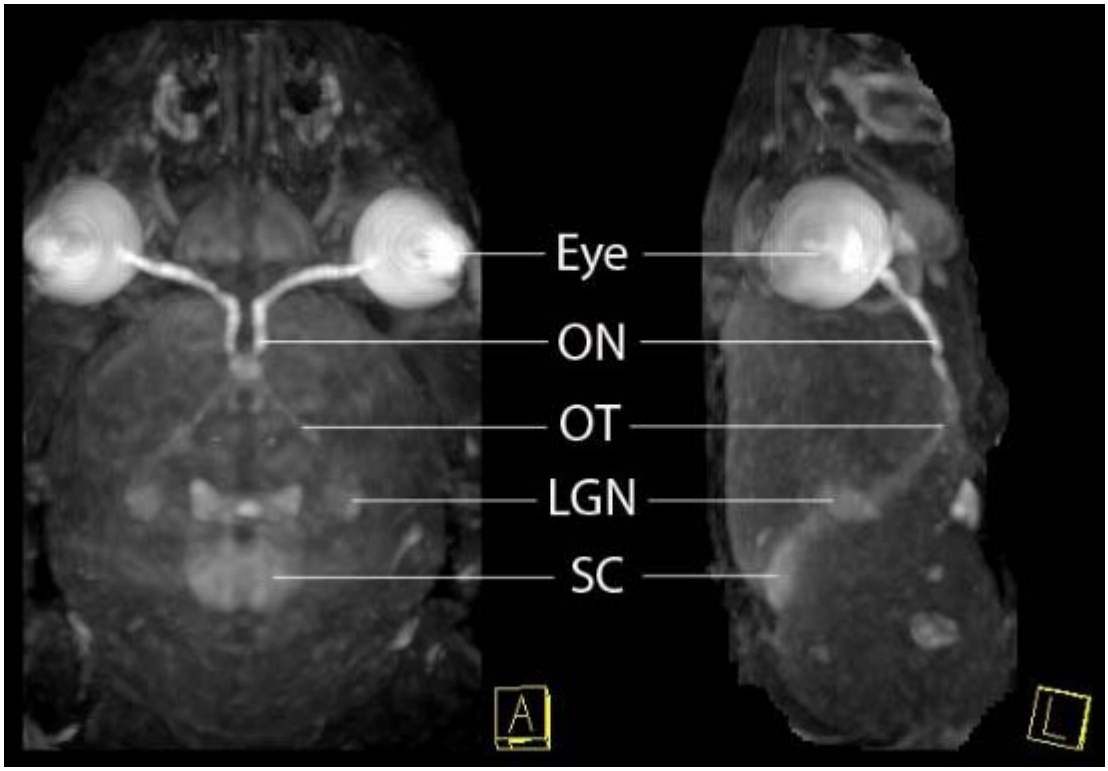
Fig. 1. Manganese-enhanced MR-imaging of the visual system in a wild-type mouse depicting eye, optic nerve (ON), optic tract (OT), lateral geniculate nucleus (LGN), and colliculus superior (SC). T1-weighted images with an isotropic resolution of 0.2 mm were acquired using a 3D FLASH sequence (VIBE 3D). Left: top view, right:

lateral view.



# Pattern Discrimination vs. Spatial Learning in Zebra Finches (*Taeniopygia guttata*)

Uwe Mayer[1], Shigeru Watanabe[2], Hans-Joachim Bischof[1]

[1]University of Bielefeld, Neuroethology Group, Postfach 100131, 33501 Bielefeld, Germany

[2]Keio University, Tokyo, Japan

Zebra finches (*Taeniopygia guttata*) are able to learn the location of hidden food by orienting on spatial cues in a "dry water maze", or by discriminating between food feeders with different patterns. At least two distinct brain areas are essential for these abilities: Entopallial lesions cause deficits in pattern discrimination, hippocampal lesions cause strong deficits in spatial orientation.

In the present study we tested whether intact birds prefer to learn the position of a baited feeder by spatial orientation or pattern discrimination in a task where both is possible. For this purpose, four food feeders were placed at the floor of a small aviary with extra maze cues. Only one feeder, which was marked with a different pattern, was accessible for food. When the birds had learned to find the food, the location of the differently patterned baited feeder was changed. It was then examined whether the birds preferred the previously learned position in relation to the extra maze cues or the differently patterned food feeder at the new location. The immediate early gene products c-Fos and Zenk were visualized after the test trial to determine the activity of the relevant brain areas.

Half of the birds preferred the learned location, the others preferred the differently patterned feeder at a new location. The two remaining positions were not chosen by any of the birds. Expression of the immediate early genes Zenk and c-Fos was enhanced within the hippocampus of birds that preferred location during the test trial compared with those with a pattern preference.

Our experiments show that zebra finches are using either the one or the other strategy for orientation. Hippocampal activity during the task correlates with behaviour and suggests that hippocampus is involved in processing of spatial information, but not in pattern discrimination.

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# Phosphorylation of the horizontal cell specific connexin cpCx53.8 in the fish retina: Effects of light, dopamine and all-trans retinoic acid

Sebastian Hermann[1], Katharina John[1], Nadine Mellies[1], Nina Hoyer[1], Reto Weiler[1], Ulrike Janßen-Bienhold[1]

[1]University Oldenburg, Fak V; Neurobiology, Carl-von-Ossietzky-Straße, 26129 Oldenburg, Germany

There are four types of horizontal cells (HC) in the carp (cp) retina which are coupled by homologous gap junction channels (GJCs). This coupling results in the formation of four independent neuronal networks that process incoming signals from the photoreceptors, feed forward information to bipolar cells and feedback information to photoreceptors. Several electrophysiological and tracer-coupling studies revealed that HC coupling is modulated by the ambient light and that dopamine (DA) and neuromodulators, such as nitric oxide (NO) and all-trans retinoic acid (RA) are involved. It is a matter of debate that DA and the neuromodulators exert their effects via the phosphorylation of the GJC forming proteins, the connexins (Cx), but until today this has not been shown, due to the lack of appropriate HC-specific connexin antibodies. To solve this problem, we have identified four connexins with a high degree of identity which are only expressed in carp HCs, and we have generated an antibody against one of these connexins, termed cpCx53.8. By means of immunofluorescence labelling of dissociated HCs, we could show that this connexin is expressed in all four types of HCs and therefore seems to be an important candidate involved in the coupling of the HC networks. In the present study, we wanted to find out whether light adaptation affects the phosphorylation of cpCx53.8 and its distribution within the carp retina, and which effects the application of DA and all-trans RA have on this process.

By means of immunoblotting we could show that light adaptation, as well as the incubation (45 min) of dark-adapted retinas with DA (500µM) or all-trans RA (10µM) lead to a considerable phosphorylation of cpCx53.8 visible in form of newly appearing immunoreactive cpCx53.8 (MWr 54 kDa) isoforms of MWr 55, 58 and 60 kDa. The incubation of dark-adapted retinas with cAMP (10µM) or IBMX (10µM) /Forskolin (10µM), all leading to the activation of protein kinase A (PKA), revealed an increase in cpCx53.8 phosphorylation like DA, and thus suggest that DA might exert its effects on cpCx53.8 and HC coupling via the activation of PKA. Furthermore, we could show that the DA-effect can be inhibited by preincubation (15 min) of the retina with the specific PKA-inhibitor H89 (2µM) and the DAR1-receptor-antagonist Schering 23390 (10µM). Incubation of dark-adapted retinas with Phorbol 12,13- Dibutyrate (1µM), a potent activator of protein kinase C (PKC), resulted in a very prominent phosphorylation of cpCx53.8 and identified it as a substrate for PKC. But, the most interesting finding was, that staurosporine, a potent inhibitor of PKC, was able to inhibit the all-trans RA-induced phosphorylation of cpCx53.8. This indicates for the first time that all-trans RA exerts its effects on HC coupling via the activation of PKC. By immunofluorescence labelling we could show, that light adaptation results in a significant increase in cpCx53.8 immunoreactivity in the outer plexiform layer and inner nuclear layer of the carp retina, when compared to dark-adapted retinas. Furthermore, cpCx53.8 immunoreactivity patterns obtained from dark-adapted retinas incubated with DA or all-trans RA appeared to be similar to patterns obtained from light-adapted retinas.

In summary, our data suggest that a HC specific connexin (cpCx53.8) is a substrate for PKA and PKC, and that light-adaptation affects phosphorylation of cpCx53.8 via the activation of DAR1-receptors and PKA and all-trans-RA and PKC. Thus it is likely to assume that light reduces HC coupling via two different pathways: (1) the DA, DAR1, cAMP, PKA-pathway as well as (2) the all-trans RA, PKC-pathway and the subsequent phosphorylation of cpCx53.8.

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# Physiological consequences of horizontal cell ablation in adult living mice

Karin Dedek[1], Stephan Sonntag[2], Birthe Dorgau[1], Kerstin Cimiotti[2], Konrad Schultz[1], Reto Weiler[1], Klaus Willecke[2], Ulrike Janssen-Bienhold[1]

[1]University of Oldenburg, Neurobiology, D-26111 Oldenburg, Germany
[2]University of Bonn, Institute of Genetics, D-53117 Bonn, Germany

Horizontal cells are interneurons in the outer plexiform layer of the retina which form synaptic triads with bipolar cells and photoreceptors. Synaptic triads consist of two lateral horizontal cell dendrites and one or two central ON bipolar cell dendrites invaginating the cone photoreceptor terminal. To study the functional role of horizontal cells in these synapses in detail, we generated mice expressing the primate diphtheria toxin receptor (DTR) under the promoter of connexin57 (Cx57), a horizontal cell-specific connexin. Treatment of heterozygous three-month old Cx57+/DTR mice with diphtheria toxin led to ablation of horizontal cells in living animals. Two weeks after treatment, calbindin staining revealed the complete loss of horizontal cells. Electron microscopy showed that not only horizontal cell dendrites were missing from triads but that ON bipolar cell dendrites also lost contacts with photoreceptor terminals suggesting that horizontal cells are essential for triad stability.

To test retinal signal processing, we measured electroretinograms (ERG) from diphtheria toxin-treated control and Cx57+/DTR mice six month after toxin injection. Consistent with the morphological changes, b-wave amplitudes were significantly reduced in scotopic ERGs from treated Cx57+/DTR mice, pointing to a defect in the retinal ON pathway. Also, the time from flash onset to b-wave peak (implicit time) was significantly higher for Cx57+/DTR than for control mice. To test whether these changes also impaired ganglion cell function, we performed multi-electrode recordings and measured light responses to fullfield stimulation. Interestingly, light responses of Cx57+/DTR mice were robust and showed ON, ON-OFF or OFF characteristics. However, for ON ganglion cells we found a significant increase in time to peak response in ablated retinas compared to control retinas. Thus, both ERG and multi-electrode recordings showed that light responses from Cx57+/DTR mice are slower than from control mice. Nevertheless, our results also indicate that the ON pathway is still functional, although most synaptic triads are disrupted. This hints to functional ectopic synapses formed by ON bipolar cells in the outer nuclear layer. Indeed, we observed sprouted ON bipolar cell dendrites decorated with mGluR6 receptors in the outer nuclear layer of ablated retinas.

In summary, our results show that horizontal cells are essential elements of synaptic triads between horizontal cells, bipolar cells and photoreceptors. Disruption of horizontal cells leads to disruption of the triad and ERGs show a severe impairment of signal transmission. However, ganglion cells are still responsive to full-field light stimuli and show ON, ON-OFF and OFF responses, although with subtle differences in the temporal course of the light response.

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# Response-triggered averages of retinal ganglion cells with intracellular recordings

Jing Shao[1], Tim Gollisch[1,2]

[1]Max Planck Institute of Neurobiology, Visual coding, Am Klopferspitz 18, 82152 Martinsried, Germany
[2]University Medical Center Göttingen, Dept. of Ophthalmology, Waldweg 33, 37073 Göttingen, Germany

In the vertebrate retina, light-evoked signals flow through the network from photoreceptors via bipolar cells to retinal ganglion cells in a vertical direction. This direct pathway is modified by lateral inhibitory interactions mediated by horizontal cells and amacrine cells. Retinal ganglion cells integrate excitatory and inhibitory inputs and send the results to the brain through their axons, which form the optic nerve. Studying how the activity of retinal ganglion cells results from this interplay of excitation and inhibition is thus crucial for understanding the neural processing within the retina.

To investigate how retinal ganglion cells integrate inhibitory and excitatory inputs, we performed whole-cell patch clamp recordings from retinal ganglion cells in the isolated axolotl retina. We measured the voltage and current responses of ganglion cells to a flickering light stimulus. For each cell, we then computed from the voltage measurements the cell's spike triggered average (STA), the average stimulus preceding a spike, and compared it to the current triggered average (CTA). The latter can be measured for various holding potentials in voltage-clamp mode, which allows us to separate excitatory and inhibitory inputs and explore how these affect the cell's spiking response.

# Retinal and tectal connections in the paddlefish, *Polyodon spathula*

Vanessa Kassing[1], Michael Hofmann[1]

[1]Institute of Zoology, Vergleichende Neuroanatomie, University of Bonn, Germany

Paddlefish are uniquely adapted to forage for water fleas (Daphnia) by using primarily their passive electrosensory system. There are many studies on how the electrosensory information is processed at different levels of the paddlefish brain. Major sensorimotor interface is the mesencephalic tectum and we have investigated physiological properties of electrosensory and visual units in this brain area. However, we still need detailed information about the afferent and efferent connections of the tectum. There is no description of the connections of the tectum in the paddlefish and only incomplete information in the closely related sturgeons. Sturgeons and paddlefish constitute a basal group of ray-finned fishes and information on tectal and also retinal projections are important to understand the early evolution of the actinopterygian brain. Hence, we made tracer injections into the retina and the mesencephalic tectum in the paddlefish.

Retinal targets are the suprachiasmatic nucleus, a terminal field lateral to the ventromedial thalamus, the neuropile of the dorsal posterior and central posterior thalamic nuclei, the periventricular pretectum, and the tectum, contralaterally. A smaller ipsilaterl projection is also present to the lateral part of the tectum. An accessory optic system appears to be lacking. This shows that retinal projections are more restricted in the paddlefish than in the closely related sturgeon (Ito, Brain Behav Evol 1999;53:127)

The tectum is interconnected with a variety of areas throughout the brain. Afferents from the telencephalon as present in teleosts, however, are lacking. The general connections of the tectum is similar to other ray finned fished, but more extensive. In the diencephalon, terminal fields in the pretectum, dorsal and ventral thalamus are present as well as retrogradely labeled cells in these areas. The terminal fields mostly overlap with retinal terminal fields and this has previously been interpreted as an uncomplete separation of 'geniculate' and 'extrageniculate' visual projections (Yamamoto, Brain Behav Evol 1999;53:142). However, there are some reservations to this interpretations. First the terms 'geniculate' and 'extrageniculate' imply visual pathways to the telencephalon, but we found that many units in the tectum are unimodal electrosensory and the nature of tectal efferents is not necessarily visual. Second, we also found that some dorsal thalamic nuclei in question project massively to the brain stem, which challenges the view of a telencephalic relay station. Furthermore, some terminal fields filled from the tectum may actually be collaterals of retinal fibers. More detailed studies on the organization and connections of the diencephalon in sturgeons or paddlefish are necessary in order to compare it with teleost or other vertebrates.

# Sodium-driven chloride bicarbonate exchanger NCBE in the mouse retina

Gerrit Hilgen[1], Antje K. Huebner[2], Reto Weiler[1], Christian A. Hübner[2], Karin Dedek[1]

[1] Department of Neurobiology, University of Oldenburg, D-26129 Oldenburg, Germany
[2]Institute of Human Genetics, University Hospital Jena, D-07747 Jena, Germany

In the central nervous system, regulation of intracellular pH is important for normal neuronal function. The sodium-driven chloride bicarbonate exchanger NCBE (*Slc4a10*) uses the transmembrane gradient of sodium to drive cellular net uptake of bicarbonate and to extrude chloride [$+Na^+/+2HCO3^-/-CL^-$], thereby regulating pH and excitability in CNS neurons (Jacobs et al., 2008). Here, we analyzed the expression and function of NCBE in the mouse retina.

To assess NCBE expression, we used immunohistochemistry and stained wild-type (WT) and NCBE-deficient retinas (NCBE-KO; Jacobs et al., 2008) for NCBE and various retinal cell markers. To assess the functional role of NCBE in the mouse retina, we recorded extracellular ganglion cell light responses with a single electrode and compared them between WT and NCBE-KO mice. Light stimuli consisted of spots, increasing in diameter, which were centered onto the ganglion cell receptive. Light-evoked spikes were sorted and perievent time histograms (PSTH) were calculated.

NCBE is expressed on almost every retinal neuronal cell type. Staining for calbindin revealed a strong NCBE expression in horizontal cell somata but not in horizontal cell dendrites. Using various bipolar cell markers, we found NCBE on the dendrites of OFF bipolar cells and on axon terminals of OFF and ON bipolar cells in the mouse retina. Double labeling with KCC2, a chloride exchanger, and NCBE revealed that NCBE is distributed on the same bipolar cell compartments as KCC2. In NCBE-KO retinas, no NCBE staining was found; however, the intensity of the KCC2 staining was not altered. We also labeled amacrine cells with various markers and found NCBE to be expressed on starburst and calretinin-positive amacrine cells. Ganglion cell bodies were also stained by NCBE antibodies. To assess the functional role of NCBE in the retina, we performed ganglion cell recordings. PSTH analyses of ganglion cell light responses revealed that the light-induced spike frequency of NCBE-KO ganglion cells was increased compared to WT ganglion cells; baseline activity, however, was unchanged. Furthermore, after light stimulus onset, ON ganglion cells from NCBE-KO mice showed more sustained responses than ON ganglion cells from WT littermates. Interestingly, ON-OFF ganglion cells from NCBE-KO mice showed a decreased OFF response with increasing spot sizes.

In summary, we showed that the sodium-driven chloride bicarbonate exchanger NCBE is expressed in bipolar and amacrine cells of the mouse retina and that lack of NCBE leads to temporal changes in light-evoked ON and ON-OFF ganglion cell responses. Whether these effects are caused by an altered pH regulation of retinal neurons or by changes in neuronal excitability remains to be seen.

Jacobs et al., 2008: PNAS 105, 311–316 January 8, 2008

# Spatiotemporal analysis of electrically evoked activity in the chicken midbrain slice

Stefan Weigel[1], Simon Breitenbach[1], Ralf Wessel[2], Harald Luksch[1]

[1]Technische Universität München, Chair of Zoology, Liesel-Beckmann Strasse 4, 85350 Freising-Weihenstephan, Germany
[2]Washington University at Saint Louis, Physic department, Saint Louis, USA

The dorsal midbrain (superior colliculus in mammals, optic tectum in other vertebrates) is involved in the processing of topographic sensory stimuli. Here, all spatially organized sensory modalities are integrated, relayed to further processing areas, and appropriate premotor signals are generated. Our group is interested in the architecture and function of midbrain neuronal networks, in particular in the signal processing in local networks of the optic tectum (OT) as well as between the OT and the nuclei isthmi (NI). The latter consists of three subdivisions: the nucleus isthmi pars parvocellularis (IPC), the n.i. pars magnocellularis (IMC) and the n.i. pars semilunaris (SLU). The three nuclei are interconnected and have reciprocal connectivity with the optic tectum, thus forming exclusive feedback loops of a complex architecture. In this system, visual information is conveyed retinotopically from retinal ganglion cells to the upper layers of the optic tectum. Here, retinal afferents contact a prominent neuron type – the Shepherd's Crook Neurons (SCN). These neurons project exclusively to the NI. Neurons from the IPC form homotopic, cholinergic backprojections to the OT, while neurons from the IMC form heterotopic GABAergic connections to the OT. The circuit between OT and isthmic subnuclei might implement essential mechanisms such as winner-takes-all and novelty preference.

To study these complex interactions, we need to explore the spatiotemporal activation of the system under various stimulus paradigms. Here, we present data gathered with optical imaging approaches with voltage sensitive dyes in a chicken midbrain slice preparation.

The circuit is activated by electrical stimulation of afferent layers of the OT which mimics the input from retinal ganglion cells. Circuit activation in the tectum results in a neuronal response consisting of a short (~8ms) and a long-lasting component (up to several hundred ms). To isolate the influence of the various feedback connections, we either removed the NI surgically or inactivated receptors pharmacologically. While the short component was not affected, blocking of inhibitory circuits resulted in an enhancement of the long-lasting component. Physical removal of NI did not affect the response.

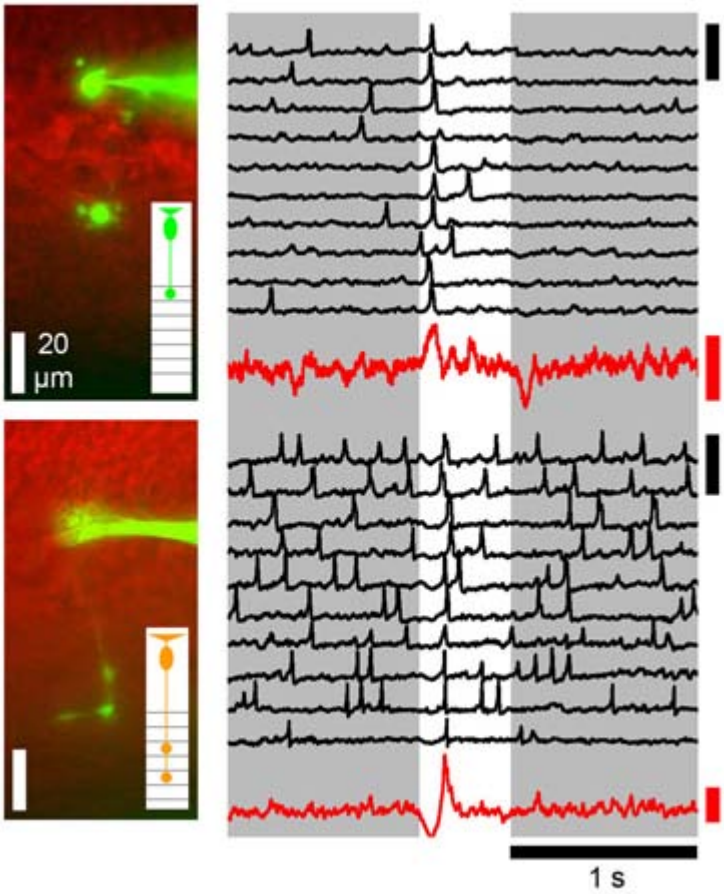
The intrinsic inhibition seems to play an important role during concurrent stimulation at adjacent sides.

# Spikes in retinal bipolar cells generate a temporally precise visual code

Tom Baden[1], Federico Esposti[1], Leon Lagnado[1]

[1]MRC-LMB, Cambridge, MRC-Laboratory for Molecular Biology, Hills Road, CB2 0QH Cambridge, United Kingdom[2] CIN - Center for Integrative Neurosciences, Institute for Opthalmic Research, University of Tübingen, Röntgenweg 11, 72107 Tübingen, Germany

Vision requires that neurons encode the timing of events in the visual scene reliably and with temporal precision. The first neurons to achieve both requirements are ganglion cells, which deliver the output from the retina to the brain with spikes displaying a trial-to-trial variance less than 5 ms. Visual signals reach ganglion cells through bipolar cells, which are thought to transfer slow and graded information. Here we show that the large majority of bipolar cells in the retina of goldfish generate spikes in the synaptic terminal, phase-locked to fluctuating light with a variance less than 5 ms. Crossing the threshold for spike generation is unreliable in individual bipolar cells because it depends on voltage noise through a mechanism of stochastic resonance, but reliability is achieved by convergence of bipolar cell synapses onto postsynaptic amacrine and ganglion cells. These results provide a new view of the functional plan of the retina and the role of noise in early visual processing.



# Stimulus Coding Strategies of Fish and Turtle Retina: A Comparative Study

Leon Mauricio Juarez Paz[1], Jutta Kretzberg[1]

[1]Carl von Ossietzky Universität Oldenburg, Computational Neuroscience, Carl von Ossietzky Str. 9-11, 26111 Oldenburg, Germany

The action potentials generated by retinal ganglion cells (RGC) are the only source of information about the visual environment available to the brain. Nevertheless, it is still not clear how different features of visual stimulus are encoded by the responses of RGC. Moreover, it is also unknown if the coding strategies are the same for different animal species. In the following study, we have recorded and analysed the extracellular activity of RGC of isolated fish (*Cyprinus carpio*) and turtle (*Pseudemis scripta elegans*) retinae that were stimulated with different light intensities and with a pattern of squares that was moving with different velocities along one axis.

Based on the recorded activity from our experiments, we have found that:
1.-Almost all fish and turtle RGC tune their activity to different light intensities.
2.-For both species there are RGC which show tuned activity to motion speed and direction. Nonetheless, the number of these kinds of cells is greater in the turtle retina.

For both species, we have analysed the recorded activity of single RGC that showed motion tuning using spike-cost based metrics [1]. This analysis method was selected in order to test the relevance of spike firing rate and spike time structure for the encoding of stimulus motion features. Our results show that for both species, stimulus velocity is mostly encoded by the spike firing rate of single RGC, whereas instant velocity changes are encoded by temporal structure of single RGC responses.

Based on our results we can conclude that in the turtle retina there are more cells responsible for the encoding of moving stimuli. Nevertheless, for both species, the coding strategies for stimulus movement seem to be very similar.

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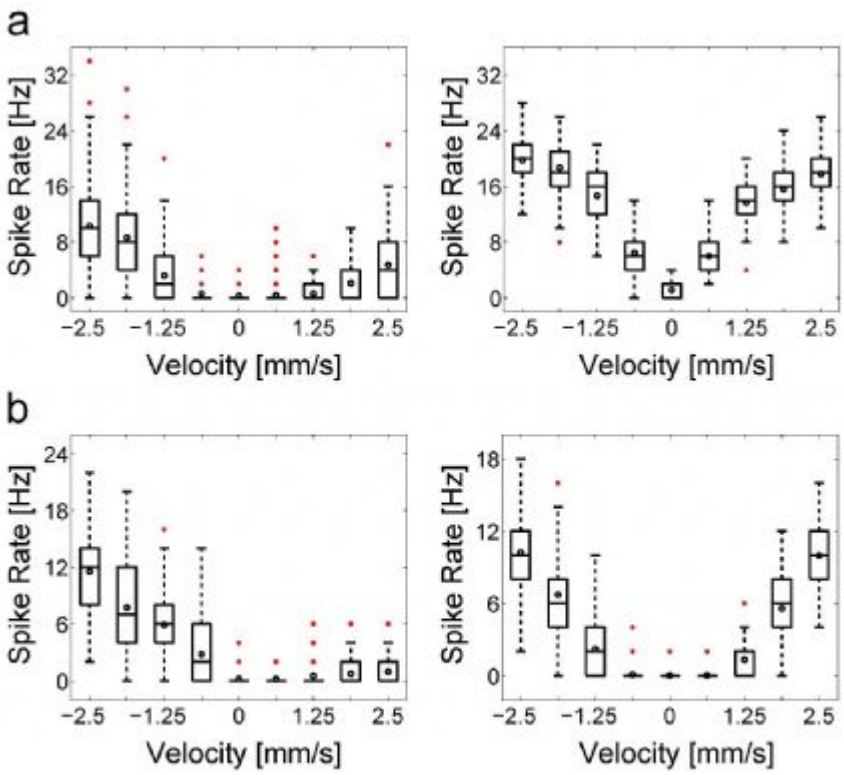


**Tuning curves** of fish (a) and turtle (b) retinal ganglion cells. Some cells show stronger responses for a prefered motion direction (left), whereas other cells tune their response depending on the motion speed.

# Subcellular determination of the BBSome in photoreceptor cells and non-ciliated retinal neurons

Benjamin Spitzbarth[1], Gabi Stern-Schneider[1], Elisabeth Sehn[1], Uwe Wolfrum[1]

Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg-University Mainz, Germany

The Bardet-Biedl Syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder. It is characterized by retinal degeneration, obesity, polydactyly, learning disabilities and hypogenitalism. To date, 14 BBS genes (BBS1-BBS14) have been identified. Although the exact function of the BBS proteins remain elusive so far, it was demonstrated that the group of BBS proteins assemble into a multiprotein complex, named BBSome. The BBSome is thought to play an essential role in the ciliary structure and the formation and function of cilia and basal bodies in general. In the mammalian retina, an association with proteins related to the intraflagellar transport (IFT) is assumed to be required for genesis and maintenance of photoreceptor cilia.

Although investigations on the BBsome are in focus of current intense research the spatial distribution of individual BBS proteins remained so far elusive. Here, we analyzed the subcellular localization of individual BBS proteins, present in the BBSome in retinal cells, in particular in the ciliated photoreceptor cells by high resolution immunofluorescence and immunoelectron microscopy.

Vertebrate photoreceptor cells are highly polarized neurons consisting of well defined morphological and functional compartments. The photosensitive outer segment resembles a highly modified sensory cilium, characterized by stacks of membranous disks and a cytoplasmic axonem. The connecting cilium connects the outer segment to the inner segment which contains all organelles for biosynthesis.

Here we show that all BBS proteins investigated are localized at the basal body and the adjacent centriole of the photoreceptor cilium, as expected from previous immunofluorescence analysis of primary cilia in cultured cells. However, we surprisingly also detected the BBS proteins in the connecting cilium and the axonem of photoreceptor cells. Latter findings indicate a functional role of the BBSome at the ciliary base but also in ciliary compartments which are thought to be related to ciliary maintenance and ciliary transport processes.

Moreover, we identified a set of BBS proteins in the outer plexiform layer of murine retina where the synaptic junctions between the photoreceptor cells and 2nd retinal neurons are located. Further analysis using molecular markers revealed that these BBS molecules are present predominantly in the post-synaptic terminals of dendritic processes of bipolar cells. The presence in the latter non-ciliated neurons indicates that the expression and function of BBS molecules are not restricted to ciliated cells.

In conclusion we provide evidence that the BBSome does not only function at the ciliary base but also in more apical compartments of photoreceptor cilia and probably in cilia in general. Moreover, we provide first evidence for a non-ciliated function of BBS molecules in the retina.

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# The USH1G protein SANS is a microtubule-binding protein and part of the cytoplasmic dynein motor in mammalian photoreceptor cells

Nasrin Sorusch[1], Nora Overlack[1], Tina Märker[1], Erwin van Wijk[2], Katharina Bauß[1], Ferry Kersten[2], Ronald Roepman[3], Hannie Kremer[2], Uwe Wolfrum[1]

[1]Cell and Matrix Biology, Inst. of Zoology, Johannes Gutenberg-University of Mainz, Germany
[2]Dept. of Otorhinolaryngology, University Nijmegen Med. Center, Netherlands
[3]Dept. of Human Genetics, University Nijmegen Med. Center, Netherlands

Vertebrate photoreceptor cells are highly polarized sensory neurons. The photosensitive outer segment is a modified primary cilium. Continuous renewal of the outer segment transduction machinery is supported by the biosynthetic active inner segment. Thus, cellular function and maintenance of photoreceptor cells require intense vectorial transport. We recently identified a periciliary protein network in photoreceptor cells that is suggested to participate in ciliary transport processes. The components of this network are related to the human Usher syndrome (USH), the most common form of combined deaf/blindness. We have shown that the USH1G protein SANS (scaffold protein containing ankyrin repeats and SAM domain) is a major scaffold protein in the periciliary protein network. Here we analyzed the putative role of SANS in transport in photoreceptor cells.

The cellular localization and microtubule depolymerisation assays revealed association of SANS with microtubules in NIH3T3 cells and retinal cells. Microtubule spindown assays confirmed direct binding of SANS to microtubules. Yeast-2-Hybrid screens with SANS central domain as bait identified $p150^{Glued}$, a dynactin-subunit and component of the cytoplasmic dynein motor complex (CDMC), as a binding partner for SANS. SANS-$p150^{Glued}$ binding was validated and confirmed *in vitro* by GST pulldowns, *in vivo* in co-transfection assays and co-expression analyses in cultured cells and in retinal photoreceptor cells. Furthermore, SANS was recovered in pulldowns by GST-dynein intermediate chain (DYN1I1) fusion protein in eukaryotic cells, indicating that SANS is a component of the cytoplasmic dynein motor complex.

In conclusion, we have shown that SANS is a microtubule-binding protein, closely associated with centrosomes and cilia. The direct interaction with the dynactin subunit $p150^{Glued}$ and association with cytoplasmic dynein motor further supports our hypothesis that SANS participates in microtubule-based transport processes in photoreceptor cells. Furthermore, SANS bridges the periciliary USH protein network to the BBSome, which is thought to be involved in the molecular sorting and delivery of cargo molecules into cilia. This further supports the USH networks role in ciliary cargo reloading at the interface between the inner segmental transport and the ciliary delivery. Molecular defects in the network may lead to a disturbed protein delivery, a known cause of ciliopathies in man.

# Trichostatin A induces apoptosis at the concentration recommended to differentiate the RGC-5 cell line

Sven Schnichels[1], Maximillian Schultheiss[1], Johanna Hofmann[1], Peter Szurman[1], Karl Ulrich Bartz-Schmidt[1], Martin S. Spitzer[1]

[1]Centre of Ophthalmology Tübingen, University Eye Hospital Tübingen, Schleichstr. 12/1 , 72076 Tübingen, Germany

The retinal ganglion cell (RGC)-5 cell line is widely used for studying chronic or acute optic neuropathies in vitro. Initially, the cell line showed several characteristics of RGCs. However, over time many RGC-characteristics have been lost. Several groups tried to (re)-differentiate the RGC-5 cells. The best results were reported using staurosporine, a non- selective protein kinase inhibitor, and Trichostatin A (TSA), a histone deacetylase inhibitor. Using these substances, RGC-5 cells showed more ganglion cell-like morphology and expressed typical RGC markers. The optimal concentration for differentiation of RGC-5 cells was determined at 316 nM for staurosporine and 500 nM for TSA, respectively. However, these concentrations are known to induce apoptosis in primary cells. We recently showed, that staurosporine induces apoptosis in every concentration that leads to "redifferentiation". The aim of our study was to investigate whether TSA at the recommended and three additional concentrations could also induce apoptosis in RGC-5 cells.

40 nM, 150 nM, 500 nM or 2000 nM of TSA were supplemented on RGC-5 cells for 1, 24, 48 hours (h). Phase contrast pictures were taken to evaluate cell morphology. Cell amount was determined by crystal violet staining. Apoptosis was detected with Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays and Caspase 3/7 activity assay. Concentrations of the cleaved Caspase 3, pro-apoptotic Bax and anti-apoptotic Bcl-2 were determined via Western blot. Cell viability was determined by an MTS assay. Cells were stained with Propidium Iodide (PI) to determine cell death via fluorescence microscopy.

Morphological changes of the RGC-5 cells were observed by phase contrast pictures 24h after treatment with concentrations of 500 nM and 2000 nM TSA. However, very sporadically single cells also changed their morphology at concentrations of 150 nM. Forty-eight hours after treatment most cells at 500 nM and all cells at 2000 nM TSA concentration showed altered morphology. Almost all cells at 150 nM TSA were unchanged. Obviously fewer cells were observable at concentrations 150 nM, 500 nM and 2000 nM compared to controls. These findings were confirmed by crystal violet staining, which showed that a TSA concentration of 500 nM reduced the amount of cells to 51 % (24 h after treatment) and to 24 % for 500 nM (48 h after treatment) compared to controls ($p<0.0005$).
We found a massive induction of apoptosis at the recommended doses 24 and 48 h after treatment compared to controls: TSA increased Caspase 3/7 activity 24 h after treatment by 5.0–fold for 500 nM TSA ($p<0.0005$) (Figure 1). The amount of cleaved Caspase 3 rose with higher concentrations and was higher than in controls of the same time-points (24 h and 48 h). In parallel, cell viability declined to 70% 24 h and 35% 48 h after treatment for 500 nM ($p<0.0005$) compared to controls. After 24 h of TSA treatment, already 3.8x more PI positive cells were observed for 500 nM TSA ($p<0.005$). For all treatments and methods no significant changes were found 1h after treatment.

In conclusion, TSA (like staurosporine) induces apoptosis in a dose -dependent manner. However, further investigations may be necessary to determine how (if at all) RGC-5 cells can be differentiated into cells with stable RGC-properties.

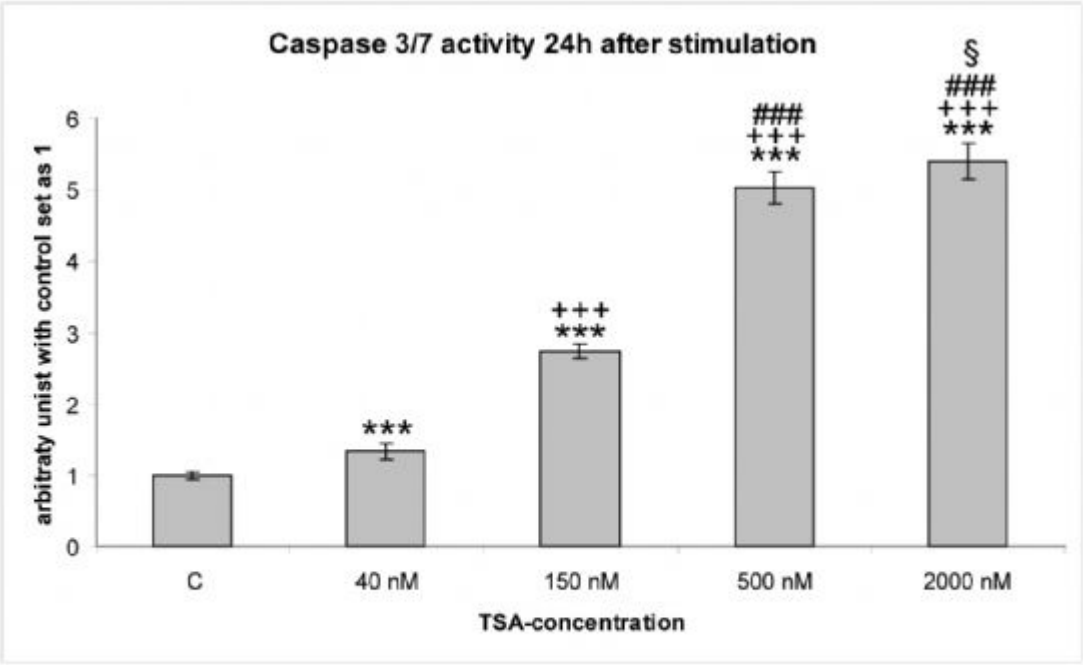


Fig1: Caspase 3/7 activity expressed as arbitrary units with controls set as 1; n=6; Data are depicted as mean ± SD, with *** $p<0.0005$ versus controls, with +++ $p<0.0005$ versus 40 nM of TSA, with ### $p<0.0005$ versus 150 nM of TSA, with § $p<0.05$ versus 500 nM of TSA

# USH1C gene repair mediated by Zinc Finger Nuclease induced Homologous Recombination

Nora-Lena Overlack[1], Tobias Goldmann[1], Uwe Wolfrum[1], Kerstin Nagel-Wolfrum[1]

Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg-University of Mainz, Germany

The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. USH is clinically and genetically heterogeneous and assigned to three clinical USH types. USH1, which represents the most severe form, is characterized by profound inner ear defects and retinitis pigmentosa. Mutations in the USH1C gene encoding for the scaffold protein harmonin cause USH1. The auditory deficit is successfully treated with cochlear implants. Unfortunately, so far no effective clinical treatment for the ophthalmic component of USH exists. However, gene based strategies are attractive for the treatment of hereditary retinal degenerations.
In the present project we are establishing a strategy for gene repair in the USH1C gene by homologous recombination. This represents a powerful method to correct genetic defects. In our study we focus on one nonsense mutation, identified in a German family, leading to a premature translational stop (p.R31X) in the USH1C gene product harmonin. We plan to replace the endogenous mutated segment of the gene by an exogenously introduced rescue plasmid encoding for the healthy USH1C gene. To increase the efficiency of homologous recombination, double-strand breaks are introduced at the mutated chromosomal region using zinc finger nucleases (ZFN). ZNF are hybrid proteins composed of an engineered zinc finger DNA-binding domain fused to the non-specific cleavage domain of the FokI enzyme.
Screening the mutated USH1C sequence for plausible zinc finger binding sites revealed six promising pairs of ZFN. We generated the identified ZFN by modular assembly, verified the nuclear localization upon transfection in HEK293T cells and demonstrated their in vitro cleavage capability. Differences in the ZFN binding activity to their specific USH1C DNA-target sequences were observed in bacterial-two-hybrid analyses. The gene repair by ZFN induced homologous recombination in cell culture is analyzed by indirect immunofluorescence, Western blot and PCR.
Gene repair by homologous recombination represents a powerful technique to correct genetic defects. The repair of a gene at the site of the mutation ensures sustained and tissue-specific expression of the gene product because it remains under control of its endogenous promoter. The restoration of some functional harmonin protein might be sufficient to cure or at least slow down the progression of the retinal degeneration which would greatly improve the life quality of USH1 patients.

Supports: DFG, EU FP7 “SYSCILIA” and “TREATRUSH”, FAUN-Stiftung, Forschung contra Blindheit, Foundation Fighting Blindness

# USH1C TRANSCRIPTS AND HARMONIN PROTEIN EXPRESSION IN PRIMATE PHOTORECEPTORS

Mirjana Becker[1], Tobias Goldmann[1], Kerstin Nagel-Wolfrum[1], Nico Fuhrmann[1], Ulrike Maas[1], Elisabeth Sehn[1], Christina Müller[2], Jan M. Vetter[2], Uwe Wolfrum[1]

[1]Cell and Matrix Biology, Institute of Zoology, Johannes Gutenberg University of Mainz, Germany
[2]Dept. of Ophthalmology, University Clinic of Mainz, Germany

The human Usher syndrome (USH) is the most frequent cause of inherited combined deaf-blindness. 9 identified USH genes encode proteins of diverse protein families. Previous studies revealed that all known USH1 and USH2 proteins are organized in protein networks within protein networks, the USH protein interactome. The USH1C scaffold protein harmonin is one of the major organizers in those networks. The USH1C gene is expressed in form of various alternative spliced variants. The resulting proteins are subgrouped into a, b and c isoforms based on their modular composition of PDZ domains, a PST domain and coiled-coil domains. So far, the specific functions and the cellular expression profiles of the various isoforms are unknown. Here we investigate whether all groups of harmonin isoforms are expressed in human retina and furthermore analyse the localization of harmonin in primate retinas.

The expression of harmonin isoforms in human retina was analyzed by RT-PCR and Western blot analyses. RT-PCRs revealed expression of harmonin a, b and c isoforms in human retina using different primer sets. Western blot analyses confirmed the presence of diverse isoforms in the human retina. The localization of harmonin in the primate retinas was studied by a combination of high resolution immunofluorescence and immunoelectron microscopy. The investigation of the subcellular localization of harmonin in human retina revealed that harmonin was differentially expressed in primate rod and cone synapses. In addition, harmonin was found in the outer limiting membrane within the photoreceptor cells, but not in Müller cells. Furthermore, harmonin was localized in inner and outer segments of photoreceptor cells. However, harmonin was not localized in the connecting cilium or in the periciliary membrane complex where a USH protein complex scaffold by other USH proteins was previously described. Further analyses of harmonin´s photoreceptor localization by co -staining of anti-harmonin and cone-specific markers (e.g. fluorescent peanutagglutinin) as well as immunoelectron microscopy demonstrated that harmonin´s expression was restricted to outer segments of primate rod photoreceptor cells.

In conclusion, the present data reveal that different harmonin isoforms of all types are expressed in human retina. We assume that harmonin functions as an organizer of protein networks in the outer segments of primate rod photoreceptor cells and in primate cone synapses.

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# Identification of connexins in photoreceptors of 129/Sv mice

Petra Bolte[1], Regina Herrling[1], Ulrike Janssen-Bienhold [1], Reto Weiler[1]

[1]Department of Neurobiology, Carl-von Ossietzky-Str. 9-11, 26111 Oldenburg, Germany

Gap junction channels between rod and cone photoreceptors enable transmission of rod-mediated signals via the cone pathway to the central nervous system. The structural components of gap junction channels in vertebrates are connexin proteins. The connexin family encodes for twenty different genes in the mouse genome. Our lab showed connexin36 expression in cones (Feigenspan et al., 2004) but, despite many efforts and physiological evidence for rod-cone coupling (Trümpler et al., 2008) and rod-rod coupling (Tsukamoto et al., 2001), the connexin expressed in rods has not yet been identified.

With the present study we aim to identify the corresponding rod connexin in photoreceptors of 129/Sv mice, in which rod-cone coupling has clearly been demonstrated (Trümpler et al., 2008). We tested for mRNA expression of all known connexin genes in photoreceptors of this mouse strain using RT-PCR. To determine whether the connexin genes that probed positive in cDNA samples are in fact expressed in rods, immunohistochemistry using different anti-connexin antibodies was carried out in vertical retina slices.

In RT -PCR experiments we detected six (Cx32, Cx36, Cx43, Cx45, Cx50 and Cx57) out of twenty mouse connexin mRNAs in samples of photoreceptors. The alignment of the sequenced PCR products revealed 100% identity of the amplified fragments with the respective connexins. The immunohistochemical analysis of connexin protein expression in mouse retinal sections showed that in contrast to Cx36, which was localized in cone pedicles, neither Cx32, Cx43, Cx50 nor Cx57 proteins could be localized in photoreceptors of 129/Sv mice. Thus, it is unlikely that these four connexins are expressed in rods as the possible counterpart of Cx36 mediating rod-cone coupling, and leaves Cx45 as the only possible candidate protein. However, preliminary immunohistochemical experiments performed with anti-Cx45 antibodies do not yet allow a clear exclusion of Cx45 as a candidate connexin protein expressed in rods.

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# Poster Topic

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*Konrad Lehmann, Naira Yeritsyan, Oliver Puk, Jochen Graw, Siegrid Löwel*

# A minimal model for saccadic inhibition

Theda Backen[1], Stefan Treue[1], B. Suresh Krishna[1]

[1]Bernstein Center for Computational Neuroscience and German Primate Center, Cognitive Neuroscience Laboratory, Kellnerweg 4, 37077 Goettingen, Germany

A distractor flashed during the preparatory period of a saccade induces a robust, transient, short-latency slowing of the subsequent saccade in humans and monkeys: this phenomenon is known as saccadic inhibition (Reingold and Stampe 2002, Blanchard et al. 1985). The physiological mechanism by which the distractor interferes with the saccadic preparatory process remains unknown. In order to achieve a better characterization of the time-course of the distractor effect, we developed a minimal, yet physiologically plausible model of saccadic inhibition. We started with the LATER model (Carpenter and Williams 1995) which posits that the saccadic latency on each trial is the (random) time taken by a particle to travel over a fixed (arbitrary) distance when the particle's velocity is drawn randomly on each trial from a normal distribution with mean μ and standard-deviation s; μ and s are model parameters. The LATER model has been found to characterize human saccadic latencies to single targets quite well; it also captures the responses of saccade-related neurons in the frontal eye field (FEF) of macaque monkeys (Hanes and Schall 1996). We then postulated that the flashed distractor reduces the velocity of the particle by a value V for a period of time equal to D ms, starting at a fixed latency L after distractor onset; V, D and L are model free parameters. After D+L ms following distractor onset, the velocity goes back to its original value for that trial. Initial testing showed that such a model captures all the key qualitative aspects of saccadic inhibition that have been previously reported. We then tested the model with novel data obtained from 10 naive human subjects using a distractor flashed for 200 ms during the preparatory period of a delayed visually-guided saccade to a target 10 degrees away along the horizontal axis. The distractor appeared at three different eccentricities 93 ms after the disappearance of the fixation point, which served as the go- signal to make the saccade towards the visible visual target. We found good quantitative agreement between the model fits and the experimental data. Based on the model, we calculate that the flashed distractor interferes with saccadic preparation for a surprisingly short time: on average, the distractor hindered saccadic preparation for only 40 ms starting 80 ms after distractor onset. This phenomenological model should facilitate direct comparison of behavioral data with the physiological effects of the distractor on saccade -related neurons in brain areas like the superior colliculus, lateral intraparietal area and FEF. Saccadic inhibition has the potential to serve as a rapidly-measurable assay of distractibility in both normal human subjects and patient populations.

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# A model for the influence of adaptation on the representation of instantaneous speed changes in macaque area MT.

Andreas Traschütz[1], Bonne Habekost[1,2], F. Orlando Galashan[1], Andreas K. Kreiter[1], Klaus R. Pawelzik[2], Udo E. Ernst[2], Detlef Wegener[1]

[1]University of Bremen, Center for Cognitive Science, Institute for Brain Research, P.O. Box 33 04 40, 28334 Bremen, Germany
[2]University of Bremen, Center for Cognitive Science, Institute for Theoretical Neurophysics, P.O. Box 33 04 40, 28334 Bremen, Germany

Motion signals are of particular relevance for successful behavior in natural environments. Extensive experimental and modelling data exist regarding the representation of continuous motion, but changes in motion, e.g. in direction or speed, have rarely been analyzed. We here describe neuronal responses of motion direction-selective neurons recorded in macaque area MT to a large range of instantaneous positive and negative speed changes. We show that the temporal dynamics of their response to different speed change amplitudes can be explained by a simple adaptation model.

To perform recordings, two macaque monkeys were trained on a dimming task at fixation. Visual stimulation consisted of Gabor gratings shown to the receptive fields of the recorded units. All neurons were extensively tested for direction, spatial, and temporal frequency tuning, and for their responses to different speed changes. For the latter, we used Gabor gratings with an intrinsic speed of either 2.1 or 6.3 deg/s and seventeen different speed change factors ranging from 0.33 to 3.0 (8 decelerations, 8 accelerations, 1 no- change). Direction and temporal frequency of the Gabors were adjusted according to the tuning characteristics of the units which were measured prior to the main experiment.

MT neurons respond to speed changes with a rapid transient change of their firing rate, followed by a somewhat lower sustained response, and an offset response with higher firing rates for non-preferred motion directions. Fitting neuronal responses with a von Mises function revealed no differences in the width of the direction tuning curve regarding transient and sustained responses to speed changes. However, transient and sustained responses differ significantly regarding speed tuning. The transient response is best correlated with the amplitude of the speed change, while the sustained response represents the absolute speed of the stimulus. This finding was consistent not only for accelerations but for decelerations as well. We present a model relying on adaptation mechanisms that is capable to explain the temporal dynamics of the response profile regarding the representation of speed and speed changes to different directions of motion.

# Blindsight depends on the lateral geniculate nucleus

Michael C Schmid[1], Sylwia Mrowka[2], Janita Turchi[2], Melanie Wilke[2], Richard Saunders[2], Andrew Peters[2], Frank Ye[2], David Leopold[2]

[1]Ernst Strüngmann Institut, Deutschordenstrasse 46, 60528 Frankfurt, Germany
[2]National Institutes of Health, 49 Convent Drive, Bethesda, MD, 20892, USA

Injury to the primary visual cortex (V1) leaves human patients and macaque monkeys with remarkable visual detection capacities, usually in the absence of visual awareness (blindsight). On the neural level, the common interpretation of these behavioral observations is that visual information must bypass V1, reach extrastriate areas, where information is processed to the extent that enables meaningful behavioral responses. Although a number of studies have aimed to delineate the brain circuits that contribute to blindsight, there exists to date little consensus which extrastriate areas are visually responsive in the absence of V1 input and what V1-bypassing pathways drive activation in these areas. The goal of our experiments was to 1) delineate the extent of V1-independent fMRI activity in extrastriate areas, and 2) test whether a direct projection from the lateral geniculate nucleus (LGN) can account for V1-independent visual functions.

We used a combination of fMRI, behavioral testing, and pharmacological inactivation methods to assess visual functions of macaque monkeys with chronic V1 lesions. A small part of V1 gray matter between 2 and 7 degrees visual eccentricities was surgically aspirated. Monkeys were scanned in a dedicated 4.7T primate magnet while they performed a passive fixation task during which various visual stimuli were presented either inside or outside the visual field affected by the lesion (scotoma). Complimentary behavioral assessment tested the monkeys' ability to detect visual stimuli inside the scotoma. Experiments were conducted with and without MRI-guided reversible inactivation of the LGN using the GABA-A agonist THIP.

Significant V1-independent fMRI activation was found in a number of extrastriate visual areas and was associated with the monkeys' ability to detect visual targets presented in the visual field corresponding to the lesion (scotoma). Repeating the same set of experiments while inactivating the LGN eliminated both fMRI and behavioral responses to stimuli presented in the scotoma.

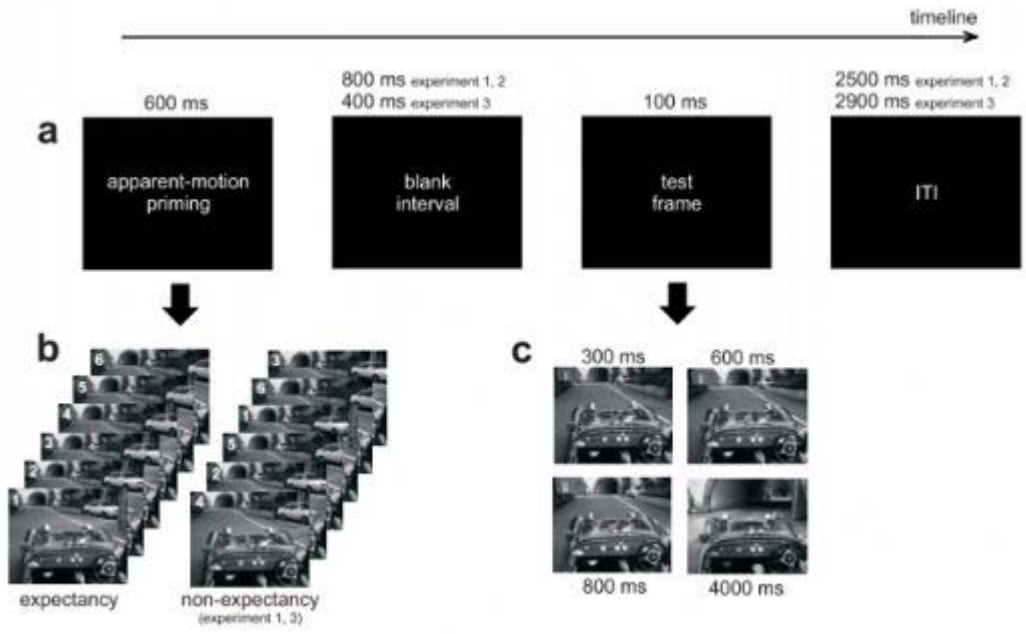
These results, taken together, demonstrate that the LGN is critical for relaying visual information to extrastriate visual cortex and guiding residual visual behavior following V1 injury. The direct projection from the LGN to extrastriate areas may play an important role in mediating fast neural and behavioral responses during normal vision.

# Contextual association network and the prediction of movements in natural visual scenes

Lars Muckli[1], Fraser Smith[1], Fabiana Carvalho[1]

[1]University of Glasgow, Centre for Cognitive Neuroimaging (CCNi), Institute of Neuroscience and Psychology , 58 Hillhead Street, G12 8QB Glasgow, United Kingdom

We propose that one of the core functions of the cortical visual system is to predict upcoming visual stimulation based on repeating spatio-temporal patterns and regularities from our environment (Hawkins, 2004; Bar, 2007; Friston 2010). In a recent study, we showed spatial-temporal predictions along the apparent motion path in V1 (Alink et al. 2010). Here, we set out to test the notion of predictive coding in natural visual scene movies and found evidence in the default mode network (DMN), or contextual association network (Bar, 2007), on addition to retinotopic visual areas. We induced the percept of apparent motion in natural scenes with a prime sequence of six frames (extracted from 16 different movie-clips) followed by a gap of 800 ms (Exp.2: 400ms). We tested visual prediction after the gap with a test stimulus taken from the movie at either 300 ms, 600ms, 800ms or 4000ms (Exp2: 300ms and 4000ms). We looked for mental extrapolation effects by testing BOLD-signal attenuation in response to the different test stimuli (fMRI at 3T-SiemensTimTrio). Strongest attenuation was found for a 600ms test stimulus (Exp2. 300ms), confirming that the cortex anticipates the future (but with a mental shrinkage effect of approx. 25%). We found MTL, MPC, and pre-SMA, areas of the "contextual association network" (or DMN), that are strongly involved in motion specific predictions. Our findings suggest that the contextual association network (DMN) is involved in predicting future upcoming stimulation. Moreover, our evidence suggests feedback of predictions extends to early visual areas including V1.



# Correlations of simultaneously recorded neural activity in macaque prefrontal cortex

Florian Pieper[1,2], Adam Sachs[1,3], Julio C. Martinez-Trujillo[1]

[1]Cognitive Neurophysiology Laboratory, Dept. of Physiology, Faculty of Medicine, McGill University, Montreal, Quebec, Canada
[2]Universitaetsklinikum Eppendorf, Institut fuer Neuro- & Pathophysiologie, Martinistr. 52, 20246 Hamburg, Germany
[3]Division of Neurosurgery, Ottawa Hospital, Ottawa, Ontario, Canada

Simultaneously recorded activity of many single neurons have revealed new insights in the structure of temporal correlations in visual and (pre-)motor cortical areas of the primate brain. This, however, has been less investigated in areas of the prefrontal cortex that play a more active role in executive control during cognitive tasks.
We recorded spiking activity of single neurons using a 100- microelectrode (10x10) array (Blackrock Inc.) in prefrontal area 8a (Petrides, 2005) of two awake monkeys (Macaca fascicularis) while they performed a contrast detection task. The array's individual electrodes were 1.5 mm long and equispaced at 400 µm, covering ~13 mm² of the cortical surface. During the experiments the monkeys started each trial of the experiment by fixating a spot at the center of a gray projection-screen. After 650 ms a small circular sine-wave grating (2° diameter) of variable contrast appeared randomly at one of 40 possible locations (5 concentric configurations of 8 positions 45° apart, at 5 different eccentricities 3° apart). Another 650 ms later the fixation spot disappeared, and the monkey made a saccade towards the perceived target location to obtain a juice reward.
We computed noise correlation (CC) via the z-transformed neuronal activity (spike rate) of more than 2300 pairs of 163 offline sorted single neurons. During the 500 ms preceding the target onset the CC of single-neuron pairs, ranged from -0.3 to 0.5. The average CC (~ 0.03) was slightly positive (0.045) for the shortest distance (400µm) between the neurons' source electrodes and decreased with increasing electrode distance (Kruskal-Wallis: $p<0.005$). For distances larger than ~2.2 mm the CCs were statistically not different from zero. Additionally, the variance of the CCs decreased with electrode distance. During the 500ms period after the stimulus onset the overall average CC slightly increased to ~0.06. Again, significant positive correlations deceased beyond ~2.2 mm inter-electrode distance (Kruskal-Wallis: $p<0.005$).
71 of the recorded neurons at 58 positions were spatially selective during the period of stimulus presentation being apparently randomly distributed across the electrodes of each array. We evaluated the CCs of the subset of 538 neuron-pairs, where each neuron was recorded from a different probe. Already with no stimulus present on the screen, the activity of neurons with similar spatial preferences (<90° preferred position difference) was significantly covarying (range: 0.07 - 0.03), however, neuron pairs with further preference discrepancy were not covarying (range: 0.015 - -0.007). This changed with the onset of the stimuli. In similar tuned pairs correlations strongly further increased up to on average 0.27; dissimilar tuned neurons strongly decreased their CCs up to -0.13 for 135°-180° distance. For all except the 60°-90° distance-class the effects were significant, indicating a push-pull mechanism on the responses between spatially selective neurons in area 8a.
Our results demonstrate that responses of pairs of neurons in area 8a are on average weakly positively correlated, however, the strength and variance of the correlation declines with increasing distance between neurons. Task- and stimulus-conditions can dynamically change correlation structure between individual neurons in the prefrontal cortex.

# Cross-frequency coupling of eye-movement related LFP activities of freely viewing monkeys

Junji Ito[1], Pedro Maldonado[2], Sonja Gruen[1,3]

[1]RIKEN Brain Science Institute, Laboratory for Statistical Neuroscience, 2-1 Hirosawa, Wako, 351-0198 Saitama, Japan

[2]CENI and Programa de Fisiologia y Biofisica, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, Chile

[3]Theoretical Systems Neurobiology, Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany

Living organisms do not only passively receive sensory stimuli from the external world, but also actively explore their surroundings using their sensory organs such as e.g. sniffing for odor sensation, whisking for touch sensation, and eye-movements for visual sensation. While neuronal activities underlying active sensing in olfaction and vibrissa sensation have been studied in detail, the activities related to active vision have remained largely unknown. In a recent study, we studied local field potentials (LFPs) recorded from the primary visual cortex of monkeys while they perform visual exploration of natural scene images with self-paced, voluntary eye-movements [1]. We found that the LFPs exhibit oscillatory activity in the beta frequency band (10-25 Hz) in relation to the initiation of saccadic eye-movements [2].

It has widely been recognized that brain activities show oscillations on multiple time scales [3]. While previous studies tended to focus on a single, specific frequency band, recent studies have reported strong interactions between activities in different frequency bands [4]. Such interaction is referred to as cross-frequency coupling, and the most common type of coupling is phase-amplitude coupling, also known as nested oscillations [5], where the phase of the slower oscillations modulates the amplitude of the faster oscillations.

In the present study, we investigate cross-frequency coupling in the eye-movement related LFPs of freely viewing monkeys. We identify three distinct frequency components characterized by different types of temporal locking to the onset of saccadic eye-movements: A delta-theta band component which is phase-locked to fixation-onset, a beta-gamma band component that is evoked by saccade-onset, and an induced component in the high gamma band. We find a close relationship between the frequency of the slowest component (in the delta-theta frequency band) and the frequency of self-initiated, exploratory eye movements. Interactions between this slow component and the other faster components are studied in terms of phase-amplitude coupling measured by the modulation index (MI) [6]. We show that the amplitudes of the faster frequency components (in the beta-gamma and high-gamma frequency bands) are modulated by the phase of the slow component (delta-band), as reflected in high MI values for the respective frequencies. Furthermore, we find positive correlations between the degree of the phase-amplitude coupling and the amplitude of the slow component.

These results represent the first evidence of mutual entrainment of different LFP frequency components during natural, active viewing behavior. Spontaneous, self-initiated eye-movements are accompanied by a reset of slow oscillations and by evoked beta oscillations, which modulate the high-gamma oscillations induced by visual input. This suggests a structuring role of slow oscillations in the processing of external visual stimuli.

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# Dopamine and serotonin involvement in nictation behavior in the nematode *Pristionchus pacificus*

Maria Mónica Rodriguez[1], Federico Brown[12], Ralf Sommer[2]

[1]Universidad de los Andes, Evo-Devo, Carrera 1 N° 18A - 12 , 4976 Bogotá, Colombia

[2]Department of Evolutionary Biology, Max-Planck Institute for Developmental Biology, Tübingen, Germany

How is behavior genetically controlled? Which neural pathways are involved? The nematode *P. pacificus* has been developed as a satellite model organism for evolutionary studies. The model nematode *C. elegans* develops a specific larval stage for survival during harsh conditions, called dauer. Dauer metabolism is low, the pharynx is closed and nematodes develop a hard cuticule. During this stage it is possible to observe nictation, a specific behavior in which worms stand on their tail and move upwards. This behavior is seen in a small proportion of the population. I want to investigate how this behavior is genetically regulated by the nervous system, therefore we study two neural pathways: serotonin and dopamine. Preliminary studies with exogenous amines show a difference in the effect on nictation between these pathways in *C. elegans* and *P. pacificus*. I want to know if the difference persists by reducing levels of endogenous amines. For this purpose I carried a deletion mutagenic screen targeting thirteen neural genes. We screened approximately 1200000 gametes, and found a dopamine related mutant for lgc-53 that carries a mutation of around 60bp between intron 11 and 12 (exon 12 is deleted). The mutation removes the third transmembrane domain of the protein. lgc-53 that encodes an ion channel activated by the biogenic amine dopamine, and is a member of the Cys-loop family of ion channels. This mutant may allow us to study whether the endogenous dopamine disruption is directly involved in nictation behavior. Future studies will address the question whether an evolutionary difference can be observed between the effect of serotonin and dopamine on *C. elegans* and *P. pacificus* behaviors.

# Ephrin-A5 Affects the Laminar Organisation of the Neocortex

Katrin Gerstmann[1], Theresa Köbe[1], Jürgen Bolz[1], Geraldine Zimmer[1]

[1]Friedrich-Schiller Universität Jena, Allgemeine Zoologie und Tierphysiologie, Erbertstraße 1, 07743 Jena, Germany

The phylogenetic evolution of the six-layered mammalian neocortex can only be understood in the light of ontogenetic development. During evolution, the surface of the neocortex expanded, while the thickness remains unchanged. Moreover, the relative size of the supragranular layers increased. Excitatory cortical neurons are generared in transient proliverative regions (ventricular zone and subventricular zone) at the ventrucular surface of the dorsal embryonic telencephalon. Postmitotic neurons migrate radially into the developing cortex and form successively the cortical layers. Thereby, neurons destined for the infragranular layers are born first, while supragranular neurons are born at later stages. Various key molecules have already been identified to orchestrate developmental processes like neurogenesis, cell cycle exit, neuronal differentation and migration, including proneural genes, transcription factors and guidance molecules. Members of the Eph/Ephrin-system are expressed in the developing neocortex and were already identified to regulate proliferative processes, axonal guidance and migration. Analysis of the somatosensory cortex in ephrin-A5 deficient mice revealed alterations in the organisation of the cortical layers. The relative size of the infra- and supragranular layers are altered in the ephrin-A5 knockout animals, although the thickness of the cortex remains unchanged. In addition to a reduced cell cycle exit at E16, the fraction of Pax6+ cells is increased, while Tbr2+ cells are decreased in the ephrin-A5 deficient embryoes. Thus, the knockout of ephrin-A5 affects the progenitor pool, resulting in expanded infragranular layers and reduced supragranular layers.

# Experience dependent plasticity of orientation preference in mouse visual cortex

Anne Kristina Kreile[1], Tobias Bonhoeffer[1], Mark Hübener[1]

[1]Max Planck Institute of Neurobiology, Department of Cellular and Systems Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

Neuronal response properties in the visual cortex are shaped by a combination of intrinsic factors and sensory experience. For example, orientation preference develops independently from visual input, but restricting experience to a single orientation by stripe rearing has been shown to cause an overrepresentation of the experienced orientation and an underrepresentation of the orthogonal orientation. However, due to methodological limitations in earlier studies, the mechanisms underlying the stripe rearing effect could not be completely resolved: According to the permissive hypothesis neurons initially tuned to non-experienced orientations loose responsiveness, while the instructive hypothesis states that single neurons change their tuning towards the experienced orientation.

We investigated the plasticity of orientation preference in mouse visual cortex with two-photon calcium imaging, providing single cell resolution and allowing direct testing of these hypotheses. We induced plasticity by stripe rearing visually experienced mice from postnatal day 25 onwards for three weeks with cylindrical lenses (167dpt) of four different orientations fitted to skull mounted goggles. Following stripe rearing, the goggles were removed, the calcium indicator OGB1 -AM was injected into the monocular visual cortex and the responses to drifting gratings were measured with two-photon imaging.

In control mice, we found a horizontal bias in the distribution of preferred orientations which decreased with increasing depth in layer 2/3. In stripe reared mice, the distributions of preferred orientations showed a clear shift towards the experienced orientation. Response amplitudes and tuning widths were not affected. The fraction of responsive neurons decreased slightly, indicating that a weak permissive component may be present. The size of the permissive effect, however, was not correlated with the magnitude of the shift in the distribution of preferred orientations in individual animals. Moreover, in lower layer 2/3 there was a clear shift towards the experienced orientation, but no drop in the fraction of responsive neurons.

Thus, diverse mechanisms contribute to the changes in preferred orientation following stripe rearing, but the effect is at least partially mediated by an instructive process, causing individual neurons to change their orientation preference.

# fMRI-based retinotopic mapping at 7 Tesla magnetic field strength – Conservative thalamo-cortical projections in patients with abnormal optic nerve projections

Falko Kaule[1], Anil Kumar[2], Irène Gottlob[2], Jörg Stadler[3], Barbara Wolynski[1], Oliver Speck[4], Martin Kanowski[5], Synke Meltendorf[1], Wolfgang Behrens-Baumann[1], Michael B. Hoffmann[1]

[1]Otto-von-Guericke-University Magdeburg, Visual Processing Laboratory, Department of Ophthalmology, Leipziger Str. 44, 39120 Magdeburg, Germany

[2]Ophthalmology Group, University of Leicester, Leicester Royal Infirmary, Robert Kilpatrick Clinical Sciences Building, Leicester LE2 7LX, United Kingdom;

[3]Leibniz Institute for Neurobiology, Brenneckestraße 6, 39118 Magdeburg, Germany;

[4]Department Biomedical Magnetic Resonance, Institute for Experimental Physics, Otto-von-Guericke-University Magdeburg , Leipziger Str. 44, 39120 Magdeburg, Germany;

[5]Department of Neurology, Otto-von-Guericke-University, Leipziger Str. 44, 39120 Magdeburg, Germany

Purpose: In albinism the visual cortex receives due to an abnormal crossing of the temporal retina input from the ipsilateral visual hemifield. Remarkable, this abnormal cortical representation is organised as an orderly retinotopic map that is superimposed onto the normal representation of the contralateral visual hemifield [1]. Strikingly, in another patient group, hypochiasmia, there is also input from the ipsilateral field, but here it is due to an abnormal projection of the nasal retina to the ipsilateral hemisphere. We applied fMRI-based retinotopic mapping [2] and compared the cortical visual field representations in controls, albinism, and hypochiasmia to investigate, whether similar principles govern the self organisation in this spectrum of input to the visual cortex.

Methods: In seven subjects (4 controls, 2 albinotic, 1 hypochiasmatic) T2*-weighted images were acquired at a magnetic field strength of 7 Tesla (voxel size: $2.5^3$ $mm^3$; TR: 2.4 s; number of slices: 42; number of acquired volumes per scan: 105). During the scans the subjects monocularly viewed a contrast-inverting section of a circular checkerboard-stimulus that moved through either the left or right visual hemifield as an expanding ring for eccentricity mapping and as a rotating wedge for polar angle-mapping. The data were distortion- and motion-corrected online. After Fourier analysis and the correlation of each voxel's time-series with the stimulus fundamental frequency, the stimulus driven fMRI signals were projected to the flattened representation of T1 weighted images of the occipital lobe.

Results: We report three main findings: (1) In the visual cortex there was an abnormal but retinotopic representation of the ipsilateral visual field in albinism contralateral and in hypochiasmia ipsilateral to the eye stimulated. (2) For both albinism and achiasmia, the normal and abnormal representations were identified within similar boundaries of the early visual areas. (3) Not only in albinism, but also in hypochiasmia the abnormal cortical representation of the ipsilateral visual field is mirror-symmetrically superimposed onto the normal representation of the contralateral visual field in both striate and extra-striate cortex.

Conclusion: In both albinism and hypochiasmia the abnormal input to the visual cortex does not undergo a topographic reorganisation of the geniculo-striate projection. These findings underline that this conservative mapping of large-scale abnormal input appears to be a general principle to make the abnormal visual input available for cortical processing in primates. This clearly contrasts with the heterogeneity of solutions reported in various non-primate animal models of misrouted optic nerves [3].

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# Functional organization of extraocular muscles in Xenopus laevis

Michael Faust[1], Anja K. Horn[2], Hans Straka[1]

[1]Institure of Anatomy, LMU Munich, Pettenkoferstr. 11, 80336 Munich

[2]LMU Munich, Dept. II, Faculty of Biology, Grosshaderner Str 2, 82152 Planegg, Germany

The range of different types of naturally occurring eye movements requires dynamic specializations of extraocular muscles that allow making persistent contractions to compensate tonic head deviations as well as ultrafast contractions for saccades or resetting fast phases during optokinetic or vestibulo-ocular reflexes. Accordingly, extraocular muscles consist of of several subtypes of muscle fibers that differ in various interrelated morpho-physiological properties. Functionally matching connectivity of individual extraocular motoneurons and postsynaptic muscle fibers are necessary to induce appropriately timed contractions that are adequate for particular types of eye movements, respectively. Here we investigated the morphological organization of eye muscles and their motor innervation in larval Xenopus laevis. The muscle fiber organization was studied on semithin cross-sections of individual eye muscles and by various histochemical methods in dissected whole eye muscles and head preparations. The morphological identification and characterization included fluorescent staining with phalloidin, DAPI and immunostaining for "myosin heavy chain slow type". The motoneuronal innervation profile of the eye muscles was determined by a -bungarotoxin binding to visualize motor endplates. This repertoire of identification methods allowed obtaining quantitative data on number, size and spatial arrangement of muscle fiber subtypes as well as innervation patterns of extraocular motoneuronal populations. Confocal imaging allowed computer-aided 3D-visualization and post-processing of the datasets for in-situ morphological analyses. Xenopus larvae at stage 50 were used as a reference for characterizing major structural features. Cross-sections of the lateral rectus muscle, as a representative for the six eye muscles, covers a total area of 2500 µm² and is composed of fibers that cover a large spectrum with mean diameters of 1-7µm (cross-sectional area: 3-150 µm²). A specific group of very thin fibers with a mean diameter of 2.2 µm (cross-sectional area: 15 µm²) were identified as myosin heavy chain slow (MHCs) type immuno-positive and comprise up to 20% of all muscle fibers at the central regions and the two distal ends of the muscle. The majority (>85%) of these fibers are arranged as a dispersed external layer that surrounds the population of particularly thick fibers in the center. MHCs-immuno-positive muscle fibers are contacted by thin nerve fibers with multiple innervations at rather distal positions of the muscle. In contrast, thick muscle fibers are innervated with single terminal boutons from the thickest nerve fibers at more central areas. This organizational pattern is compatible with the general structural design of the ocular motor plant as an assembly of fibers with a wide range of dynamic properties. Physiologically matching connectivity of motoneurons and muscle fibers comply with an arrangement into parallel frequency-tuned pathways that extend e.g. for vestibulo-ocular reflexes from the sensory periphery to the motor effector. The observed organizational scheme is the basis for the large spectrum of dynamically different eye movements. Different developmental stages of Xenopus larvae and adults will now allow tracking the assembly and progression of muscle growth as well as motor innervation and correlation with the functional needs during changing locomotor dynamics before and after metamorphosis.

# Identification and mapping of synaptic inputs to dendritic spines

Onur Gökce[1], Tobias Bonhoeffer[1], Volker Scheuss[1]

[1]Max Planck Institute for Neurobiology, Department of Cellular and Systems Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany

Dendritic spines are prominent cellular structures closely associated with excitatory synapses in the cortex and hippocampus and are therefore favorable targets for experimental studies. However, almost always the presynaptic inputs to the studied spines remain unknown. Therefore it is unclear for example, (i) to what degree heterogeneity in dendritic spine properties arises from a difference in the synaptic input, (ii) in what way spine formation and retraction as observed during experience-dependent plasticity rewires cortical circuits, and (iii) what role dendritic computations play in the information processing in the cortex.

Here, we present an approach to identify functional synapses from specific types of presynaptic neurons onto the dendrites of their target cells in mouse visual cortex. Channelrhodopsin-2 was expressed in presynaptic neurons, either in layer 2/3 by *in utero* electroporation in wild-type mice, or in layer 5 in a transgenic mouse line (Thy1-ChR2; Arenkiel et al., Neuron, 2007). Postsynaptic neurons in layer 5 were patched and filled with a calcium indicator. Functional synapses were identified by two-photon imaging of photostimulation-evoked postsynaptic calcium signals in the dendritic spines of the postsynaptic cell.

This method allows the precise localization and characterization of spines receiving input from a specific type of presynaptic neuron. Such information will advance our understanding of dendritic computations as well as the rewiring of cortical circuits during experience-dependent plasticity.

# Judgments of Amounts of Randomly Distributed Colored Dots are Nonlinear

Werner Georg Karl Backhaus[1]

[1]University of Technology Berlin, AG Psychophysiology, Einsteinufer 25, Flachbau HFT-CO, 10587 Berlin, Germany

The model of neuronal color coding and spatial color sensations (NCC/SCS, Backhaus, 1998, 2001, 2008; see Fig. 1) describes the color sensations in man to consist of a heterogeneous mixture of the six elementary color sensations red, green, blue, yellow, black, and white steered by three types of color coding neurons. The amounts of the elementary colors can be judged, in any color sensation, in terms of numbers (direct scaling). Compared to the much simpler yes/no-judgments (indirect scaling) judgments by number (direct scaling) are more complex and thus must always be suspected to be nonlinearly related to the subjective entities judged. Thus, in general, the numbers cannot be assumed to describe, e.g. the actual amounts of elementary colors 1:1. The model (Fig. 1), therefore, describes the nonlinearities of the judgment process related to the amounts of elementary colors in addition to the relationships of the excitations of the color coding neurons and the amounts of elementary colors.

Now, judgments have been investigated that are not related to the amounts of elementary colors but are instead related to the amounts of randomly distributed colored dots. The judgments are directly comparable, because the large numbers of densely neighbored colored dots are too large to be counted and thus must be judged in terms of area-densities, as in the case of the amounts of elementary colors in our color sensations.

Results: 1) The measured relationships of the judged numbers to the actual amounts of randomly colored dots turned out to be generally nonlinear. The measured nonlinearities were 2) independent of the color, but 3) depend on the number of different colors (2 - 4) involved and were 4) less pronounced when an 1-dimensional array (1 Pixel wide) was presented, instead of a 2-dimensional square-array.

Conclusions: 1) It became obvious from the presented results that the amounts (area-densities) of randomly distributed dots can generally not be judged 1:1 by numbers. 2) This means for the case of the amounts of elementary colors that the numbers judged in color content analytical experiments have to be transformed according to the results of additional experiments with each observer, which are related to the judgment process, in order to determine the actual amounts of elementary colors by respective model calculations. 3) We will continue these investigations in order to achieve experimental designs that allow to avoid judgment induced nonlinearities as far as possible.

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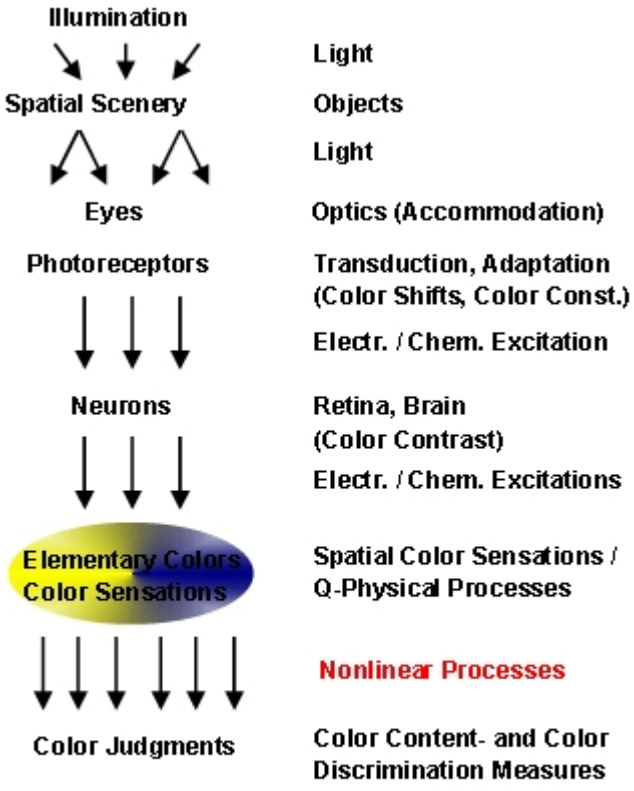


**Fig. 1** Sceme of spatial color vision in man as described by the NCC/SCS-model (Backhaus, 1998, 2001, 2008).

# Localization of flashed stimuli during accelerated smooth pursuit eye movements.

Jan Hüsers[1], Frank Bremmer[1]

[1]Philipps-University Marburg, Neurophysics Department (AG Bremmer), Karl-von-Frisch-Straße 8a, 35043 Marburg / Lahnberge, Germany

The oculomotor system uses Smooth Pursuit Eye Movements (SPEM) to keep moving objects of interest in the fovea, the area with the highest spatial acuity in our retina. During SPEM the world around us is perceived as stable, even though the retinal input changes constantly. This perceptual stability, however, is not complete. Visual perceptual stability and its limitations have been analyzed in various psychophysical and physiological studies. Van Beers et al. (2001) showed that briefly flashed stimuli are mislocalized in the direction of pursuit. The strength of mislocalization shows a retinal topography (van Beers et al. 2001, Koenigs & Bremmer 2010). A possible influence of target velocity on mislocalization is as yet a controversial issue (Brenner et al. 2001; Kerzel et al. 2006). Although target velocity alters the perception of briefly flashed targets, its exact functional role is still not fully understood.

Visual targets in everyday life (e.g., a disturbing fly an observer wants to catch) constantly change their velocity and direction. In physical terms, this is equivalent to an accelerated motion. Surprisingly, little attention has been paid to the relationship between pursuit acceleration and the ability to localize briefly flashed targets during SPEM. In this study we determined if and how acceleration modulates localization performance of briefly flashed targets.

Visual stimuli were projected via a CRT-projector on a tangent screen. In each trial - except for a fixation control condition - a red dot (fixation target) was accelerated with one of five different accelerations values [-50; -25; 0; 25; 50 /°/s²] while moving from left to right at eye level. The timing of the stimuli was adjusted such that the target velocity in the centre of the screen was always at 20°/s irrespective of the acceleration condition. When the target reached the screen centre, a white rectangle (size: 0,5° * 4°) that served as localization stimulus was presented for 10 ms at one of five horizontal positions ranging from -10° to 10°. Thereafter subjects had to indicate the perceived position of the flash using a ruler stimulus.

Localization error heavily depended on eye acceleration. During acceleration phases, localization errors were systematically reduced as compared to stimuli presented when the eyes decelerated or were pursuing a target at a constant velocity. In line with previous reports we found a retinal topography of the localization error. Eye velocity did not have a modulatory effect on localization errors.

Area MT of the macaque monkey plays a crucial role in generating and maintaining SPEM (see Ilg & Thier 2008 for review). Recent data (Schlack et al. 2007) suggests that velocity history changes the sensitivity for acceleration in area MT. These findings might be related to our current results. Further studies, however, are necessary, for clarifying this relationship and especially for determining the position of visual receptive fields in area MT during accelerated pursuit as compared to the RF-locations during steady fixation.

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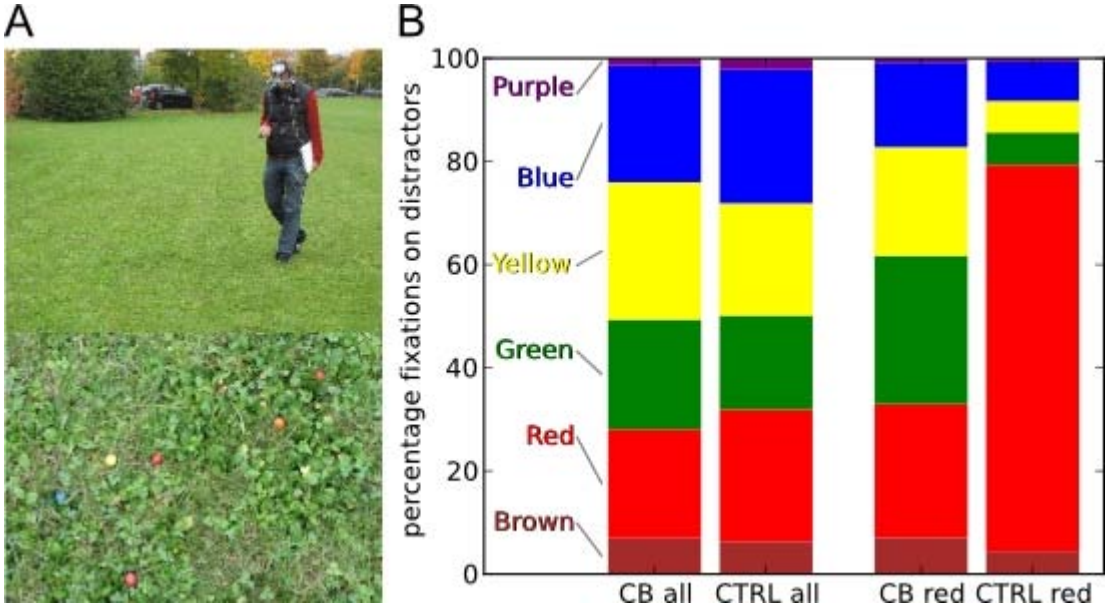
# Looking For Candy: Real-world, feature based search

Günter Kugler[1], Bernard Marius 't Hart[2], Klaus Bartl[1], Stefan Kohlbecher[1], Frank Schumann[3], Wolfgang Einhäuser [2], Thomas Brandt[1], Erich Schneider[1]

[1]Institute of Clinical Neurosciences, University of Munich Hospital
[2]Neurophysics, Philipps-University Marburg
[3]Institute of Cognitive Science, University of Osnabrück

Searching for resources like ripe berries and fruits probably played a large role in the survival of early humans and other hominids. The evolution of a third cone can then be explained by facilitated discrimination between ripe and unripe fruits through the improved red-green color resolution. We here study color and shape-based search, similar to search for berries or fruits, in a natural setting. To do so, we distributed target candies ("Smarties", 3.2%, 0.70/$m^2$) and distractor candies ("m&m's", 96.8%, 21/$m^2$) evenly on a field (800 $m^2$, see Fig 1A). Both sets of items come in a variety of colors. Targets can be discriminated from distractors by their shape. Two groups of observers participated; one red-green "colorblind" group consisting of observers with color vision deficiency (deuteranomaly or deuteranopy, as measured by the Farnsworth-Munsell 100 Hue Test) and age- and sex-matched "controls". We used the EyeSeeCam to record a high resolution video stream of the center of gaze. The first task for the observers was to search for target candies of any color amongst the distractors and point at located ones. Both groups fixated a comparable percentage of distractors of each color (see Fig 1B). Observers in both groups pointed at a similar number of targets. In a second task, the observers were instructed to search for red targets and point at located ones. Compared to the first task, the percentage of red distractors that were fixated rose from ~21% to ~26% in the colorblind group, and from ~26% to ~75% in the controls. In contrast to the controls, the percentage of fixations on green distractors in the colorblind increased (from ~21% to ~29%). Also, in the second task the colorblind observers found fewer red targets in comparison to the control group. These results imply that human observers in real life can preselect possible targets based on a single low-level feature, as they do in laboratory tasks. Avoiding possible targets based on their color would also be a viable strategy for colorblind subjects, which would enable them to reduce the number of yellow and blue distractors inspected. The percentage of fixations on distractors of these colors does not drop to the same level as in the controls, though. This suggests that low-level based preselection of targets relies on positive selection only and does not incorporate an avoidance strategy (or negative selection) by ruling out obviously false targets.



# Multisensory Integration in Intermodal Areas of the Rat Brain

Michael T. Lippert[1], Kentaroh Takagaki[1], Christoph Kayser[2], Frank W. Ohl[1,3]

[1]Leibniz-Institute for Neurobiology, Akustik Lernen Sprache, Brenneckestr. 6, 39118 Madeburg, Germany
[2]Max-Planck-Institute for Biological Cybernetics, Tübingen, Germany
[3]Otto-von-Guericke University, Magdeburg, Germany

The world we live in abounds with sensory stimuli. These rarely occur in one sensory modality alone. Therefore, brain function depends on proper integration and segregation of multiple cues in order to master processing a complex scenery. To perform this task, neural activity evoked by separate modalities has to be integrated across space and time. Previous reports indicate that intermodal areas, that are localized between primary sensory areas, may play an important role in this process[1]. One advantage of such intermodal cortical fields is that not all afferent activity needs to be routed via long range connections and thalamic input, but instead, propagating network activity from proximal primary modalities can directly influence local circuit function[2]. We used intrinsic optical imaging and electrophysiological methods to investigate the spatiotemporal properties of multisensory integration in such an intermodal cortical area. First, we mapped the neurovascular response of rat neocortex in response to light flashes and whisker deflection, and indentified unisensory and multisensory areas. In the same animals, we inserted laminar electrodes to record local field potentials, current source densities and multiunit activity within this optically identified multisensory cortical field. Our results suggest that multimodal integration in these intermodal cortical fields relies primarily on sublinear processes, with multisensory response being less than the sum of unisensory responses. These findings support previous studies which question a predominant role of superadditivity for multisensory integration in the cortex[3].

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# Neural model for the visual tuning properties of action-selective neurons in monkey cortex

Martin A. Giese[1], Vittorio Caggiano[2], Falk Fleischer[1]

[1]Hertie Institute for Clinical Brain Research and Center for Integrative Neuroscience, Section Computational Sensomotorics, Dept. Cognitive Neurology, Tübingen, Germany

[2]McGovern Institute for Brain Research, MIT, Dept. Brain and Cognitive Sciences, Cambridge, MA.

The recognition and understanding of actions of conspecifics is crucial for survival and for successful social interaction. Experimental and theoretical studies on biological motion recognition have emphasized the role of the visual analysis of form and motion patterns specified by action stimuli. In contrast, most current models on the recognition of transitive (goal-directed) actions have been dominated by the idea that motor representations play a central role in action recognition. Specifically, such models have assumed that mirror neurons are central for action recognition by supporting an internal simulation of motor patterns. However, these models do not specify how visual information can be processed by physiologically plausible mechanisms in order to extract information that can be compared with such motor representations. This raises the question how such visual processing might be accomplished, and in how far motor processing is critical in order to account for the visual properties of mirror neurons.

We present a neural model for the visual processing of transient motor actions that is consistent with physiological data and that accomplishes recognition of grasping actions from real video stimuli. The model explains the recognition of transitive actions by an extension of established models for the recognition of not goal-directed body movements, by addition of specific neural circuits modeling the behavior of neurons in parietal and premotor cortex. The recognition of effector and goal object is accomplished by a view-dependent hierarchical neural architecture consistent with many established models from object recognition. Opposed to object recognition models, shape recognition is not completely position-invariant, retaining some information that can be exploited by subsequent stages for the extraction of retinal positions of the goal object and the effector. Effector recognition includes a simple predictive neural circuit that results in temporal sequence selectivity.

The proposed novel circuits associate the information about the goal object with the one about the effector and its movement. For this purpose, position information is read out from the visual recognition pathway exploiting a population code, and a neural map of the relative positions of object and effector in a retinal frame of reference is constructed by a gainfield-like mechanism. Based on this relative position map, the subsequent neural levels decode the correct spatial matching between effector and object, and the matching of the affordances of the object with the effector action. The highest level of the model accomplishes a view- and position-invariant recognition of grips.

We demonstrate that this model reproduces a variety of electrophysiological findings on the visual properties of action-selective neurons in the superior temporal sulcus, parietal cortex and of mirror neurons in premotor area F5. Specifically, the model accounts for the fact that a majority of mirror neurons in area F5 show view dependence. The model predicts a number of electrophysiological results. Partially, those predictions could be confirmed in recent electrophysiological experiments.

We conclude that the visual recognition of transitive motor acts can be accounted for by well-established, predominantly visual neural processes. An involvement of internal motor simulation seems not critical in order to account for the basic visual tuning properties of action-selective neurons.

# Nystagmus generated by positive visual feedback system in healthy humans

Chien-Cheng Chen[1], Dominik Straumann[1], Melody Ying-Yu Huang[1,2]

[1]University Hospital Zurich, Department of Neurology, frauenklinikstrasse 26, CH-8091 Zurich, Switzerland
[2]University of Zurich, Institute of Moleculor Life Science, Winterhurerstrasse 190, CH-8091 Zurich, Switzerland

Infantile nystagmus syndrome (INS), which appears at birth or during the first six months of life, is characterized by involuntary oscillations of the eyes. The involuntary eye movement produces special waveforms that can be distinguished from other types of eye movement disorders. The causes of INS are still unknown. One possible cause is the axonal misrouting of the optic nerve which may make the ocular motor system unstable.
In this study, instead of studying patients with INS, we exposed normal humans to a visual stimulus, controlled by a positive or negative feedback of the eye velocity. We projected vertical sine wave gratings on a diffusive screen in front of the subject. Simultaneously, we recorded the eye movement of the subject with a video eye tracking system and used the eye position as the input to determine the moving direction of the grating. The positive visual feedback condition consisted of a grating moving in the same direction as the eye, but at different velocities. Our experimental results show that the positive feedback stimulus can elicit waveforms in normal humans that are similar to the ones in INS.
Based on the experimental results, we propose that the artificially generated positive feedback in a normal visual system causes instability of the eye movements. Moreover, the similarity of the resulting eye movement waveforms to INS support the hypothesis that INS could result from a positive feedback in the ocular motor system, such as caused by axonal misrouting of the optic nerve.

# Optical Imaging of Retinotopic Maps in a Small Songbird, the Zebra Finch

Nina Keary[1], Joe Voss[1], Konrad Lehmann[2], Siegrid Löwel[2], Hans-Joachim Bischof[1]

[1]Universität Bielefeld, Verhaltensforschung, Postfach 100131, D-33501 Bielefeld, Germany

[2]Friedrich-Schiller-Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstr. 1, D-07743 Jena, Germany

The primary visual cortex of mammals is characterised by a retinotopic representation of the visual field. It has therefore been speculated that the visual wulst, the avian homologue of the visual cortex, also contains such a retinotopic map. We examined this for the first time by optical imaging of intrinsic signals in zebra finches, a small songbird with laterally placed eyes. In addition to the visual wulst, we visualised the retinotopic map of the optic tectum which is homologue to the superior colliculus in mammals. For the optic tectum, our results confirmed previous accounts on topography based on anatomical studies and conventional electrophysiology. Within the visual wulst, the retinotopy revealed by our experiments has not been shown convincingly before. The frontal part of the visual field (0° ± 30° azimuth) was not represented in the retinotopic map. The visual field from 30°-60° azimuth showed stronger magnification compared with more lateral regions. Only stimuli within elevations between about 20° and 40° above the horizon elicited neuronal activation. Activation from other elevations was masked by activation of the preferred region. Most interestingly, we observed more than one retinotopic representation of visual space within the visual wulst, which indicates that the avian wulst, like the visual cortex in mammals, may show some compartmentation parallel to the surface in addition to its layered structure. Our results show the applicability of the optical imaging method also for small songbirds. We obtained a more detailed picture of retinotopic maps in birds, especially on the functional neuronal organisation of the visual wulst. Our findings add to the notion of homology of visual wulst and visual cortex by showing that there is also a functional correspondence between the two areas.

# Perceptual and fMRI Evidence for Filling-In of the Rod Scotoma Under Scotopic Conditions

Alyssa A Brewer[1], Brian Barton[1]

[1]University of California, Irvine, Department of Cognitive Sciences, 3151 Social Sciences Plaza, UCI, 92697 Irvine, USA

INTRODUCTION: The phenomenon of perceptual filling-in, where a region of the retina does not transmit visual information, yet we perceive visual information based on what information is available from nearby retinal locations, has been studied extensively at the blind spot (e.g., Ramachandran, 1992) and after inducing "artificial scotomas" (retina-stabilized adaptation) in the periphery (e.g., Ramachandran & Gregory, 1991; Ramachandran,1993). However, very little work has been done examining filling-in at the rod scotoma in the central fovea, likely because it has been assumed with cursory examination that it does not happen. Hubel (1997) reported that a simple line passing through the rod scotoma does not complete (as happens in blind spots and artificial scotomas), and Hadjikhani and Tootell (2000) reported no perceptual or functional magnetic resonance imaging (fMRI) evidence of filling-in using standard retinotopic mapping stimuli. However, filling-in and scotopic afterimages of an extended surface have been recently reported (Hubel, Howe, Duffy, & Hernandez, 2009).

METHODS: We measured angular and eccentric retinotopic organization across human visual cortex using fMRI under scotopic conditions. Retinotopic stimuli consisted of black and white, drifting radial checkerboards comprising (1) wedges and rings (3°, 7.4°, and 11° in radius) or (2) drifting bars (11° in radius). The data were analyzed using population receptive field (pRF) modeling (Dumoulin & Wandell, 2007).

RESULTS/DISCUSSION: Here, we report new perceptual and fMRI evidence for perceptual filling-in at the rod scotoma under scotopic conditions using drifting bar stimuli. In contrast, when viewing standard flickering checkerboard rotating wedges and expanding rings, no such filling-in occurs.

# PERCEPTUAL SENSITIVITY TO STATISTICAL REGULARITIES IN NATURAL IMAGES

Holly E. Gerhard[1,2,3], Thomas Wiecki[3], Felix Wichmann[4,5], Matthias Bethge[1,2,3]

[1]Werner Reichardt Center for Integrative Neuroscience [2]Bernstein Center for Computational Neuroscience Tübingen [3]Max Planck Institute for Biological Cybernetics [4]Bernstein Center for Computational Neuroscience Berlin [5]Technical University of Berlin

Introduction

A long standing hypothesis is that neural representations are adapted to environmental statistical regularities (Attneave 1954, Barlow 1959), yet the relation between the primate visual system's functional properties and the statistical structure of natural images is still unknown. The central problem is that the high-dimensional space of natural images is difficult to model. While many statistical models of small image patches that have been suggested share certain neural response properties with the visual system (Atick 1990, Olshausen&Field 1996, Schwarz&Simoncelli 2001), it is unclear how informative they are about the functional properties of visual perception. Previously, we quantitatively evaluated how different models capture natural image statistics using average log -loss (e.g. Eichhorn et al, 2009). Here we assess human sensitivity to natural image structure by measuring how discriminable images synthesized by statistical models are from natural images. Our goal is to improve the quantitative description of human sensitivity to natural image regularities and evaluate various models' relative efficacy in capturing perceptually relevant image structure.

Methods

We measured human perceptual thresholds to detect statistical deviations from natural images. The task was two alternative forced choice with feedback. On a trial, two textures were presented side-by-side for 3 seconds: one a tiling of image patches from the van Hateren photograph database, the other of model-synthesized images (Figure 1A). The task was to select the natural image texture.

We measured sensitivity at 3 patch sizes (3x3, 4x4, & 5x5 pixels) for 7 models. Five were natural image models: a random filter model capturing only 2nd order pixel correlations (RND), the independent component analysis model (ICA), a spherically symmetric model (L2S), the Lp-spherical model (LpS), and the mixture of elliptically contoured distributions (MEC) with cluster number varied at 4 levels (k = 2, 4, 8, & 16). For MEC, we also used patch size 8x8. We also tested perceptual sensitivity to independent phase scrambling in the Fourier basis (IPS) and to global phase scrambling (GPS) which preserves all correlations between the phases and between the amplitudes but destroys statistical dependences between phases and amplitudes. For each type, we presented 30 different textures to 15 naïve subjects (1020 trials/subject).

Results

Figure 1B shows performance by patch size for each model. Low values indicate better model performance as the synthesized texture was harder to discriminate from natural. Surprisingly, subjects were significantly above chance in all cases except at patch size 3x3 for MEC. This shows that human observers are highly sensitive to local higher-order correlations as the models insufficiently reproduced natural image statistics for the visual system. Further, the psychometric functions' ordering parallels nicely the models' average log-loss ordering, beautifully so within MEC depending on cluster number, suggesting that the human visual system may have near perfect knowledge of natural image statistical regularities and that average log-loss is a useful model comparison measure in terms of perceptual relevance. Next, we will determine the features human observers use to discriminate the textures' naturalness which can help improve statistical modeling of perceptually relevant natural image structure.

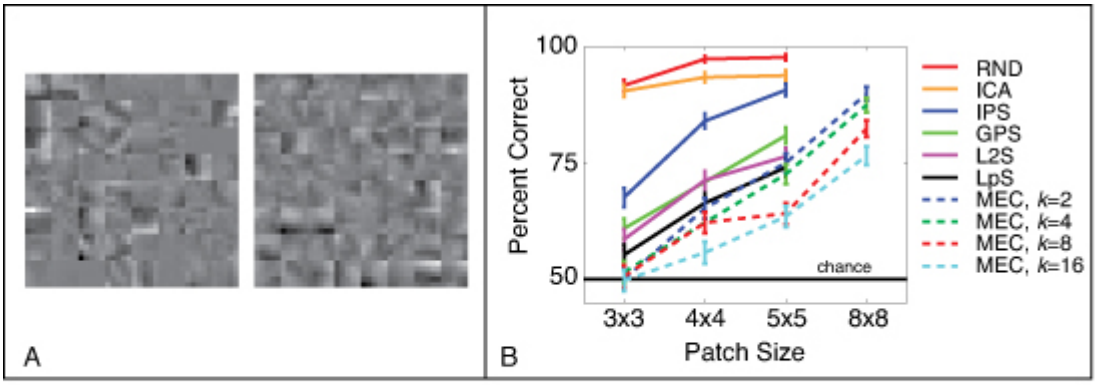



**Figure 1A.** Example stimulus with 5x5 patches generated from natural images (left) and from the ICA model (right). Textures subtended 3.2-10.4 degrees of visual angle on a side, depending on patch size.

**Figure 1B.** Percent correct ±SEM for each model as a function of patch size. The closer to chance level, the less distinguishable the image model is from the true statistics of natural images.

# Pinwheel Cartography: Visual Field Map Clusters in Ventral-, Medial-, and Lateral-Occipital Cortex

Brian Barton[1], Alyssa A. Brewer[1]

[1]University of California, Irvine, Cognitive Science, 3151 Social Sciences Plaza, 92617 Irvine, USA

Introduction: One of the more important larger scale organizing principles of visual cortical organization is the visual field map: neurons whose visual receptive fields lie next to one another in visual space are located next to one another in cortex, forming one complete representation of contralateral visual space. As increasing numbers of visual field maps have been defined in human visual cortex, one question that has arisen is whether there is an organizing principle for the distribution of these maps across visual cortex. A basic approach that has worked for early visual cortex has been to define strings of visual field maps along contiguous strips of occipital cortex, with adjacent portions of the maps representing similar portions of space, but performing different computations. In V1, at an order of magnitude smaller than the visual field map, there exist orientation pinwheels, which are orderly organizations of neurons that have preferred tuning systematically spanning the full range of oriented lines (e.g., Bartfeld & Grinvald, 1992). We investigate the possibility that visual field maps are organized into similar circular clusters, which are replicated across visual cortex, oriented independently to one another, and subserve similar computations within a cluster.

Methods: We measured angular and eccentric retinotopic organization and population receptive fields across visual cortex using fMRI and population receptive field (pRF) modeling (Dumoulin & Wandell, 2008). We model pRFs as 2-dimensional differences of Gaussians with preferred centers (x, y) and spreads (sigma), convolve the predicted response to the stimuli with the haemodynamic response function, and fit the best population receptive field independently to each voxel via a least-squares method. Retinotopic stimuli consisted of black and white, drifting bar apertures comprised of flickering checkerboards, 11Â° in radius.

Results/Discussion: We identify 14 new visual field maps in medial, lateral, and ventral occipital cortex, organized with previously defined visual field maps into 6 visual field map clusters: Occipital Pole (OP), V3A/B, Temporal-Occipital (TO), Inferior Temporal-Occipital (ITO), Ventral-Occipital (VO), and posterior Superior Temporal Sulcus (pSTS). We propose that these pinwheel clusters are a fundamental organizing principle of the human visual system, extending from low-level to higher-order visual processing areas.

# Reduced cortical plasticity and impaired sensory learning in young adult Bassoon mutant mice.

Bianka Goetze[1,2], Karl-Friedrich Schmidt[1], Wilko D. Altrock[3], Eckart D. Gundelfinger[3], Carmela Giampà[4], Francesca R. Fusco[4], Siegrid Löwel[1,2]

[1]Institut für Allgemeine Zoologie und Tierphysiologie, Friedrich-Schiller-Universität Jena, Germany

[2]Bernstein Focus for Neurotechnology (BFNT) and School of Biology, Georg-August-Universität Göttingen, Germany

[3]Leibniz-Institut für Neurobiologie, Magdeburg, Germany

[4]Santa Lucia Foundation IRCCS Hospital, Department F and Laboratory of Neuroanatomy, Santa Lucia Foundation IRCCS at the European Center for Brain Research, Rome, Italy

In C57Bl/6 mice, monocular deprivation (MD) induces a shift in the ocular dominance (OD) of binocular neurons towards the open eye in the visual cortex of juvenile (4 days MD at postnatal day (PND) 25) and young adult mice (7 days MD at PND 80-100), called OD-plasticity. Since Bassoon-/- (Bsn-/-) mice have a modified excitation-inhibition balance and inhibition plays a major role in this form of plasticity, we studied whether Bsn-/- mice display modified OD-plasticity and/or sensory learning. We i) visualized cortical activity maps in V1 of both Bsn+/+ and Bsn-/- mice after 4 (juvenile) respectively 7 days of MD (young adult animals) using optical imaging of intrinsic signals, and ii) measured visual acuity of the open eye in a virtual-reality optomotor system. While there was a significant change in the OD-index after MD in juvenile Bsn-/- mice, OD-plasticity was absent in young adults in contrast to their wild-type littermates (t-test, $p<0.01$). In the same juvenile Bsn-/- mice, visual acuity increased significantly less compared to Bsn+/+ mice (increase on baseline 10% vs. 20%, t-test, $p<0.001$) after MD. In young adult Bsn-/- mice, sensory improvement was again significantly reduced compared to Bsn+/+ littermates (15% vs. 30%, t-test, $p<0.01$).

Since it was recently shown that young adult Bsn -/- mice display an increased number of parvalbuminergic interneurons in the striatum[1] and increased inhibition could be an explanation for the absent OD-plasticity of our young adult Bsn -/- mice, we performed immunohistochemistry for parvalbumin in V1. Preliminary data indicate that the number of parvalbuminergic interneurons was indeed increased in V1 of Bsn-/- mice, especially within supragranular layers.

Taken together, these data indicate that there is a loss of both OD-plasticity and sensory learning after MD in young adult Bsn-/- mice. Whether the reduced plasticity is due to an increasing frequency of epileptiform seizures as previously shown for the striatum[1] or to an increased number of inhibitory parvalbuminergic neurons is the subject of ongoing experiments.

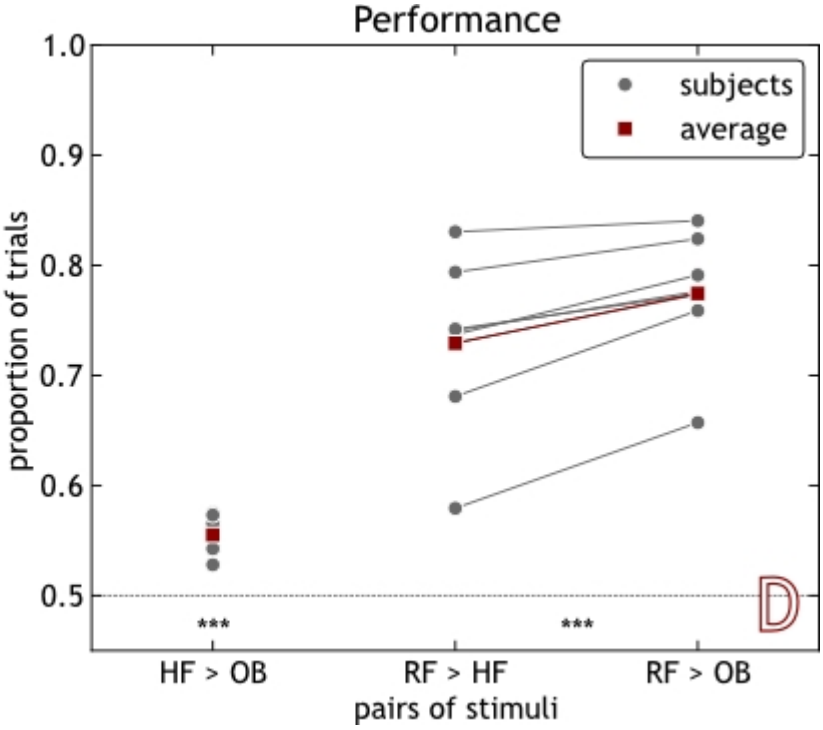
[1]Ghiglieri et al. (2009) EJN 29:1979

# Similar errors in human and computational face-detection

Tilman Gerrit Jakob Abresch[1], Bernard Marius 't Hart[1], Wolfgang Einhäuser[1]

[1]Philipps-University Marburg, Neurophysics, Karl-von-Frisch-Str. 8a, 35032 Marburg, Germany

Faces appear to be processed by the human visual system as a special class of stimuli. Augmenting an attention model with the Viola and Jones (2001) face-detection algorithm to improve its prediction of fixated locations has recently supported this view. To what extent faces constitute an 'elementary' feature of early processing has, however, remained controversial. Here we compare the Viola-Jones algorithm's output to human errors in ultra-rapid face detection. Stimuli were gathered from first person perspective movies and split into three sets: real faces (hits of the algorithm, fig 1a), 'hallucinated' faces (false positives, fig 1b), and non-faces (correct rejections, fig1c). Observers were shown two stimuli, each from a different category, for 20 ms, followed by a mask, and were instructed to make a saccade to the face as quickly as possible. When non-faces were paired with hallucinated faces, observers had a significant bias to choose the hallucinated face (fig 1d). In pairings with real faces, hallucinated faces were erroneously chosen significantly more often than non-faces (i.e., hallucinated faces were more effective distractors). In a second experiment we presented pairs consisting of a real face and a hallucinated face. We tested the effect of orientation of features by rotating either stimulus by 90° counterclockwise, and we tested the effect of color by having each subject perform the task both with colored and black and white stimuli. Observers' performance decreased for rotated real faces, whereas it increased for rotated hallucinated faces. Performance was better for colored than for black and white stimuli in all conditions. While features that are invariant to spatial transformations - such as color - clearly may improve face detection performance, our data show that human rapid face detection recruits features comparable to the Viola-Jones algorithm: localized, oriented luminance transitions.

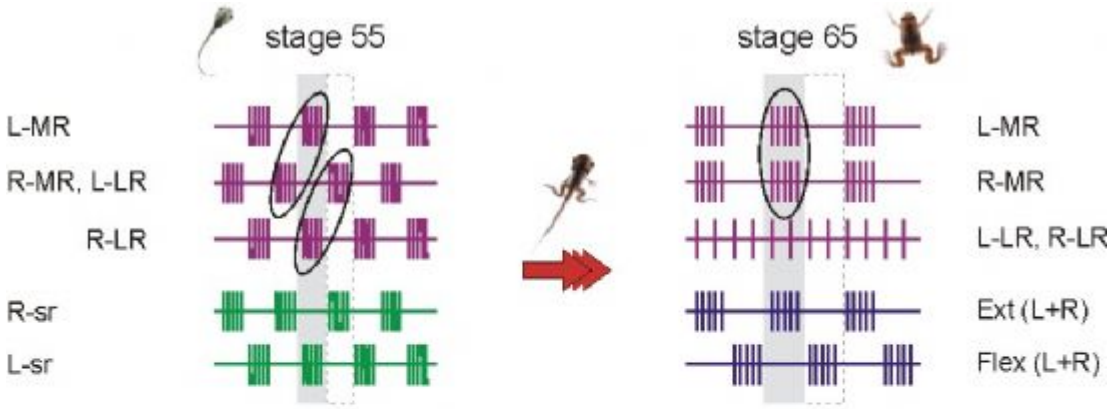
# Spinal efference copy signaling and gaze stabilization during locomotion in *Xenopus* frogs: developmental plasticity of spino-extraocular motor coupling during metamorphosis.

Géraldine von Uckermann[1], François Lambert[2], Didier Le Ray[1], Hans Straka[3], John Simmers[1], Denis Combes[1]

[1]Université de Bordeaux, CNRS UMR 5227, Laboratoire Mouvement-Adaptation-Cognition, 146, rue Léo Saignat, 33076 Bordeaux, France
[2]Université Paris Descartes, CNRS UMR 7060, 75006 Paris, France
[3]LMU Munich, Faculty of Biology, 82152 Planegg, Germany

Retinal image stabilization is critical to all vertebrates, whether running, swimming or flying, as they are confronted with the disruptive effects of their locomotory actions on the ability to perceive the surrounding environment. Consequences of self-generated body motion are head movements that cause retinal image drift with a resultant degradation of visual information processing. In order to maintain visual field acuity, retinal image displacement is counteracted by dynamic compensatory eye and/or head adjustments that are classically attributed to the concerted actions of vestibulo-ocular and optokinetic reflexes. We have recently discovered (Combes et al. 2008, Curr Biol 18:R241) that intrinsic copies of rhythmic locomotor commands produced by central pattern-generating (CPG) circuitry in the spinal cord of larval *Xenopus* are able to drive extraocular motor output patterns appropriate for image-stabilizing eye movements during undulatory tail-based swimming. Such CPG efference copy signaling to the brainstem extraocular nuclei provides an intrinsic predictive mechanism for driving appropriate dynamic eye adjustments during locomotion, pre-empting the indirect engagement of movement-encoding sensory pathways.

During metamorphosis, *Xenopus* switches its mode of locomotion from larval tail-based undulatory swimming to bilaterally-synchronous hindlimb kick propulsion in the adult, traversing intermediate stages where functional larval and adult locomotory systems co-exist within the same animal. The change in locomotory mode leads to body motion dynamics in young adult frogs, which require non-conjugate eye motion patterns for adequate retinal image stabilization. We are investigating spino-extraocular motor coupling during this developmental transition in order to understand how and when gaze control processes are altered in accordance with the animal's change in body plan and movement strategy. Our experiments were performed on semi-isolated brainstem/spinal cord preparations of decerebrated *Xenopus laevis* (stage 55 to 66). Simultaneous recordings of extraocular motor nerves, spinal ventral roots and/or limb motor nerve branches (innervating the *tibialis* and *gastrocnemius* muscles) were made during episodes of spontaneous "fictive" swimming activity. Our results show that there is indeed a developmental transition in spinal efference copy control of extraocular motor output during *Xenopus* metamorphosis that corresponds to the change in locomotor strategy. In contrast to fictive axial swimming, which drives alternating bursts in bilateral *medial rectus* (MR) motor nerves in a pattern that *in vivo* is compatible with the production of oppositely-directed horizontal conjugate eye movements, during fictive limb extensions, the MR nerves are synchronously active in correspondence with a need for convergent eye movements during the linear head accelerations produced by forward propulsion. At intervening metamorphic stages, when functional larval and adult locomotory systems co-exist within the same animal, spino-extraocular motor coupling can be mediated by both axial and hindlimb CPG systems, although the latter's influence becomes progressively dominant as metamorphosis progresses. Thus, neural plasticity during metamorphosis allows spinal CPG-derived extraocular motor activity to remain functionally adapted to the respective spatial and dynamic requirements for compensatory eye motion in the two distinct locomotory strategies.




Patterns of spino-extraocular motor output coupling before and after metamorphosis. Left side: Pre-metamorphic stage 55 tadpole. Bilaterally-alternating bursting activity in left (L-) and right (R-) *medial rectus* (MR) and *lateral rectus* (LR) motor nerves are coordinated with alternating bursts in left and right spinal ventral roots (sr) during "fictive" locomotion that ***in vivo*** drives undulatory tail-based swimming. Right side: Post-metamorphic stage 65 froglet. During fictive limb extensions that ***in vivo*** drive bilaterally-synchronous hindlimb kicking, both MR nerves are active in synchrony, in correspondence with a need for convergent eye movements during forward linear head accelerations.

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# Stimulus strength modulates the synaptic correlation between individual neurons and population activity in macaque area MT

Laura Busse[1,2], Mohammad Reza Daliri[2,3,4,5], Steffen Katzner[1,2], Stefan Treue[2,3]

[1]University Tübingen, Centre for Integrative Neuroscience, Tübingen, Germany
[2]German Primate Center, Cognitive Neuroscience Laboratory, Göttingen, Germany
[3]Bernstein Center for Computational Neuroscience, Göttingen, Germany
[4]Biomedical Engineering Dept., Iran University of Science and Technology, Tehran, Iran
[5]School of Cognitive Science, Institute for Research in Fundamental Science, Tehran, Iran

It has previously been shown that stimulus contrast affects the degree to which local neuronal populations in visual cortex are synchronously active: as assessed by the amplitude of the spike-triggered local field potential (LFP), increasing contrast decreases correlations (Nauhaus et al., 2009). Is this effect due to stimulus contrast only or does it depend, in a more general way, on stimulus strength?
We used a multi-electrode system and recorded single-unit activity and LFPs from area MT of three awake, behaving monkeys while presenting stimuli varying in strength. We compared neuronal activity during the absence of a visual stimulus inside the receptive field, and during the presentation of a null- or preferred- direction random dot pattern (RDP). These conditions provided no drive, weak drive, and strong stimulus drive, respectively.
We assessed correlations of the local neuronal population by computing the spike-triggered LFP and found that its amplitude decreased with increasing stimulus strength. In all three stimulus conditions the spike-triggered LFP consisted of a negative-going deflection peaking at 5 ms after spike occurrence. This peak negativity was largest for the condition without sensory stimulus (0.90 ± 0.04 s.e., z-score), intermediate for the condition with the null-direction (0.64 ± 0.03), and smallest for the preferred-direction stimulus (0.39 ± 0.02).
The effect of stimulus drive on local neuronal correlations cannot simply be explained by differences in the number of spikes serving as triggers for averaging LFPs. Reducing the number of trigger spikes by random removal of half of the spikes in the spike train did not influence the amplitude of the spike-triggered LFPs. Therefore, independent of the absolute number of trigger spikes, local neuronal correlations are stronger in stimulus conditions with low firing rates and weaker in conditions with high firing rates.
We also found that local correlations fall off over a distance of ~300 m. Using spikes from one electrode as trigger points, we determined the amplitude of the averaged LFP from the same and up to four neighboring electrodes. The amplitude of the spike-triggered LFP was largest when the LFP was recorded from the same electrode as the trigger-spikes, and was already significantly reduced for LFPs recorded at the inter-electrode distance of 300 m.
We conclude that local neuronal correlations decrease with increasing stimulus strength. Contrast seems to be only one stimulus feature that influences synchronous activity in a local neuronal neighborhood. Beyond contrast, other stimulus features modulating firing rates can also affect correlations of neuronal populations.

The project was supported by a grant (01GQ0433) of the German Federal Ministry of Education and Research to the Bernstein Center for Computational Neuroscience, Goettingen.

# Surround suppression of across-trial variability in macaque LIP

B Suresh Krishna[1], Annegret L Falkner[2], Michael E Goldberg[3]

[1]Bernstein Center for Computational Neuroscience and German Primate Center, Cognitive Neuroscience Laboratory, 4 Kellnerweg, 37077 Goettingen, Germany

[2]Mahoney Center for Brain and Behavior, Department of Neuroscience, Columbia University College of Physicians and Surgeons, New York, New York 10032, USA.

[3]Mahoney Center for Brain and Behavior, Department of Neuroscience, Columbia University College of Physicians and Surgeons, and New York State Psychiatric Institute, New York, New York 10032, USA.

We have recently shown (Falkner, Krishna and Goldberg, 2010) that the receptive fields (RFs) of most macaque LIP neurons possess extensive suppressive surrounds. Neuronal responses to a distractor flashed at the RF center during the delay period of a delayed visually-guided saccade task are substantially reduced when the saccade target lies outside the RF. By systematically varying the saccade target location, we showed that surround suppression is spatially tuned in individual neurons and extends to locations up to 30 degrees away from the RF center. Here, we show that saccades to the surround also induce systematic and strong variations in the across-trial variability of LIP neurons during the pre-distractor epoch, as quantified by the Fano factor. For saccade targets outside the RF, lower neuronal firing-rates are accompanied by lower Fano factors. This relationship between mean firing-rate and Fano factor becomes stronger as the distance of the saccade target from the RF center increases. Thus, the reduction of firing-rate by surround suppression in LIP is accompanied by reduced across-trial variability. Importantly, the relationship between firing-rate and Fano factor that we observe cannot be mediated by the known effects of firing-rate on the Fano factor via the refractory period, because that mechanism impacts the Fano factor in the opposite direction, producing Fano factor *increases* when the firing-rate becomes smaller. The effect of surround stimulation on variability can potentially further improve the precision of the LIP priority map, in addition to the improvement induced by the suppressive effects on mean firing-rate (Paradiso 1988). Additionally, increasing the monkey's motivation (via a higher reward expectation) also further reduces across-trial variability, in addition to its effect of enhancing the impact of surround suppression on mean firing-rate (Falkner, Krishna and Goldberg, 2010). These results may have strong implications for our understanding of visual representation and selection processes in the attentional and oculomotor priority map network.

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# The representation of visual space in macaque areas V1 and V4 during saccade adaptation

Steffen Klingenhoefer[1], Markus Wittenberg[1], Thomas Wachtler[1,2], Frank Bremmer[1]

[1]Philipps-University Marburg, Neurophysics, Karl-von-Frisch-Straße 8a, 35043 Marburg, Germany
[2]Ludwig-Maximilians-Universität München, Deptartment Biology, Großhaderner Straße 2, 82152 Martinsried, Germany

The ability to produce accurate fast eye movements (saccades) is essential for humans and non human primates due to the foveal structure of their retinas. The saccadic system is capable of rapidly adapting to changes in the oculo-motor system (e.g. changes in the mechanics of the eyeball) that otherwise would lead to movement inaccuracy and poor vision - an effect usually referred to as saccade adaptation. In the laboratory this situation is typically emulated by a consistent displacement of the saccade target while the eyes of the subject are in flight. In this paradigm subjects initially correct for the imposed targeting errors by means of corrective saccades; but then, the amplitude of the first targeting saccade is gradually adjusted in order to bring the eyes immediately to the position of the displaced target.

Saccade adaptation challenges the mechanisms that guarantee visual perceptual stability across eye-movements – the neural basis of which is currently unknown. In the saccade adaptation paradigm, a dissociation is introduced between a driving visual signal (the original saccade target) and a motor output (the adapted saccade vector). We hypothesized that perceptual stability during saccade adaptation would require a modulation of the visual RFs with respect to the fovea to compensate for the mismatch between sensory and motor signals. In our current study we therefore mapped visual receptive fields (RFs) in areas V1 and V4 of the macaque monkey during a backward saccade adaptation task.

Preliminary data suggest that, in both areas RF were different during saccade adaptation trials as compared to fixation or non-adapted saccade trials. In adaptation trials, RF locations calculated from stimuli presented immediately after saccade offset were shifted in the direction of the saccade (i.e. opposite to the direction of the target displacement) compared to control trials. This displacement was larger for RFs mapped in V4 than in V1. In both areas, however, the magnitude of RF shift decreased slightly during the course of adaptation.

We conclude that eye movement signals do have an influence on spatial information processing in areas V1 and V4 - a mechanism probably involved in the maintenance of perceptual stability across saccadic eye movements.

# The time scales of neural coding in auditory and visual cortices of the primate

Christoph Kayser[1], Alberto Mazzoni[1], Nikos K Logothetis[1], Panzeri Stefano[1]

[1]Max Planck Institute for Biological Cybernetics, Physiology of Cognitive Processes, Spemanntrasse 38, 72076 Tuebingen, Germany

How neurons in sensory cortices represent the environment still remains a matter of investigation. With regard to individual neurons, it is of particular interest on what time scales their responses carry information, and hence what are the time scales that define the neural code. Given that the responses of sensory neurons are temporally modulated by the environment, and given that the sensory environment can change on different time scales, however, it might well be that there is no unique time scale of neural coding in a particular sensory area. Analyzing data recorded in primate auditory and visual cortices, we show that this is indeed the case.

Specifically, we recorded the responses of neurons in primary auditory cortex of alert macaque monkeys listening passively to complex and natural sounds. In addition, we analyzed the activity of neurons recorded in primary visual cortex of anesthetized macaques during the presentation of naturalistic movies. For each neuron and stimulus set, we quantified the amount of stimulus information carried by firing rates at different temporal scales (bins) and systematically compared the information derived from progressively longer time bins. From this we determined the shortest time scale (i.e. precision of neural code) that still provided all stimulus information.

We found that the critical time scale of neural coding depends on the temporal stimulus dynamics. In auditory cortex, we compared responses to sequences of random tones (stimulus auto-correlation scale of below 10ms) to responses to a sequence of natural sounds (auto-correlation of more than 30ms). The temporal precision required to obtain maximal information was shorter for the tone sequence (median 5ms) than for the natural sounds (median 12ms). In visual cortex, we compared responses to natural movie epochs containing fast temporal modulation to responses to epochs of slower modulation (the temporal auto-correlation of the latter being about 2-3 times longer). We found that the time scale of optimal coding was shorter for the faster movie epochs (about 12ms) than for the slower epochs (about 20ms).These results demonstrate that the time scale of neural coding can only be specified in the context of a particular sensory environment.

# Vision and visual plasticity in BALB/c mice

Konrad Lehmann[1], Naira Yeritsyan[2], Oliver Puk[3], Jochen Graw[3], Siegrid Löwel[1,4]

[1]Friedrich Schiller-Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstr. 1, 07743 Jena, Germany

[2]Leibniz-Institut für Neurobiologie, Brenneckestr. 6, 39118 Magdeburg, Germany

[3]HelmholtzZentrum München, German Research Center for Environmental Health, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany

[4]present address: Georg-August-Universität Göttingen, Bernstein Focus for Neurotechnology and School of Biology, von Siebold-Str. 4, 37075 Göttingen, Germany

The albino mouse strain BALB/c is being increasingly used as an animal model of neuropsychological disorders owing to their specific behavioural characteristics such as increased anxiety level, reduced exploratory activity, neophobia and low social approach. Assessments of these characteristics can – however - be confounded by poor vision, one of the key syndromes of oculocutenous albinism in all mammalian species. Nevertheless, the visual abilities of BALB/c mice have not yet been characterized sufficiently. We therefore analyzed visual performance and visual cortical activity in 3-month-old BALB/c mice using a combination of behavioural analyses and optical imaging of intrinsic signals.

Visual acuity in BALB/c mice was 0.12 cyc/deg as measured by a virtual-reality optomotor system, and 0.3 cyc/deg in a forced-choice visual discrimination task (visual water task=VWT). Most notably, BALB/c mice showed reflexive head movements against the direction of the rotating stimulus in the optomotor system. Contrast sensitivity peaked at 2.2 (=45% contrast) at the optimal spatial frequency of 0.064 cyc/deg. In contrast, pigmented C57BL/6 mice reached a visual acuity of 0.39 cyc/deg in the optomotor setup, 0.59 cyc/deg in the VWT, and a contrast sensitivity of 17.5 (=5.6% contrast). Thus, visual performance of BALB/c mice was significantly weaker both in the virtual-reality optomotor system (visual acuity: $p<0.001$, t-test, contrast sensitivity: $p<0.001$, ANOVA with repeated measurements) and in the VWT ($p<0.001$, t-test) than in C57BL/6 mice.

Visual cortical retinotopic maps induced by stimulation of the dominant contralateral eye appeared normal: both cortical activation strength and retinotopic map quality were indistinguishable between the strains (activation: elevation $p=0.61$, azimuth $p=0.996$; quality: elevation $p=0.74$, azimuth $p=0.12$, t-test). In contrast, visual stimulation of the ipsilateral eye induced activity maps that differed significantly in shape from those in C57BL/6: The relative activity of a region representing between 3° and 19° degrees elevation was weaker than in the pigmented strain, with a significant difference from 15° to 19° ($p<0.05$, Bonferroni-corrected t-test). As expected for albinotic animals with their reduced number of uncrossed optic nerve fibers, the average ocular dominance index (ODI) was 0.36 and thus significantly higher than in C57BL/6 mice (0.23, $p<0.01$, t-test). We therefore wondered whether monocular deprivation (MD) of the dominant contralateral eye could nevertheless induce a shift of the ocular dominance towards the weak ipsilateral eye. This was the case: the average ODI was significantly reduced to 0.07 after 7 days of MD ($p<0.001$, Bonferroni-corrected t-test), predominantly due to a reduction of contralateral eye responses, and thus resembling the mechanism of OD-plasticity characteristic for critical period C57Bl/6 mice.

Our results show that BALB/c mice, like the albinotic animals of other species, have reduced visual acuity, a disturbance in the horizontal nystagmus, and a weak and modified cortical representation of the ipsilateral eye that may impair stereopsis. Thus, the results caution against disregarding vision as a confounding factor in behavioural tests. They are furthermore in line with results that suggest that the visual system of BALB/c mice retains an immature state.

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T17-11C Hyperacute directional hearing and phonotactic steering in the Mediterranean field cricket
*Berthold Hedwig, Stefan Schoeneich*

# A synchrony population-code for communication signals in weakly-electric fish depends on social context

Henriette Walz[1], Jan Grewe[1], Jan Benda[1]

[1]Ludwig-Maximilians-Universität München, Department Biologie II, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

Weakly electric fish use their electric organ discharge (EOD) for electrolocation as well as for communication. When two fish interact, both their EODs superimpose to a beat, an amplitude modulation of their frequency difference. The EOD frequency of each fish depends on its gender, size and social status. Hence, different beats reflect different social encounters. Communication signals, so-called chirps, cause an amplitude, frequency, and phase modulation of the beat. Both, beats and chirps, are encoded in electroreceptors (p-units).

In *Apteronotus leptorhynchus*, the p-unit population response depends strongly on the frequency of the beat. At intermediate beat frequencies (~60Hz), the population is synchronized while it is desynchronized at slow and fast beats. In agonistic contexts, fish emit type II chirps, small frequency excursions of their EOD of a short duration. They are emitted mostly at low beat frequencies up to 30Hz, where they are known to be encoded by a strong synchronization of the p-unit population. Until now, it was unknown, how the receptors respond to chirps at other beat frequencies that are also behaviorally relevant. For example, it has been shown recently that behavioral responses to type II chirps increase with beat frequency. Also, chirps cause different modulations of the beat depending on the sign of the difference between the EOD frequencies of two communicating fish. Our single-cell recordings of p-units demonstrate firstly, that small chirps desynchronize the population at high beats. Secondly, the effects of chirps on the p-unit response are reversed if the fish carrying the lower EOD frequency chirps: while we see a synchronization for large beat frequencies (>80Hz), lower beat frequencies desynchronize the receptor population.

The effects of chirps on the synchronization of electroreceptors at all beat frequencies can be explained by the synchronization properties of the population response at various stimulus frequencies. Chirps briefly change this frequency and thus transiently move the population into a different response regime. With a simple integrate-and-fire neuron model we are able to reproduce our data. By means of the model we explore how the synchronization properties of the receptor population are influenced by spike-frequency adaptation, intrinsic noise, and heterogeneity in their baseline firing-rates.

# Age-related hearing loss (ARHL) in *Drosophila melanogaster*

David Piepenbrock[1], Martin Göpfert[1]

[1]Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Georg-August-Universität Göttingen, Department for Cellular Neurobiology, Herman-Rein-Straße 3, 37075 Göttingen, Germany

Hearing impairment is the most common sensory deficit in humans. Age-related hearing loss (ARHL) is influenced by environmental and genetic factors. So far, 17 genes have been associated with ARHL, and it seems likely that many more genes are involved in ARHL. In the interest of identifying genes that are involved in ARHL, we tested whether *Drosophila melanogaster* can be used for dissecting ARHL. *Drosophila* uses antennal hearing organs for courtship song detection. By analyzing mechanical and electrical sound-responses, we discovered that the function of these hearing organs deteriorates with age: After 2 weeks, auditory sensitivity is reduced by ca. 30% in wild-type flies when compared to one-day old controls. First genes that affect the time course of this deterioration have been identified. Our results show that ARHL occurs in short-lived animals like *Drosophila*, making it possible to exploit the power of fly genetics to dissect fundamental aspects of ASHL.

# Auditory mechanics of bushcrickets in-vivo

Arun Palghat Udayashankar[1], Manfred Kössl[1], Manuela Nowotny[1]

[1]Goethe University , Institute for Cell and Neurobiology, Siesmayerstr. 70A, 60311 Frankfurt am Main, Germany

The vertebrate cochlea has been and continues to be the classic model system used to study mechano-electrical transduction (MET) in auditory systems. However there are several questions that have been difficult to address using the vertebrate cochlea. These include the biophysical origins of cochlear amplification and acoustic emissions among others. By employing what is commonly referred to as the reductionist approach in biology we used bush-crickets as model organisms to understand MET at the organ level. The hearing organ in bush-crickets is located in their forelegs and is known as the crista-acustica(CA). A characteristic feature of the hearing organ is that it is lined with an array of auditory receptors in a tonotopic fashion with lower frequencies(6 kHz) processed at the proximal part and higher frequencies (60 kHz) at the distal part. The receptors, graded in size, are directly involved in the mechanics of transduction along with the basilar membrane (BM) like acoustic trachea that supports the receptor cells. Each receptor is associated with a sensory neuron that is thought to convey information to the auditory ganglion and higher processing centers. Functional similarities between the CA and the vertebrate cochlea such as frequency selectivity and distortion product otoacoustic emissions have been well documented.

In this study we probe the mechanics of the CA using laser Doppler vibrometry (LDV). We observed traveling waves that were tonotopically ordered along the length of the CA. Distinct amplitude peaks were observed that could be clearly localized at the position of the graded receptors of the auditory organ. The tonotopic representation was exponential with the traveling waves propagating from the high frequency (distal) to the lower frequency (proximal) area similar to the situation in the vertebrate cochlea. Vibration of the organ reached detectable levels (>1μm/s) only at 30 dB SPL. This corresponds to a sensitivity 20 dB lower than that of the gerbil BM. Velocity magnitude increased more than seven fold (>17 dB) for an 80 dB increase in SPL. This indicates a smaller non-linear compression as opposed to a 45 dB increase in the gerbil BM velocity for the same change in SPL. Our results indicate that bushcrickets can be a good model system for experimental studies of auditory mechanics in-vivo.

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# Cercal Wind-Sensing System of Crickets: Investigating the Sensory Neurons.

Andreas N. Vollmayr[1], Jan-Moritz P. Franosch[1], J. Leo van Hemmen[1], Michael Gebhardt[2]

[1]Technische Universität München, Physik Department, T35, James-Franck-Str., 85748 Garching, Germany
[2]Technische Universität München, Lehrstuhl für Zoologie, Hochfeldweg 2, 85350 Freising-Weihenstephan, Germany

Crickets, like other insect taxa, possess a pair of posterior sense organs, the cerci, which bear sensory hairs for the detection of air flow. These so-called filiform hairs consist of a long thin shaft viscously coupled to the motion of the surrounding air. Hair deflections are detected by a mechanosensory neuron, which relays the resulting spike trains to the terminal ganglion of the central nervous system. It has been demonstrated in behavioral studies that the wind-elicited escape response of crickets can be fast and reliable. Special attention has been paid to the neural mechanism of the evaluation of stimulus direction by the ascending first-order interneurons, which are believed to control the escape response. Some basic questions, however, still remain open. What features of a stimulus can be resolved and at which accuracy? To derive hypotheses for the function and capabilities of the system it is necessary to know the performance of a single sensor. We have therefore designed a setup to record the afferent responses of a filiform-hair sensory neuron of Gryllus bimaculatus. The setup consisted of a moving box producing the air flow stimulus with the cricket placed inside. We have measured both the filiform-hair activity by means of an extracellular electrode and the hair motion in 3D simultaneously through two high-speed cameras. This has allowed us to correlate air motion, hair motion, and sensor activity. First analyses show that inter-spike intervals were distributed differently in different sensory neurons. Furthermore, inter-spike intervals appear to be a rather complex function of stimulus velocity and acceleration as well as spike history, i.e., preceding spike intervals.

# Cubic and quadratic distortion-product otoacoustic emissions (DPOAE) in awake and anesthetized short-tailed fruit bats.

Désirée Schlenther[1], Cornelia Voß[1], Manfred Kössl[1]

[1]Institute of Cell Biology and Neuroscience, Goethe University, Frankfurt 60323, Germany

Sensitive hearing organs emit otoacoustic emissions (OAEs), sound signals which are considered by-products of active and nonlinear amplification processes in the inner ear. They arise either in the absence (Spontaneous OAEs) or in the presence of external acoustical stimulation (e.g. Distortion -product OAEs). DPOAEs are evoked by simultaneous stimulation of the ear with two pure tones (f1 < f2) and are measured in the outer ear channel with a sensitive microphone. Two types of DPOAEs are distinguishable, the cubic and the quadratic distortion tones. The cubic distortion tone (CDT), e.g. 2f1 -f2, depends on the slope of the transfer function of cochlear amplification. The quadratic distortion tone (QDT), e.g. f2-f1, reflects shifts of the transfer function.

CDT and the QDT were recorded in 15 awake and 15 anesthetized *Carollia perspicillata* within a stimulus frequency range of 15-90 kHz. For both the CDT and QDT, DPOAE growth functions, obtained by increasing the level of the two stimuli, have a discontinuity or notch in the stimulus level range between 35 and 50 dB SPL, which is a typical feature for vertebrates. Anaesthesia leads to a reduction of the CDT level at low to medium stimulus levels. At higher stimulation levels, the CDTs are not affected by anaesthesia, whereas the QDT levels reach higher values than in awake bats. Such high QDT levels could indicate that under anaesthesia hair cell amplification acts more asymmetrically.

Using the DPOAE growth functions, the -10 dB SPL threshold was determined for each tested frequency by calculating that L2 level which was sufficient to evoke a -10 dB SPL distortion level. The CDT threshold curve obtained in awake animals lies between 8 to 20 dB SPL for the entire frequency range from 15-90 kHz. In the anaesthetised condition, the threshold becomes more insensitive by 6-14 dB for frequencies above 55 kHz. The QDT threshold curve of awake bats has a distinct minimum of ~7 dB SPL at 50 kHz. Under anaesthesia, the most sensitive range of the threshold curve shifts down to 40 kHz.

Both CDT and QDT were influenced, but affected differently and frequency-specific by the use of anaesthesia. The here shown effects on the distortion tones are consistent with the assumption that anaesthesia causes a shift of the operation point of the underlying transfer function of the cochlear amplifier.

# Differential Expression and Localization of Glycine Transporters GlyT1 and GlyT2 in the murine cochlea

Stefanie Buerbank[1], Julia Dlugaiczyk[2], Bernhard Schick[2], Holger Schulze[1]

[1]University Hospital Erlangen, Dept. for Otorhinolaryngology, Head and Neck Surgery/Experimetal Otolaryngology, Waldstr. 1, 91054 Erlangen, Germany

[2]University Hospital Homburg/Saar, Dept. for Otorhinolaryngology, Head and Neck Surgery, Kirrberger Str., 66421 Homburg, Germany

## Introduction

Olivocochlear efferences originate from the lateral and medial olive and contact acoustic nerve fibres as well as outer hair cells. They are known to participate in fine-tuning of auditory nerve activity, perceiving sound in background noise and protecting the cochlea against acoustic trauma.
We recently described and functionally characterized glycine receptors (GlyRs) as candidate targets of efferent innervation in the murine inner ear.
In addition to GlyRs but also glycine transporters (GlyT) are necessary for balanced glycinergic transmission. So far two isoforms (GlyT1 and 2) have been described in the mouse model. There GlyT1 as well as GlyT2 proteins can be subdivided into the splice variants a-c. Whereas GlyT2, predominantly expressed in neurons, is responsible for re-uptake of transmitter into glycinergic synapses, GlyT1 participates in the clearing of glycine from the synaptic cleft. Thus GlyTs are influencing glycinergic transmission in opposing ways and may therefore play a role in regulating glycinergic signalling in the cochlea. Moreover, GlyT1 has also been found at glutamatergic synapses. There it is supposed to modulate NMDA receptor activity by regulating local concentration of the receptor co-agonist glycine.
Here we characterize the expression of GlyT isoforms and splice variants in the murine cochlea during development and give first insights into their localization during development and in the adult animal.

## Material and Methods

Cochleae were removed from C57/Bl6 mice at different stages of development (P0-P22) and subjected to fixation, cryo-embedding, and slicing (9µm). Immunofluorescence staining served to visualize GlyT as well as glial and neural proteins.
Additionally, RNA was isolated and cDNA was synthesized by reverse transcription. RT-PCR and quantitative RT-PCR (qPCR) using standard dilution series were used to characterize GlyT isoforms and splice variants on mRNA level.

## Results

For the first time we were able to identify GlyT1 and GlyT2 mRNA in the murine inner ear by RT-PCR using specific primers and sequencing of PCR products. In particular we found several isoforms and splice variants of GlyT mRNA which are expressed differently during development.
According to changes in structure and localization of efferent synapses, immunofluorescent signals of GlyT2 shift during maturation of the murine cochlea. Co-staining of GlyT2 with Synaptotagmin, a marker of efferent synapses strongly suggests GlyT2 to be located at efferent (possibly glycinergic as suggested by GlyR co-staining) synapses in the organ of corti.
GlyT1 partly co-localizes with vimentin and other glial markers, which corroborates the theory that GlyT1 is predominantly expressed in glial cells.

## Conclusions

During the last few years selective blocking of GlyTs became a promising target for treating neuropsychiatric disorders related to glycinergic and glutamatergic signalling. Thus our results provide important contribution to the understanding of glycinergic transmission in the murine cochlea.

Therefore our ongoing studies on expression and function of GlyT in the cochlear coupled with pharmacological intervention might lead to new therapeutic approaches to inner ear derived hearing impairments.

This work was supported by the Interdisciplinary Center for Clinical Research (IZKF) at the University Hospital of the University of Erlangen-Nuremberg, Germany

# Does the auditory nerve activity reflect the tympanal membrane motion in bushcrickets?

Jennifer Hummel[1], Manfred Kössl[1], Manuela Nowotny[1]

[1]Goethe Universität Frankfurt, Institute for Cell Biology and Neuroscience, Siesmayerstr. 70A, 60323 Frankfurt, Germany

The hearing organs of bushcrickets are located inside the tibia of the forelegs. In contrast to mammals, the two eardrums (tympanal membranes) of the bushcrickets are situated on each side of the foreleg and are not the main entrance for sound input. The main sound entrance is a spiracle opening at the thorax. From this opening the sound proceeds through the auditory trachea inside the foreleg and induces a motion in the hearing organ. Therefore, questions about the role of the tympana in the transmission process arise. In contrast to other insects, like Locusts and noctoid moths, the sensory cells are not directly attached to the tympana, but are located within a hemolymph filled channel. The tympana border parts of this hemolymph channel as well as a part of the air filled auditory trachea. It is assumed that outward movement of the tympana during sound transmission leads to (i) a stretching of the acoustic trachea, which has the hearing organ on top of it and (ii) a pressure changes that propagate into the hemolymph channel containing the hearing organ. By these two properties the specific activity of the auditory receptor cells could be influenced by the movement pattern of the tympanal membranes.
In the present study we examined the correlation of auditory nerve activity and tympanal vibration pattern in the tropical bushcricket *Mecopoda elongata* using a combination of electrophysiological recordings and Laser-Doppler-Vibrometer measure¬ments. For far field stimulation (4-78 kHz), comparable with natural hearing situation of bushcrickets, the tympanal membrane motion exhibits two maxima at about 7 kHz and 16 kHz. The 16 kHz maximum matches the frequency of most sensitive hearing obtained from electrophysiological recordings. A correlation of the second maximum of the tympanum displacement at lower frequencies (~7 kHz) with enhanced hearing sensitivity was not found on lower SPLs. Manipulation of the sound entry (closing or separate stimulation) produces different and sound-frequency dependent effects on tympanal vibration and auditory nerve response. Consequently, the tympanal membrane motion cannot be used as a detector of the auditory nerve activity when the acoustic characteristics are altered. Nevertheless, under far field stimulation, comparable with natural hearing situation of bushcrickets, the tympanum vibration could be used as a non-invasive method for hearing analysis in *M. elongata*, especially the most sensitive hearing range.

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# Effect of temperature on auditory receptors, local and ascending interneurons in the locust

Monika J. B. Eberhard[1], Frederic A. Roemschied[2,3], Bernhard Ronacher[1,3], Susanne Schreiber[2,3]

[1]Humboldt-Universität zu Berlin, Department of Biology, Behavioural Physiology Group, Invalidenstr. 43, 10115 Berlin, Germany

[2]Humboldt-Universität zu Berlin, Institute for Theoretical Biology, Computational Neurophysiology Group, Invalidenstr. 43, 10115 Berlin, Germany

[3]Bernstein Center for Computational Neuroscience Berlin, Humboldt-Universität zu Berlin, Philippstr. 13, 10115 Berlin, Germany

In ectothermic animals, performance of muscles or nerve cells depends strongly on temperature. Temperature shifts influence basic properties of nerve cells such as spike rate, conduction velocity, and spike amplitude (Burrows 1989, Franz & Ronacher 2002). Changing temperatures particularly affect acoustic communication systems where decoding of temporal characteristics of conspecific calls is required for species and mate recognition. In grasshoppers, processing of auditory input starts within the metathoracic ganglion, where the first stages of song pattern recognition and analysis of sound direction are accomplished. Altogether, three processing levels are located here, namely receptor neurons, local (segmental) interneurons, and ascending interneurons, which constitute a hierarchically organized feedforward network (Vogel & Ronacher 2007). Intracellular recordings of locust auditory receptors and interneurons at different temperatures revealed an improvement of temporal resolution at higher temperatures due to a higher precision of spike timing (Franz & Ronacher 2002). However, neurons of the three processing levels were not equally affected by changing temperatures, suggesting different temperature dependence.

To further investigate this, the responses of locust auditory receptors, local and ascending interneurons were analyzed in terms of their reaction to two different temperatures (30° and 20°). Intracellular recordings were conducted to obtain spike rate vs. intensity curves, using short acoustic broad-band stimuli (1 – 40 kHz) of 100 ms duration delivered five times each at various intensities rising in steps of 8 dB (32-88 dB). From the resulting curves, temperature coefficients ($Q_{10}$) were determined and compared.

Our results showed an increase of spike count with higher temperature for most neurons. However, the overall response pattern (tonic or phasic) and the shape of the intensity-response curve did not change considerably. Ascending interneurons exhibited lower $Q_{10}$ values than local neurons (and receptors). This might hint at a temperature compensation which differs between the processing levels, with the third stage being least affected by changing temperatures.

From a theoretical point of view, we aim to use Hodgkin-Huxley type neuronal models to reproduce the observed behaviour at each processing stage. This could reveal whether temperature compensation arises from specific cell-intrinsic mechanisms or merely from the given feedforward network structure of the locust metathoracic ganglion. In the latter case, the role of excitation and inhibition within the network is of special interest, as this has been used to reproduce invariance phenomena in the past (Marino et al. 2005, Creutzig et al. 2010).

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# Effects of elevated cGMP levels on the biophysical properties of BK currents in mature mouse inner hair cells

Barbara Disteldorf[1], Niels Brandt[1], Jutta Engel[1]

[1]Saarland University, Department of Biophysics, Prof. Jutta Engel, Kirrberger Straße, Gebäude 76, 66424 Homburg, Germany

The NO-cGMP signalling pathway has recently been found to protect hair cells in the inner. Hearing function and cochlear phenotype of mice with a gene deletion of the cGMP-dependent protein kinase type I (cGKI) were significantly disturbed following noise trauma, pointing to an otoprotective role of cGMP-cGKI mediated processes. Moreover, blocking the hair-cell specific phosphodiesterase, which leads to elevated cGMP levels, also protected hair cells in noise (Jaumann et al., submitted).

We aimed at defining targets of the cGMP-mediated protective processes. One potential target is the big conductance, voltage and Ca2+-activated K+ (BK) channel. In smooth muscle cells of various tissues, elevated cGMP activate BK channels and thus counteract depolarization of these cells.

As BK currents are the most prominent K+ currents that repolarize inner hair cells, we tested the action of a non-hydrolyzable analogue of cGMP, 8-bromo-cGMP, on K+ currents in mature mouse inner hair cells. An analysis of the action of 8- bromo-cGMP on BK current amplitudes, parameters of voltage -dependent activation and on activation kinetics will be presented.

# Eps8 regulates hair bundle length and the functional maturation of mammalian cochlear hair cells

Walter Marcotti[1], Valeria Zampini[1, 2], Lukas Rüttiger[3], Stuart L Johnson[1], Christoph Franz[3], David N Furness[4], Hao Xiong[3], Carole C Hackney[1], Matthew C Holley[1], Nina Offenhauser[5], Pier Paolo Di Fiore[5], Marlies Knipper[3], Sergio Masetto[2]

[1]University of Sheffield, Biomedical Science, Western Bank, S10 2TN Sheffield, United Kingdom
[2]Department of Physiology, University of Pavia, Pavia, 27100, Italy
[3]Department of Otolaryngology, University of Tübingen, 72076 Tübingen, Germany
[4]Institute for Science and Technology in Medicine, Keele University, Keele, ST5 5BG, UK
[5]IFOM, Fondazione Istituto FIRC di Oncologia Molecolare, 20139, Milan, Italy

Mammalian cochlear hair cells are specialized for the dynamic coding of sound. The transduction of sound waves into electrical signals depends upon mechanosensitive hair bundles that project from the cell's apical surface. Each stereocilium within a hair bundle is composed of uniformly polarized and tightly packed actin filaments. Several stereociliary proteins have been shown to be associated with hair bundle development and function, and are known to cause deafness in mice and humans when mutated (Petit, Richardson 2009 Nat Neurosci Rev 12:703-10). The growth of the stereociliar actin core is dynamically regulated by elongation at the actin filament barbed ends in the stereociliary tip. However, the control of actin dynamics in stereocilia is still largely unknown.

We used a combination of single -cell electrophysiology, immunolabelling, electron microscopy and in vivo physiology to investigate the role of Eps8, a protein with actin binding, bundling and barbed-end-capping activities (Di Fiore, Scita 2002 Int J Biochem Cell Biol 34:1178-83), in the cochlea. Using control and Eps8 knockout mice we show that this protein is a novel component of the hair cell hair bundle. Eps8 was localized predominantly at the tip of the tallest row of stereocilia and was essential for their normal growth and for maintaining the normal mechano-sensitivity of the transducer apparatus. Moreover, we found that Eps8 knockout mice are profoundly deaf and that IHCs, but not OHCs, fail to develop the adult-like characteristics required to become functional sensory receptors.

We propose that Eps8 directly regulates actin dynamics in hair cell stereocilia and also plays a crucial role in the physiological maturation of mammalian cochlear IHCs. Together, our results indicate that the multifunctional nature of Eps8 is critical for coordinating the maturation and functionality of mammalian auditory hair cells.

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# Functional connectivity and temporal selectivity depend on carrier frequency in the auditory system of crickets

Jan Clemens[1], Florian Rau[1], K. Jannis Hildebrandt[1,3], R. Matthias Hennig[1,2]

[1]Humboldt-Universität zu Berlin, Institut für Biologie, Verhaltensphysiologie, Invalidenstr. 43, 10115 Berlin, Germany
[2]Bernstein Center for Computational Neuroscience, Humboldt-Universität zu Berlin, Philippstr. 13, Haus 6, 10115 Berlin
[3]University College London, Ear Institute, 332 Gray's Inn Road, London, WC1X 8EE

How does the functional connectivity in a network depend on stimulus properties? We addressed this question using the auditory system of crickets as a model. The cricket's auditory environment is divided behaviorally into a low-frequency channel associated with courtship signals and a high-frequency channel linked to predator signals. This clear behavioral partition is reflected in the simple layout of the auditory system: in the prothorax lay two ascending neurons (AN1 and AN2, see figure) that are excited at different carrier frequencies and receive inhibition from a local neuron (ON1).

We recorded simultaneous responses of both ANs to amplitude- modulated stimuli at either single carrier frequencies or at a mixture of different, independently modulated carriers. The functional connectivity between the two ascending neurons was quantified by calculating cross-correlation functions from their spike trains. Noise correlations were surprisingly weak but signal correlations changed strongly with carrier frequency.

Selectivity of both ascending neurons for temporal features of the envelope - the spike-triggered average stimulus (STA) - was modulated by carrier frequency as well. A comparison of the STA's of AN2 at single carriers with those at a mixture of different carriers - as given by the spike-triggered average spectrogram (a.k.a. STRF, see right figure) - revealed a nonlinear interaction between carrier frequencies. Also, synchronous population events in both cells differed significantly from those associated with non-synchronous spikes in either cell.

A simple model of the network allowed us to reproduce the experimental findings. Thereby, we could show that changing functional connectivity and temporal selectivity emanate directly from the fixed structure of a network through the interplay of frequency selectivity and inhibition.

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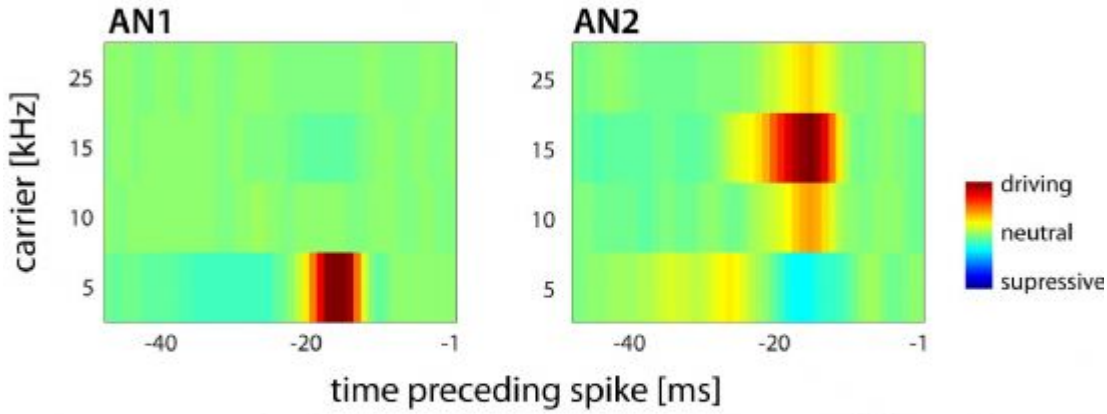
# spike-triggered average spectrogram (STRF)




**Spectrotemporal selectivity of both ascending neurons:** AN1 (left) was driven only at 5 kHz - the carrier frequency of courtship signals - and exhibited a weak suppression at 15 kHz - the "bat-frequency". In contrast, AN2 (right) was selective at all carriers. It was driven between 10 and 25 kHz; low frequency stimuli evoked a fast suppression which was followed by a slow driving effect.

# In vitro and in vivo Studies on Biodegradable Polymers as potential Carrier's Coating for Cochlear Implants

Piera Ceschi[1], Henning Rohm[3], Anne Roock[3], Katrin Sternberg[3], Klaus-Peter Schmitz[3], Manfred Kietzmann[2], Thomas Lenarz[1], Timo Stöver[1,4], Gerrit Paasche[1]

[1]Medical University of Hannover, Department of Otolaryngology, Hannover, Germany
[2]School of Veterinary Medicine Hannover [Foundation], Department of Pharmacology and Toxicology, Hannover, Germany
[3]Rostock University, IBMT, Rostock, Germany
[4]KGU, Department of Otolaryngology, Frankfurt, Germany

A new approach to the interventional treatment of inner ear disease could be the local application of drugs into the cochlea. For this purpose, we propose the modification of the cochlear implant electrode array's by biodegradable polymers able to deliver drugs directly into the scala tympani.
Polymeric coatings have been used to improve the performance of coronary stents, but little is known about their effects in the more closed environment of the inner ear. This is the reason why we tried to better understand the effects of these potential coatings both in vitro and in vivo in an inner ear model.
Survival rates of fibroblasts and spiral ganglion cells (SGC) on poly(L-lactide) (PLLA) as potential drug carrier in comparison to silicone were determined. Discs (0.1 mm thick and 6 mm in diameter) of PLLA and silicone were placed at the bottom of 96-multiwell culture plates. Cells were cultivated on these samples and, in addition, on the untreated bottom of the plates as controls.
NIH-3T3 fibroblasts were seeded at a density of 10.000 cells/well in Dulbecco´s Modified Eagle's Medium (DMEM) supplemented with 10 % fetal calf serum. After 48 hours cells were stained using neutral red, incubated for other 3 hours and, after several washing steps, they were solubilised. The supernatant, containing the stain released from the cells, was transferred into new wells and absorption was measured at 570 nm. In each experiment 5 wells were treated identically and results were averaged. The screening was repeated at least four times.
SGC were freshly isolated from Sprague-Dawley rats (p3-5). Viable cells were suspended in DMEM supplemented with 10 % fetal calf serum and 50 ng/ml BDNF and were seeded at a density of 1.5x10.000 cells/well. Before seeding wells and discs were coated with ornithin and laminin. After a cultivation time of 48 hours, cells were stained with anti-neurofilament antibody (according to the manufacturer's protocol) and counted on the discs. In all experiments at least 4 wells were treated in the same manner and the experiment was repeated four times.
Subsequently, in order to investigate the effects of the degradation of PLLA in the scala tympani, a prototype consisting of a silicone carrier coated with PLLA was developed and implanted bilaterally in guinea pigs for 1, 2, 3, and 6 months. As controls uncoated silicone samples were used. Cochleae were embedded in epoxy with the samples in situ, stained, grinded and documented for evaluation of surviving SGC.
In vitro data demonstrated that fibroblasts and SGC grow on the PLLA surfaces as on silicone. The in vivo study showed that only after two months the survival rate of SGC in the PLLA-treated group is reduced in comparison to the respective controls whereas SGC density compared to not implanted cochleae is only reduced in PLLA groups but not in control groups. These reductions in SGC survival are limited to the basal and first middle turn of the cochlea.
As on a long term there was no difference between coated and uncoated silicone samples, PLLA seems to be a possible candidate for coating of cochlear implants.

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# Interaural-canal effects in the chicken with closed sound systems in vivo

Christine Köppl[1]

[1]Carl von Ossietzky Universität, Institut für Biologie und Umweltwissenschaften, Fak. V, Carl von Ossietzky Straße , 26111 Oldenburg, Germany[1]

Avian middle ears are not enclosed in bony bullae but are instead open to multiple skull spaces, collectively termed the interaural canal. This physical interaural connection forms a pressure-gradient receiving system which potentially creates a directional sensitivity of eardrum motion and may enhance binaural level and timing cues. However, the extent of these effects is likely to differ between species and is very sensitive to experimental conditions.

The aim of the present experiments was to assess the extent of interaural-canal effects in anaesthetized chickens, aged P21 and older, under the experimental conditions commonly used for invasive neurophysiology. Closed, individually calibrated sound systems were used to deliver monaural and binaural tone-burst stimuli. Surgical openings in the skull provided middle -ear ventilation. Cochlear microphonic potentials (CM) were recorded simultaneously from both ears with wire electrodes near the round windows. Recordings were repeated after injection of liquid petroleum jelly to block the interaural canal.

With monaural stimulation, a comparison of ipsi- and contralateral CM amplitudes indicated the most pronounced interaural transmission at frequencies between 1.5 and 2.5 kHz. Blocking the interaural canal significantly reduced this cross -talk. Phase differences between ispi - and contralateral CM, when converted to interaural time differences (ITD), showed a pronounced decline with increasing frequency. Large ITDs of more than 1 ms were observed at 100 and 333 Hz. We also presented binaural stimuli with varying ITD. Under these conditions, both CM clearly modulated in amplitude with ITD. Blocking of the interaural canal abolished this modulation.

These measurements demonstrated clear interaural canal effects in chickens with nearly mature head sizes. Interaural transmission occurred with minimal attenuation at frequencies around 2 kHz.

# Neurotrophins and calcium channels function in the auditory system: help from conditional knock out mouse models.

Annalisa Zuccotti[1], Wibke Singer[1], Lukas Rüttiger[1], Sven Moosmang[2], Eckhard Friauf[3], Thomas Schimmang[4], Marlies Knipper[1]

[1]Haering Research Center Tübingen, Molecular Physiology of Hearing, Elfriede-Aulhorn strasse, 5, 72076 Tübingen, Germany
[2]Institut für Pharmakologie der Technischen Universität, Biedersteiner Str. 29, 80802 München, Germany
[3]Technische Universität Kaiserslautern, Tierphysiologie Biologie, Erwin-Schroedinger-Straße 13/572, 67653 Kaiserslautern, Germany.
[4]CSIC y Universidad de Valladolid, Istituto de Biologia y Genetica Molecular, C/Sanz y Forés s/n, 47003 Valladolid, Spain.

In addition to the well described L -type voltage gated calcium channel (L -VGCCs) CaV1.3, also CaV1.2 is expressed in the adult mouse cochlea (Green et al., 1996; Waka et al., 2003). Due to lethality of constitutive CaV1.2 KO mice, the function of this ion channel as well as the putative relationship to the brain derived neurotrophic factor (BDNF) in the auditory system is entirely elusive. BDNF, a neurotrophic factor crucial for the survival of neonatal neurons of the central nervous system (Barde et al., 1982), modifies the efficacy of synapses, including short and long term potentiation of synaptic transmission (Korte et al., 1995; Bibel and Barde, 2000). The transcription of BDNF itself is dependent on the activity of the neuron, being activated in a calcium (Ca2+) selective and neuron-specific manner, depending on L-VGCCs, (West et al., 2001). We asked if described alteration of BDNF levels in the auditory system (Rüttiger et al., 2006, Tan et al., 2007; Panford-Walsh et al., 2008, Meltser et al., 2010 a, b) is a process controlled by L -VGCC. So far a function of BDNF for the mature inner ear was hypothesized to be restricted to the vestibular system, a suggestion that could not be validated so far as BDNF mice mutants are lethal postnatally (Ernfors et al., 1994; Jones et al., 1994). Moreover CaV1.2 mice mutants die in utero before embryonic day 15 (Seisenberger et al., 2000). The elusive function of both CaV1.2 and BDNF in the mature cochlea and auditory system more in general was investigated by generating conditional CaV1.2 and BDNF KO mouse lines.
A combination of functional and molecular approaches will be presented to described these conditional mouse lines, with the aim to achieve a more comprehensive hypothesis of the role of L-VGCC and neurotrophins in the mature auditory system.

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# Patterning of spontaneous action potentials in immature inner hair cells is determined by acetylcholine and varies as a function of cochlear location

Stuart L. Johnson[1], Tobias Eckrich[1], Valeria Zampini[1, 2], Stephanie Kuhn[1], Christoph Franz[3], Kishani M. Ranatunga[4], Sergio Masetto[2], Marlies Knipper[3], Corné J. Kros[4], Walter Marcotti[1]

[1]Department of Biomedical Science, University of Sheffield, S10 2TN Sheffield, UK
[2]Department of Physiology, University of Pavia, Pavia, 27100, Italy
[3]Department of Otolaryngology, University of Tübingen, D-72076 Tübingen, Germany
[4]School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK

Patterned spontaneous action potential (AP) activity occurs during critical periods of mammalian sensory system development and is hypothesized to drive the refinement of synaptic connections before the onset of sensory-induced activity (Katz, Shatz 1996 Science 274: 1133-8). Position dependent patterning of AP activity has been observed prior to the onset of hearing in chick auditory brainstem neurones (Lippe 1995 Brain Res 703: 205-13). It is likely that such activity is driven by AP firing in cochlear inner hair cells (IHCs) (Kros et al 1998 Nature 394: 281-4). It remains uncertain whether IHC AP activity is intrinsically generated or initiated by waves of ATP released from nearby supporting cells (Tritsch et al 2007 Nature 450: 50-5).

AP activity was investigated in IHCs of altricious rodents maintained at 35-37°C in perilymph-like extracellular solution using whole-cell current clamp and cell-attached voltage clamp recordings. We show that during the first postnatal week, AP activity is intrinsically generated by IHCs and its frequency and pattern differ as a function of position along the cochlea, with apical IHCs exhibiting bursting as opposed to more sustained firing in basal cells. The difference in pattern is likely to be determined by the efferent neurotransmitter ACh, which by fine-tuning the IHC's resting membrane potential (Vm) is crucial for the bursting pattern present in apical IHCs. Endogenous extracellular ATP also contributes to maintaining the required Vm of both apical and basal IHCs via the activation of SK2 channels.

We hypothesize that the difference in IHC firing pattern along the cochlea during the first postnatal week guides the functional differentiation of IHCs along the tonotopic axis (Johnson et al 2008 J Neurosci 28: 7670-8) and refines tonotopic maps along the auditory pathway before the onset of sensory experience (Kandler et al 2009 Nat Neurosci 12: 711-7).

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# Shaking a Leg for Evolution

Jan Heusler[1], Anja Rösler[1], Lakes-Harlan Reinhard[1]

[1]Animal Physiology, Integrative Snsory Physiiology, Wartweg 95, 35390 Giessen, Germany

Many insect orders possess a vibration sensitive organ, the subgenual organ, in the proximal tibia of the legs. Especially in the orthopteroid insects, this organ is part of a larger sensory complex. From this complex, the Tettigoniidae and the Gyrllidae have evolved a tympanal hearing organ. However, the functional significance of the organ complex in atympanate species is still not understood, in the light of other species having much simpler sensory structures.
Therefore we investigate midlegs of different species for their anatomy and physiology. To test the vibration properties, the leg is stimulated with different vibratory stimuli. With laser vibrometry the resulting cuticular vibrations can be measured. The data show that, different sites of the leg vibrate differently to the same stimulus. These results are also frequency dependent. In further experiments, electrophysiological responses are registered in response to different vibratory stimuli. These data are correlated to the anatomy of the leg.
The results will give insights into the physiological parameters which can be registered by the complex sensory organ and allow speculations about the evolutionary selection pressures.

# Slow currents mediating spike-frequency adaptation shape spike-timing variability

Karin Fisch[1], Andreas Herz[2], Jan Benda[3]

[123] Ludwig-Maximilians-Universität München, Department Biologie II, Division of Neurobiology, Grosshaderner Str. 2, 82152 Planegg-Martinsried, Germany

Channel noise is a dominant intrinsic noise source in sensory neurons and plays an important role for the spike-timing variability. To characterize the stochastic properties of a neuron, direct somatic recordings are well suited. In many systems, however, such recordings are difficult to achieve without severely damaging the sensory transduction machinery. Here, we show that higher -order interspike interval (ISI) statistics of the spike-train responses can be used to indirectly assess the stochastic dynamics of sensory neurons.

Intracellular recordings were performed from axons of auditory receptor neurons of *Locusta migratoria* during simultaneous acoustic stimulation with pure tones of various intensities. The obtained ISIs show high variability with CVs up to 0.8 depending on sound intensity. With increasing spike frequency the shape of the ISI histograms changes from an inverse Gaussian to a more peaked probability density. Additionally, the ISI correlations show a shift from slightly negative values to positive coefficients with increasing spike rate.

By means of simulations of single-compartment conductance-based models we tested different assumptions about possible noise sources which could account for the observed transitions of the ISI histogram shape and correlation. Using mixed case models where both fast fluctuations and slow adaptation channel noise are present we are able to reproduce the ISI statistics of the locust receptor cell responses.

The mechanism underlying the spike-frequency adaptation in the locust auditory system has not been established so far. We currently perform blocking experiments to determine the specific adaptation mechanism and test our indirect findings that slow adaptation currents contribute to spike-response variability.

# Spatial responses of lateral line units in the midbrain of *Xenopus laevis* depend on the frequency of incoming surface waves

Francisco Branoner[1], Zhivko Zhivkov[1], Ulrike Ziehm[1], Oliver Behrend[12]

[1]Humboldt Universität zu Berlin, Department of Biology, Aquatic Bioacoustics, Invalidenstr. 43, 10115 Berlin, Germany
[2]Ludwig-Maximilians-Universität, Munich Center for Neurosciences, Großhaderner Str. 2, 82152 Martinsried, Germany

The African clawed frog employs a mechanosensory lateral line (LL) system to identify and capture prey on the water surface based on incoming wave signals. In order to dissect central representations of small moving surface objects, neuronal responses were recorded to concentric waves: wave signals were varied in frequency, and source locations covered 3 distances from 4–10 cm, and 6 different angles from 30–180°.

Altogether, 339 LL -units were recorded that showed a significant response ($p<0.05$; Wilcoxon-test; Bonferroni-correct.) to surface wave stimuli of either constant or modulated frequency time course. Extracellular data were obtained from LL -nuclei of the brainstem (N=27) and midbrain areas (Torus Semicircularis; TS; N=153; Optic Tectum; OT; N=89). 41 LL -units were registered at other brainstem, tegmental and thalamic areas. 29 recording sites could not be reconstructed. A surprising number of 79 LL-units were recorded in the laminar nucleus of the TS (TL) that is known for its strong acoustic input. Indeed, 69.2% of 39 acoustically stimulated LL-units in the TL showed a bimodal response suggesting multimodal processing in the area.

LL-units showing distinct wave parameter sensitivity (i.e. for frequency, freq.-modulation, source distance/angle) based on their spike rate ($p<0.05$; ANOVA-, weighted ANOVA-, Kruskal-Wallis-test, respectively) were often inconspicuously distributed across brainstem and midbrain regions. However, the relation of units tuned to high wave frequencies >25 Hz (N=138) and low frequencies =25 Hz (N=63) differed significantly between the TS and the OT ($p<0.05$; $X^2$). Also, the relation of LL-units that selectively responded to either frequency-modulated (N=27), or non-modulated waves (N=62), respectively, differed significantly across the TS and OT ($p<0.05$; $X^2$).

Across all central areas, most LL-units were spatially tuned to source angle (93.6% of 79) and/or distance (63.8% of 218). Whereas best neuronal responses to varying wave source distances seemed evenly distributed, the distribution of best responses to either rostral-most or caudal-most source angles, respectively, deviated significantly between dorsal, and ventral midbrain layers >180 μm ($p=0.04$;$X^2$).

Many LL-units represented particular combinations of spatial and temporal wave parameters not unlike those caused by prey objects on the water surface. In subsequent recordings, significant response rate changes to both source angle, and wave frequency variation were observed in 82.5% of 80 units. Response rate changes to both source distance, and wave frequency variation were registered in 49.3% of 213 units. However, in a combined test paradigm only 7 of 21 tested angle-sensitive units showed robust best responses across 6 source angles when wave frequencies were varied. For the latter, also only 6 of 20 tested distance -sensitive units showed robust best responses across 3 source distances. In contrast, best responses across wave frequency remained, statistically, robust over three source distances (in 23 of 36 frequency-sensitive LL-units), and over 6 source angles (in 13 of 26 units; both $p<0.001$; $X^2$). The dependence of spatial neuronal representations on a temporal wave parameter like frequency suggests temporal integration might be critical to the central computation of spatial maps, whereas the central representation of wave frequencies might be less dependent on the spatial pattern and - sequence of activation of peripheral receptors.

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# Synchrony codes in a heterogeneous population of auditory receptor neurons

Charlotte Maria Pix[1], Jan Benda[1]

[1]LMU Munich, Department Biologie II, Großhaderner Str. 2, 82152 München, Germany

The ear of the desert locust *Schistocerca gregaria* consists of an ensemble of approximately 50 receptor neurons with narrow dynamic ranges but different sensitivities. In previous studies the response properties of the receptor neurons to band-pass filtered white-noise as well as natural grasshopper songs have been investigated in detail for stimuli presented well inside the dynamic range of these neurons. However, since the receptor neurons all have different sensitivities, only few neurons will be excited in their dynamic range whereas all other neurons will respond less optimal with saturated responses or very low firing rates.

We here explore how acoustic stimuli are encoded by the whole population of auditory recptor neurons. We record single-unit activities from the auditory nerve while subsequently presenting band-pass filtered white noise stimuli at a range of mean intensities. This way we mimick the different sensitivities of the population.

Using stimulus-response coherence we compare the information carried by a single neuron with the one carried by the heterogeneous population. We also explore how strong stimulus transients that are characteristic in natural grasshopper songs are able to synchronize the population response. The other way around we ask what exactly do synchronous spike encode and compare the resulting stimulus-response coherence to the all-spikes results. Differences between the coherences suggests possible parallel read-out strategies for upstream neurons, as it was recently suggested for the electrosensory system.

# Temperature dependence of DPOAEs in grasshoppers

Julian Lang[1], Manfred Kössl[1], Manuela Nowotny[1]

Goethe Universität Frankfurt, Institute for Cell Biology and Neuroscience, Siesmayerstr. 70A, 60323 Frankfurt, Germany

Otoacoustic emissions (OAEs) are products of nonlinear mechanics in auditory sense organs. In contrast to vertebrates, the source and generation of OAEs in insects are widely unknown. When two different tones were applied pronounced distortion-product OAE (DPOAEs) at 2f1-f2 kHz can be measured in insects. The resulting slope of the DPOAEs-growth-functions in grasshoppers, *Locusta migratoria*, is dependent on the used stimulus frequency (Kössl and Boyan, 1998 J. Acoust. Soc. Am. 104). Presumably this is due to different types of auditory receptor cells and the frequency tuning of the tympanum.
The aim of the presented study is to asses the DPOAE generation mechanism in *L. migratoria* by the investigation of the effect of temperature-change on DPOAEs. The DPOAEs were recorded at the most sensitive frequency (8 kHz) and the change was quantified by recording DPOAE-growth-functions for stimulus of increasing level and calculating DPOAE-threshold-curves. DPOAEs amplitude changes could be observed during cooling (about 12°C) or heating (about 33°C) the animal and the temperature effects were most pronounced at low sound pressure levels (<40 dB SPL). The DPOAE threshold curves (threshold = -10 dB SPL) became less sensitive during cooling (28.9 ± 2.6 dB SPL, n = 10) in comparison to heating (18.3 ± 7.2 dB SPL, n = 10). Thus, the sound pressure level must be increased at lower temperatures to reach the same DPOAE-amplitude as at higher temperatures. When using higher threshold criteria (10 dB SPL) the threshold values were most sensitive at 22°C and deteriorated for both, lower and higher temperature, in a the form of a bell shaped distribution. This shows that DPOAE generally mechanics are only governed by a single metabolic temperature dependence close to hearing threshold. At higher sound pressure levels multiple DPOAE sources could interact.

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# The complex tibial organ of a splay-footed cricket, *Comicus calcaris* : Comparative neuroanatomy and the reconstruction of auditory evolution in Ensifera

Johannes Strauss[1], Reinhard Lakes-Harlan[2]

[1]Stockholm University, Zoological Institute, Svante Arrhenius väg 18A, 10691 Stockholm, Sweden [2] Institute for Animal Physiology, AG Integrative Sensory Physiology, Justus-Liebig-University Giessen, Germany

Among taxa of Ensifera, the chordotonal complex tibial organ is adapted to sound perception and contains specific sets of auditory receptor neurons. For a comparative study on the organisation of the tibial organ in non-hearing Ensifera, the neuroanatomy of chordotonal receptor structures of a non-hearingmember of Schizodactylidae (*Comicus calcaris*) is documented. The complex tibial organ consists of three distinct parts: the subgenual organ, the intermediate organ, and the *crista acustica* homologue. The latter is an array of linearly organized neurons which are homologous to auditory receptors in the tibial hearing organs of bushcrickets (Tettigoniidae). The tibial organ is present in serially similar organisation in all three leg pairs, with equivalent neuron numbers in the fore- and midleg. The overall organisation is resembling complex tibial organs known from Tettigoniidae and related taxa, indicating a close phylogenetic association. Summed recordings from the sensory leg nerve show that the organ may function as receiver to vibration stimuli, while thresholds are lower in the fore- than in the midleg. Some neuronal features of the *crista acustica* homologue such as the spatial neuron arrangement and the serial organisation do not compare to that found in auditory organs of most extant Ensifera. As the question of unique or convergent ear evolution in Ensifera is still debated, the study of this non-hearing group reveals plesiomorphic atympanate features, like the relatively high number of neurons, suggesting that Ensifera were ancestrally deaf. This first investigation concerning the sensory neuroanatomy of Schizodactylidae identifies a non-auditory cordotonal organ as the precursor for auditory receptors of related tympanate taxa like bushcrickets.

# The Mesencephalon of a mormyrid – Sensory processing during active electrolocation in the weakly electric fish Gnathonemus petersii

Tim Ruhl[1], Claudia Mohr[2], Gerhard von der Emde[1]

[1]University of Bonn - Institute of Zoology, Neuroethology/ Sensory Ecology, Endenicher Allee 11-13, 53115 Bonn, Germany
[2]Oregon Health and Science University,Department for Behavioral Neuroscience, 3181 SW Sam Jackson Park Road L470 Portland, Oregon, USA

The mesencephalon of vertebrates receives multimodal sensory afferences and it is a very important centre for transfer of sensory input into motor output. In mormyrid weakly electric fish, sensory processing is performed in different areas of the mesencephalon (several toral nuclei and tectum opticum), which are also involved in recurrent feedback loops into other sensory processing structures of the mormyrid brain.
Gnathonemus petersii detects and analyzes objects in its near vicinity by emitting short electric pulses and perceiving them with epidermal electroreceptors, a process called active electrolocation. The first station of central electrosensory processing is the medullary electrosensory lateral line lobe (ELL). Its main efferences terminate via the lateral lemniscus in the nucleus preeminentialis (PRE) and in the nucleus lateralis (NL) of the torus semicircularis in the mesencephalon. In this study, we investigated the neural connections of NL by injection of the neural tracer Biocytin. Our neuroanatomical investigations indicate a descending reciprocal feedback-loop from NL onto ELL. Furthermore, NL sends projections back to the PRE, and ascending connections towards the valvula cerebelli and two regions of the diencephalon, i.e. the preoptic area and the nucleus preglomerulus.
The NL is prominently involved in electrosensory processing, and it represents a candidate for object recognition and integration of different object features, such as electric impedances, capacitive properties and distance.
Neuronal responses to objects with different features were investigated in the NL of the mormyrid Gnathonemus petersii using extracellular single-cell and field potential recordings. Objects of same sizes (8 x 8 x 8 mm) but with different electrical properties (metal cube: good conductor; plastic cube: non- conductor) and a dipole-object, whose capacitive properties could be modulated, were presented inside the receptive fields of neurons. The electrophysiological and neuroanatomical results of these experiments provide new information about electrosensory processing during object recognition through active electrolocation in mormyrids.

# Tonotopy and interneuronal interaction in the auditory brainstem is shaped by hearing experience: A c-Fos study in the rat

Nicole Rosskothen-Kuhl[1], Robert-Benjamin Illing[1]

[1]Neurobiological Research Laboratory, Department of Otorhinolaryngology, University of Freiburg, Killianst. 5, D-79106 Freiburg, Germany

The immediate-early-gene c-fos is among the first genes to be expressed following sensory-invoked neuronal activity. Its gene product c-Fos forms the limiting monomer of the heterodimeric activator protein-1 (AP-1) transcription factor in combination with c-Jun. AP-1 triggers various genes, among them the growth associated protein-43, which are involved in neuroplastic remodeling.
We investigated the pattern of c-Fos expression in the anteroventral cochlear nucleus (AVCN) and the central inferior colliculus (CIC) of anaesthetized normal hearing rats compared to neonatal deafened rats. The latter never heard due to hair cell destruction by daily kanamycin injections between postnatal day 10 and 20, which results in a rise of hearing threshold by around 80 dB.
Both experimental groups receive unilateral electrical intracochlear stimulation (EIS) for 2 h, 3:15 h and 5 h by inserting a cochlear-implant into the medial turn of the cochlea.
Following sustained EIS at 50 Hz, c-Fos expression of normal hearing mature rats (age 6-10 weeks) was limited to a region of the ipsilateral AVCN and the contralateral CIC, tonotopically corresponding to the stimulation frequency. However, in deafened mature rats the region of c-Fos positve neurons was greatly expanded (Fig. 1 A-F). By detecting and counting c-Fos positive nuclei in AVCN and CIC of normal hearing rats, we discovered temporal non-linearities in the size of the respective population of c-Fos expressing neurons: In AVCN we detected high numbers of c-Fos positive cells by 2 and 5 h of EIS and a transient minimum by 3:15 h (Fig. 1), whereas in CIC the number of c-Fos positive neurons continuous decreases towards 5 h following a local maximum at 2 h of EIS.
By comparing the two experimental groups, the number of c-Fos positive nuclei in AVCN and CIC was always significantly higher for each stimulation time of neonatal deafened compared to normal hearing rats. A linear increase of c-Fos positive nuclei has been detected in the ipsilateral AVCN, whereas in the contralateral CIC a temporally constant level of c-Fos existed (Fig. 1).
In addition to counting c-Fos positive nuclei in sections throughout AVCN, we determined the density of c-Fos positive nuclei after 2 and 5 h of stimulation seperately for lateral, central, and medial parts of AVCN that corresponded tonotopically to the intracochlear site of stimulation. In central AVCN of normal hearing rats the density of intensively stained c-Fos positive nuclei increases significantly from 2 to 5 h, whereas medial and lateral regions remain unchanged. In contrast, no significant changes of c-Fos density could be observed in the different regions of the AVCN of deafened rats related to stimulation time.
These data suggest two massive effects of hearing experience on neurons and their interaction to sustained sensory stimulation. First, tonotopy largely failed to be established in the ipsilateral AVCN as well as the contralateral CIC in the absence of hearing experience. Second, hearing experience results in an apparently different neuronal coupling, distinguishable by a non-linear growth of the population of c-Fos positive nuclei compared to its linear rise in deafened rats.

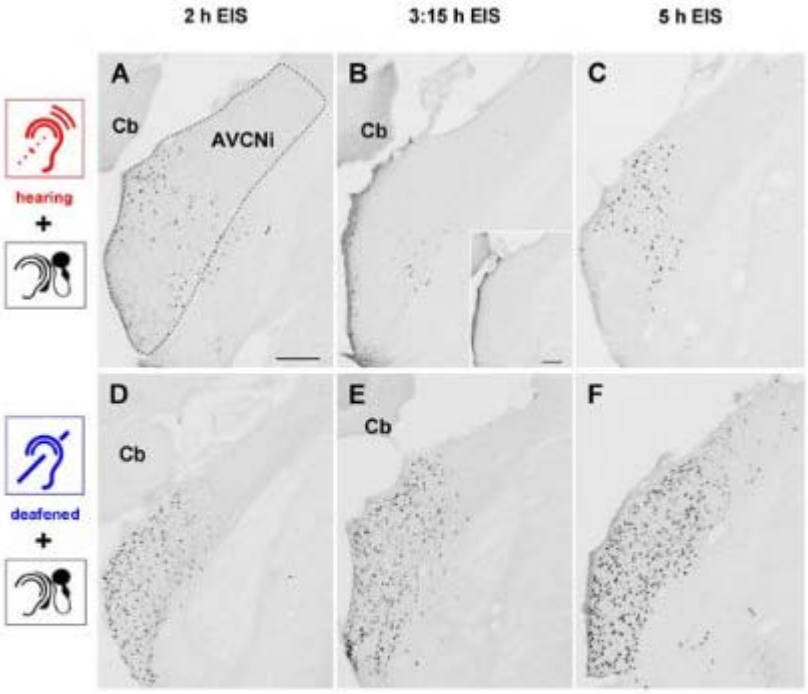



Figure 1: C-Fos expression (black dots) in the ipsilateral (i) AVCN of hearing (A-C) vs. deafened (D-E) rats by varying stimulation times. The inset shows a contralateral AVCN devoid of c-Fos staining. Scale bar: 0.2 mm. CB = Cerebellum.

# Towards autonomous large scale recordings of natural behavior of weakly electric fish

Jörg Henninger[1], Rüdiger Krahe[1,2], Jan Benda[1]

[1]Ludwig-Maximilians-Universität München, Neurobiologie, Department Biologie II, München, Germany
[2]McGill University, Department of BiologyMontreal, Quebec H3A 1B1, Canada

Weakly electric fish are a well established model system for studying sensory processing in vertebrates, providing great electrophysiological accessibility as well as a concise behavior. These preconditions make it one of the few model systems where electrophysiological data have been successfully discussed in the context of behaviorally relevant stimuli. In order to better understand tuning properties of the electrosensory system, data on the statistical structure that characterizes natural communication signals is needed. However, most behavioral data have been acquired under very restricted lab conditions. We present a novel method that allows to autonomously monitor the movement, electric organ discharge (EOD), and communication signals of individual weakly electric fish in their natural habitat, mostly small rivers in South America, as well as under controlled lab conditions. In our study, we use a setup composed of an evenly spaced multi-electrode grid, which can easily be adapted to local requirements. Our recording sites are situated in rain forests of Peru and Panama.
In a first step, we perform a spectral analysis in order to identify individual fish in proximity to the electrode array. The fit of a model of the fish's electrical field to the data yields the fish's positions, orientations and EOD strengths. In a second step, the EODs of individual fish are segregated for analysis, e.g. detection of communication signals. In a third step, the EODs of all nearby fish are integrated at a given fish's position to reconstruct the electric signals each fish receives in the context of communication. The success of the above described method strongly depends on the knowledge of the characteristics of the fish's field potential. We therefore present data on the fish's far field potential in comparison to data acquired by previous studies. For the first time, this new method allows to quantitatively analyze the interactions and communication of groups of weakly electric fish in their natural habitat. The method also provides powerful means to cover daily and seasonal changes in communication behavior as well as a novel framework for playback studies.

# Transformation of auditory information in the CNS of the grasshopper *Chorthippus biguttulus*

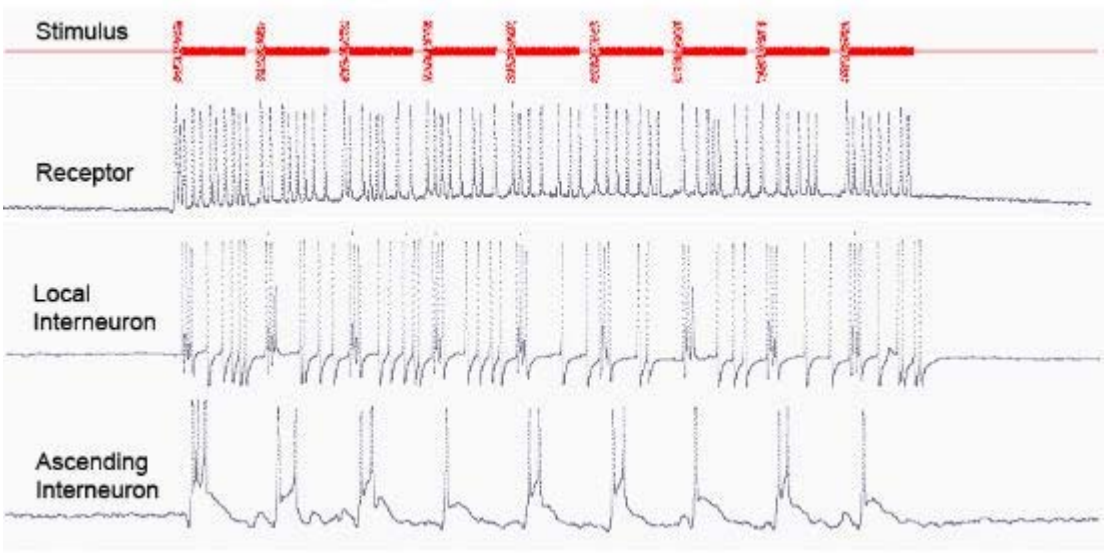
Olaf Kutzki[1], Bernhard Ronacher[1]

[1]Humboldt Universität zu Berlin, Department of Biology, Invalidenstr. 43, 10115 Berlin, Germany

*Chorthippus biguttulus* is an acoustically communicating grasshopper in which pair formation is achieved by duetting between the sexes. Females use male song patterns as a cue for species recognition and mate choice (v.Helversen, v.Helversen 1994, Fortschr. D. Zool. 39: 253-284). The females decision to answer to a conspecific male depends on a number of features, such as the syllable-pause structure and the syllable onsets and offsets (Balakrishnan et al. 2001, J Comp Physiol A 187: 255-264).

The information about the syllable pause structure reaching the tympanal organ on each side is transferred by receptors to local interneurons in the metathoracic ganglion.
Those local interneurons are connected to ascending neurons which transmit the information to the decision making stations in the brain.
Whilst the receptor cells convey the information in a synchronization code it gets transformed to a rate-population like code at the metatoracic level (Franz 2004, Mensch & Buch Verlag) so that ascending neurons preferentially code for specific features within the stimulus.

According to the sex and species specific parameters patterns of different attractiveness have been constructed by modulating the pause duration and the relative syllable onset. Those stimuli were tested in behavioral experiments. Afterwards the same stimuli were used for intracellular recordings in the brain (supra oesophageal ganglion) of the animals tested before.
We use information theoretical approaches to assign the different features to identified ascending neurons and to correlate inter-individual differences in behavior to differences in the spike patterns of the animals tested.



# Trauma-induced tinnitus in gerbils centers around the induction frequency

Martina Remus[1], Bernhard Gaese[1], Manfred Kössl[1], Manuela Nowotny[1]

[1]Goethe University Frankfurt, Institute of Cellbiology and Neuroscience, Siesmayerstraße 70 a, 60323 Frankfurt, Germany

The occurrence of tinnitus in western population has increased over the last years due to a rise in age and a higher noise level in work and spare time environments. This increase in noise level can lead to inner ear damage, which then can induce tinnitus. Therefore, an acoustic trauma model of tinnitus in gerbils was established using the modulated acoustic startle response for the detection of tinnitus. In the present study the development of tinnitus after an acoustic trauma was investigated. Important for the biomedical context of this study is that compared to other rodents, the Mongolian gerbil has low-frequency hearing which covers most of the human hearing range. To induce trauma-related tinnitus a narrow-band noise centered around 10 kHz (± 0.25 oct, 105 dB for one hour under anesthesia) was used. The resulting hearing damage and its recovery were documented by auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). A successfully induced tinnitus was detectable through a decrease in gap prepulse inhibition of the acoustic startle response (GPIAS).
Immediately after trauma induction the ABR threshold increased by about 30 dB. ABR and DPOAE thresholds were back to normal three to five weeks after the trauma. Five weeks post trauma, a significant reduction of GPIAS for high frequencies (16 and 20 kHz) was a first sign of a developing tinnitus measured by using a prepulse noise level of 75 dB SPL. At a noise level of 65 dB SPL, a decrease in GPIAS was measured at 10 kHz, the centre frequency of the trauma. After another two weeks (7 weeks after trauma) this reduction was also detectable for a 75 dB SPL noise while the effects on higher frequencies vanished.
These results point to an establishment of tinnitus in gerbils around the centre of the trauma frequency and not at its bordering frequencies, especially on the high frequency side, as often reported in the literature. These data are not compatible with the theory of lateral inhibition as the physiological basis of tinnitus. The animal model presented here can be further used in the search for medical prevention strategies for tinnitus.

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# Vibratory sense organs in the legs of the mantid *Hierodula membranacea*

Reinhard Lakes-Harlan[1], Sonja Gräbener[1], Christin Meister[1]

[1]Institut für Tierphysiologie, Integrative Sensory Physiology, Wartweg 95, 35392 Gießen, Germany

The complex tibial organ of the mantid *Hierodula membranacea* was anatomically and physiologically investigated. Two main questions were the base of the study. 1. Do have mantids a complex sensory tibial organ and how does it compare to the organs in orthopterans? 2. What are the specializations in the organ in respect to the different functions and type of legs?
Anatomically it could be shown that the complex tibial organ of the mantid consists of a subgenual organ, a distal organ and an accessory scolopidial organ. These data underline a phylogenetic relationship with Blattaria and Orthopteroids. In respect to the question of specialization, the subgenual organ of the foreleg has less sensory units than the other leg types. Physiologically the vibratory threshold is lower in the mid- and hindleg, than in the foreleg. Thus, the sensory physiology of the foreleg seems to correlate to its function for prey capture. Behavioral data of larvae underline the functional diversity of the different legs.

# Dissecting the possible role of dyneins in ciliary motility of Drosophila auditory neurons.

Somdatta Karak[1], David Piepenbrock[1], Pingkalai Senthilan[1], Daniel Eberl[2], Martin Göpfert[1]

[1]Georg August Universität, Johann Friedrich Institüt for Anthropologie and Zoologie, Abteilung Cellular Neurobiology, AG Göpfert, Hermann-Rein Str.3, 37075 Göttingen, Germany[1]

[2]Department of Biology, The University of Iowa, Iowa City, IA 52242.[2]

The hearing organ of Drosophila comprises of an arista and ca. 500 mechanosensory chordotonal (CH) neurons in the Johnston's organ (JO) in the second antennal segment. Mechanical measurements reveal that the fly ear exhibits all the key characteristics of the cochlear amplifier, an active process that boosts auditory sensitivity and frequency-selectivity and originates from the motility of sensory hair cells in the ear. Active amplification in the Drosophila ear likewise resides in the motility of auditory sensory cells in the JO. The ability of these neurons to mechanically drive the antenna is tightly linked to mechanotransduction and can be quantitatively explained by an interplay between transduction channel gating and active movements of associated adaptation motors.

CH neurons possess a single ciliated dendrite with a 9X2+0 axoneme. The axoneme shows presence of dynein arms. However, these arms are not present along the cilium's entire length: A swelling, the ciliary dilation, divides the cilium into a proximal and a distal zone, of which only the former bears axonemal dynein arms. TRPV channels, which modulate amplification in the fly's auditory system, are also restricted to the proximal zone of the cilium, whereas the TRPN1 channel NompC, which may act as auditory transducer, has been localized to the distal zone. Notwithstanding this apparent spatial segregation of transduction channels and axonemal dynein arms, it seems that dynein arms may power the active amplification that is promoted by the CH neurons of JO: first, estimates of motor activation energies suggest that dynein motors power active amplification in the antennal hearing organs of mosquitoes. And, second, in Drosophila tilB mutants, loss of active amplification is associated with the loss of axonemal dynein arms in CH neurons and with the immotility of sperm.

The Drosophila genome comprises ca. 42 genes that have been reported to encode dynein motor components (13 for heavy chains, 14 for intermediate chains and 15 for light chains). Microarray data has shown a few dyneins to be specifically expressed in the JO. For many of these genes, mutations are available, including point mutations, transposon insertions, and RNAi lines. However, from the different dynein mutants tested various different phenotypes have been observed (details in Table 1).

Thus, the motor mechanism seems to be more sophisticated than one might have thought. The current aim is towards validating the role of dyneins in ciliary motility that would give us insight regarding force generation in the axoneme.

| Dynein gene | Dynein sub family | Human Homologue | Amplification | Nerve response |
|---|---|---|---|---|
| CG17150 | Axonemal inner arm | DNAH3 | Excess | Complete loss |
| Dhc36c | Axonemal inner arm | DNAH7 | Loss | Reduced displacement sensitivity |
| CG9492 | Axonemal outer arm | DNAH5 | Loss | Complete loss |
| Dhc93AB | Axonemal outer arm | DNAH17 | Loss | Complete loss |
| Dhc62b | Axonemal inner arm | DNAH12 | Excess | Reduced displacement sensitivity |
| Dhc98D | Axonemal inner arm | DNAH10 | To be done by RNAi | |
| CG3339 | Axonemal outer arm | DNAH9 | | |
| CG6053 | Intermediate chain | DNAI2 | Loss | Reduced displacement sensitivity |

# Hyperacute directional hearing and phonotactic steering in the Mediterranean field cricket

Berthold Hedwig[1], Stefan Schoeneich[1]

[1]University of Cambridge, Zoology, Downing Street, CB2 3EJ Cambridge, United Kingdom

Auditory mate or prey localisation is central to the lifestyle of many animals and requires adequate directional hearing. However, when the incident angle of sound approaches 0° azimuth, interaural time and intensity differences gradually vanish. This poses a demanding challenge to animals especially when interaural distances are small. To cope with these limitations imposed by the laws of acoustics, crickets employ a frequency tuned peripheral hearing system. Although this enhances auditory directionality the actual precision of directional hearing and phonotactic steering has never been tested rigorously in the behaviourally important frontal range before.

We analysed the directionality of phonotaxis in female crickets (*Gryllus bimaculatus*) walking on an open-loop trackball system by measuring their steering accuracy towards male calling song presented at frontal angles of incidence. Within the range of ±30°, females reliably discriminated the side of acoustic stimulation, even when the sound source deviated by only 1° from the animal's length axis. Moreover, for angles of sound incidence between 1° and 6° the females precisely walked towards the sound source. Measuring the tympanic membrane oscillations of the front leg ears with a laser vibrometer revealed between 0° and 30° a linear increasing function of interaural amplitude differences with a slope of 0.4 dB/°. Auditory nerve recordings closely reflected these bilateral differences of tympanic membrane oscillations in the afferent response latency and intensity that provide the physiological basis for precise auditory steering.

Our experiments demonstrate how an insect hearing system based on a frequency-tuned pressure difference receiver achieves directional hyperacuity that easily rivals best directional hearing in mammals and birds. This directional accuracy of the cricket's hearing system is also reflected in the animal's precise phonotactic steering behaviour.

(Supported by the Royal Society)

# Poster Topic

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T18-17C The Cricket Auditory System Responds to Bilateral Phase-Shifts
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T18-18C The rise and fall of an experimental paradigm: orienting asymmetries and lateralized processing of sounds
*Julia Fischer, Ricarda I. Schubotz, Christoph Teufel*

# A comparative study of the torus semicircularis in actinopterygian fish

Wibke Michaela Lüdtke[1], Michael Hofmann[1]

[1]University of Bonn, Institut of Zoology, Poppelsdorfer Schloss, 53115 Bonn, Germany

The torus semicircularis is a midbrain structure that receives secondary mechanosensory lateral line and auditory information from the hindbrain. It is well investigated in a number of teleost species, but a comparative study involving species with divers auditory or lateral line specializations is lacking. We studied the cytoarchitecture of over 40 species of bony fish to find correlations with the lateral line and auditory systems.
We found that the TS consists of two major divisions, a lateral part with a single layer of small cells and deeper larger cells and a medial part with multiple small celled layers. In some species, the medial part may be missing or is inconspicuous, in some the lateral part is quite small. In general, species with a reduced lateral line system, as judged by the size of the hindbrain lateral line area, have a small lateral part (e.g. Diodon, Canthigaster) and species with a well developed lateral line system have a larger lateral part (e.g. Carassius, Holocentrus). The size of the medial part correlates with the ability to vocalize. It is especially large in species that use acoustic signals for communication (e.g. Pomacentridae, Holocentridae, Balistidae, Cichlidae). The size of the medial part, however, is not correlated with the presence of hearing specializations that connect the swim bladder with the inner ear, like in carp or goldfish where the medial part is inconspicuous. From a number of studies in different species, we know that the lateral part receives both, lateral line and auditory fibers. Since most cells in the torus semicircularis are bimodal, we suggest that the lateral part is processing complex hydrodynamic stimuli that often stimulate both, the lateral line AND inner ear. The medial part, that does not receive lateral line information may be specialized in analyzing pure acoustic communication calls. Many of these calls are in a higher frequency band above the maximum for the lateral line and unimodal acoustic processing is sufficient. Since most studies on the torus semicircularis are made in species that have a small medial part and a large lateral part, virtually nothing is known about the medial part. Currently, we are investigating the connections and the physiology of the medial part in order to confirm our hypotheses based on our comparative neuroanatomical data.

# A new approach for predicting binaural detection thresholds for sounds in quiet from the monaural thresholds

Heinrich Neubauer[1], Peter Heil[1]

1 Leibniz Institute for Neurobiology, ALS, Brenneckestr. 06, 39118 Magdeburg, Germany

The question whether the absolute threshold, measured with simultaneous input to the ears, is lower than the same threshold obtained monaurally, has been studied for a long time. A frequently cited review by Ira Hirsch entitled "Binaural summation – a centuary of investigation" was published more than 60 years ago (Psychol. Bull. 45, 193-206, 1948). But still, the magnitude of the effect and the underlying mechanism (e.g. binaural energy summation, probability summation or neuronal summation at threshold) are not fully understood. If each ear had an identical monaural threshold and if there were a perfect summation of energy at some level in the auditory system, the binaural threshold specified in dB SPL ought to be smaller by about 3 dB. A summation effect of that magnitude was found in a number of studies, but values significantly different from 3 dB have also been reported. Here, we present a new approach for predicting binaural absolute thresholds for sounds in quiet from their two directly comparable monaural counterparts, based on our model for monaural absolute thresholds. This monaural model consists of leaky integration of the stimulus amplitude, event formation, and temporal summation (LIEFTS; e.g. Neubauer and Heil, Brain Res. 1220: 208-223, 2008). The new approach allows the prediction of binaural thresholds even if the monaural thresholds are not identical and individual differences in sensitivity between the two ears are not equated. Our predictions are evaluated with a large number of measurements of monaural and binaural absolute thresholds (> 120 sessions with 6 threshold measurements for each of the 3 conditions) for tones of different envelopes from more than 30 human subjects. Based on the excellent agreement of these data with our predictions we draw specific conclusions regarding underlying mechanisms of binaural summation.

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# Activation of Primary Auditory Cortex and Posterior Auditory Field Evoked by Binaural Cues in Adult Congenitally Deaf Cats.

Peter Hubka[1], Jochen Tillein[2], Andrej Kral[1]

[1]Medical University Hannover, Laboratory of Auditory Neuroscience, 30625 Hannover, Germany [2]MedEl Company, Innsbruck, Austria

Intracochlear electrical stimulation of auditory nerve was shown to activate cortical structures in the primary auditory cortex. Further spread of the activation to nonprimary auditory fields has, however, not yet been investigated. Present study is aimed on the functional analysis of responses in the primary auditory cortex and the posterior auditory field (PAF) evoked by binaural electrical stimulation using cochlear implants. Local field potentials from four congenitally deaf cats (CDC) and three hearing controls were recorded simultaneously in AI and PAF by means of two 16- channel microelectrode arrays (Neuronexus probes). All animals were electrically stimulated; binaural responses were evoked by pulse trains (500Hz, 3 pulses) at intensities of 0-10 dB above response thresholds. In CDC, the cortical activation in PAF was repeatedly found in latency range corresponding to the activation pattern in hearing controls (peak latencies in the range of 20-30 ms) indicating propagating activation from the primary cortical fields. Evoked responses were, however, significantly smaller with different distribution of their peak latencies in PAF of CDCs. These results demonstrate activation of PAF by cochlear implants stimulation in adult deaf cats. However, substantial decrease in LFP amplitudes together with changed latency distribution in PAF indicate reduction of bottom-up propagation of activity and/or its desynchronisation in deaf animals.

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# Anatomical organisation of the auditory thalamocortical system in the Mongolian gerbil (Meriones unguiculatus)

Katja Saldeitis[1], Max Happel[2], Frank W. Ohl[2], Henning Scheich[1], Eike Budinger[1]

[1]Leibniz Institute for Neurobiology, Dept. Auditory Learning and Speech, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Leibniz Institute for Neurobiology, Research Group Neuroprostheses, Brenneckestr. 6, 39118 Magdeburg, Germany

The anatomical research on the auditory thalamocortical system is fundamental to understand auditory information processing in the brain. In the Mongolian gerbil, a valuable and frequently used model organism in auditory research, the detailed anatomical organisation of the auditory thalamocortical system is still unexplored.
Thus, we aimed to investigate the afferents arising from the three subnuclei of the medial geniculate body (MGB), namely the ventral (MGv), dorsal (MGd) and medial (MGm) subdivision, to the auditory cortex with respect to the following questions: How are the thalamocortical axonal terminals distributed with regard to the auditory cortical fields, cortical layers and their origin in one of the subdivisions of the MGB? Do strict point-to-point connections exist between the tonotopic maps of the thalamus and cortex or do the projections terminate in a rather divergent manner?
The issue was approached by injections of the anterograde tracer biocytin into a given subdivision of the MGB, using two different application methods. Firstly, larger amounts of the tracer were delivered by stereotactic pressure injections to reveal the global thalamocortical projection patterns. Secondly, smaller injections were made by microiontophoresis to label individual electrophysiologically characterized auditory thalamic neurons. In each case, the tracer was visualized by the ABC-Peroxidase reaction with diaminobenzidine as the chromogene to allow a light microscopic analysis. The distribution of labelled axonal terminals was analysed qualitatively and quantitatively mainly by means of the Neurolucida system.
Our data reveal previously unknown details on the auditory thalamocortical connections. They show highly specific features regarding their origin in one of the subnuclei of the MGB as well as their termination patterns in the auditory cortical fields and layers. In addition to tonotopically organised connections we found a high number of axons diverging across the tonotopic gradients of the auditory cortex. For example, magnocellular neurons of the MGm project in a columnar fashion to several auditory fields thereby forming various small- and medium-sized boutons in all but mainly infragranular cortical layers, but also forming hitherto unknown giant terminals in layers V and VI.
In conclusion, the results provide insights into a highly complex and specific organisation of the auditory thalamocortical connectivities in the Mongolian gerbil. The obtained data, together with our knowledge about corticothalamic and corticocortical circuitries, will be used for the establishment of an anatomically based model of information processing in the auditory thalamus and cortex.

# Auditory gating in the striatum and auditory cortex: Dynamics of cortical and striatal interactions and the effects of discrimination learning

Marie L Woldeit[1], Andreas L Schulz[1], Frank W Ohl[1,2]

[1]Leibniz-Institut für Neurobiologie, Dept. Neuroprosthesen, Brenneckestr. 6, 39118 Magdeburg, Germany.
[2]Otto-von-Guericke Universität Magdeburg, Institut für Biologie, Universitätsplatz 2, 39106 Magdeburg, Germany

In this study we investigated how auditory information is processed in the ventral striatum. We recorded local field potentials in chronically implanted Mongolian gerbils to study basal interaction between auditory cortex and the ventral striatum during auditory processing,using the sensory gating paradigm. Sensory gating is a process by which a sensory event modulates neuronal signals of subsequent sensory stimuli of the same or different sensory modality. Gating could be a filter mechanism preventing the brain from an overflow of distracting information. Functionally, the striatum is also thought to be a reward-evaluating structure. Therefore we were interested in how auditory processing and gating are altered once an auditory stimulus is associated with meaning. To investigate learning effects, animals were trained in a Go/NoGo task using trains of frequency-modulated tones. Neuronal responses were compared before and after the training. Even today it is not clear if auditory evoked potential gating is transmitted from one non-lemniscal site to another one, or if inhibition is carried out locally. To elucidate this question we additionally recorded and analyzed multi-units in both areas simultaneously.
Additionally, we were interested in whether neural interactions between auditory cortex and striatum of the ongoing activity are influenced by the discrimination learning procedure. Therefore we performed a coherence analysis between local field potentials prior and past of a conditioned stimulus (CS) and investigated the change of the coherence measure over training sessions.

# Auditory interactions during direction discrimination of frequency-modulated tones in humans

Alla Brodski[1], Bernhard H. Gaese[1]

[1]Goethe University Frankfurt, Institute of Cell Biology and Neuroscience, Siesmayerstraße 70a, 60323 Frankfurt am Main, Germany

Frequency modulations (FM) are a crucial feature in animal communication sounds as well as in human speech. They are essential, for example, for the discrimination of phonemes like 'ga' and 'da' or even whole words in tonal languages. Naturally, such FM tones do not appear in isolation, but are embedded in sequences of various other sounds. Therefore, perception of FM tone direction might very well be influenced by auditory events occurring immediately before or after it.

In order to investigate the influence of a preceding pure tone on FM direction discrimination, human subjects were presented binaurally with a higher or lower (±0.5 octaves) pure tone before a FM sweep in a 2-alternative-forced-choice-task (2AFC). Subjects were instructed to ignore the first tone and to attend to and determine the direction of FM modulation (upward or downward). We found a specific interaction between pure tone and the following FM: FM discrimination performance was significantly dependent on pure tone frequency. Higher hit rates occurred in case of a continuous arrangement in frequency developing between both tones (i.e. low pure tone and FM upward, or high pure tone and FM downward). In contrast, a discontinuous development of frequency (i.e. high pure tone and FM upward, or low pure tone and FM downward) resulted in lower discrimination performance. Furthermore, we quantified the subjects' performance according to signal detection theory, by calculating d' as a measure for "absolute" sensitivity and ß- norm as a measure for response bias. Differences in response bias depending on the preceding pure tone occurred in parallel to differences in discrimination performance.

Additional experiments revealed that this interaction between preceding pure tone and FM tone could not be abolished by an interspersed noise burst. Moreover, the specific interaction pattern could even be demonstrated in case of a frequency modulated first tone. We observed that in this case the frequency span between the two tones, not the modulation direction of the first tone was significantly influencing discrimination performance.
Using a multi-harmonic first tone with a missing fundamental revealed that the pitch of this complex affected FM direction discrimination for fundamental listeners in the same way as the pure tone in the first experiment. As for these listeners the fundamental frequency determines the perceived pitch, in spite of being physically absent, this indicates that this perceived pitch, not the spectral components were decisive for the described interaction. Consequently, it is very likely that this interaction takes place at a central, presumably cortical, level of the auditory pathway.

In summary, these results indicate that FM direction discrimination is strongly influenced by the acoustic context. The observed interaction between successive tones probably facilitates FM direction discrimination in natural conditions. This might have a general relevance in speech perception and animal communication.

# Birdsong and Melatonin

Susanne Seltmann[1], Lisa Trost[1], Rene Jansen[2], Sebastien Deregnaucourt[1], Andries ter Maat[1], Manfred Gahr[1]

[1]Max Planck Institute for Ornithology, Department Behavioural Neurobiology, Germany
[2]Vrije Universiteit Amsterdam, the Netherlands

Birdsong is a complex motor behavior that is learned in early life. The specific song patterns are shaped during a sensitive period in which auditory feedback guides vocal learning. In zebra finches the song motif of adult males is highly stereotyped and develops in two steps. In an early sensory phase a song memory is formed on the basis of a tutor song produced by an adult male zebra finch. Thereafter, the juvenile bird adjusts its song to the tutor's song during several weeks of sensorimotor learning. The final version of the individual song, the crystallized song, undergoes no further changes.
Responsible for this song learning process is the Vocal Control System, a neural network of song nuclei.
Two sensorimotor areas of this descending song control circuit are crucial for the production of the song pattern, HVC (nucleus hyperstriatalis ventrale, pars caudale) and RA (nucleus robustus archistriatalis. During singing, RA neurons show a song-specific firing pattern. Interestingly, in sleeping birds the firing pattern in RA neurons is also similar to that shown during singing. Thus, RA neurons produce a form of song „replay" during sleep.
The hormone melatonin is well known for its role in entrainment of circadian rhythms and melatonin receptors are therefore found in areas of the circadian system such as the retina, the suprachiasmatic nucleus and neuroendocrine areas of birds and mammals including humans. Melatonin 1B receptors are expressed by HVC and RA neurons. Since we are interested in a possible function of melatonin in sleep-related activity of neurons in the song circuit, we treated animals with melatonin and studied its effect on song production. In an earlier study we found that systemic administration of a melatonin-1B antagonist at the beginning of the night shortens the song and the motif length, and affects the song syllable lengths produced the next day. Furthermore, we found that the application of melatonin to brain slices decreases the firing-rate of RA-neurons. Because RA and HVC have no known clock function, we hypothesize that melatonin has a direct function in the neural control of the song motor pattern altering the song of the zebra finch.
Here, we report that melatonin treatment during constant light has the same effect on the vocal control system as night-sleep. Melatonin treatment affects both the neuronal brain activity and the song structure.
Neuronal activity was studied using chronically implanted electrodes in RA of male zebra finches equipped with wireless transmitters to obtain multi and single unit. The lightweight (~1g) and wireless design of the transmitters allows "normal" behavior patterns almost without limitation, mainly to roam freely in the cage and to interact with other zebra finches. Songs and calls are recorded as well.
Collected data were analyzed using spike sorting and song analysis software. Results will be discussed in view of recent findings concerning melatonin treatment, constant light and song replay during sleep.

# Characterization of a GFP-expressing pseudorabies virus (PRV-152) as a potential viral vector in Mongolian gerbils

Christian Porres[1], Ida Siveke[1], Alexander Kaiser[1], Benedikt Grothe[1,2], Felix Felmy[1]

[1]Ludwig-Maximilians-University, Division of Neurobiology, Munich
[2]Bernstein Center for Computational Neuroscience Munich

The development of optogenetic tools stimulating and monitoring neuronal activity offers a variety of new analytical strategies in neuroscience. A major challenge is the introduction of these optogenetic tools into specific neurons. In Mongolian gerbils genetic approaches are limited and viral transfection is thought to be an appropriate way to alter the genetic content of cells. A promising viral vector is pseudorabies virus (PRV), which shows a high specificity to neurons and spreads transsynaptically. PRV-152 is a GFP-expressing PRV, based on the attenuated PRV-Bartha strain that crosses synapses only retrogradely. Recent *in vitro* studies suggest changes in electrophysiology of infected neurons ~18 hours post infection. To further characterize PRV-152 and its feasibility of use in Mongolian gerbils we focused on the time course of viral spread after *in vivo* infection, neurospecificity and electrophysiological changes in infected neurons.

PRV-152 was stereotactically injected into the inferior colliculus or the dorsal nucleus of the lateral lemniscus in juvenile and adult Mongolian gerbils. Incubation times ranged between 12-54 hours. For histochemical analysis animals were transcardially perfused with 4% PFA. Electrophysiological characterization of infected neurons was performed by whole-cell current-clamp recordings obtained from acute brainstem slices of postnatal day 16 gerbils at room- and high temperature (~35 °C).

We show that PRV-152 infects neurons of different nuclei of the auditory brainstem in juvenile and adult gerbils. Neurospecificity is high and GFP-expression in infected neurons is intense. The time course of transneuronal spread is ~26 hours per crossed synapse indicating that after ~54 hours third order neurons are infected. Infected neurons show already after 12 hours post infection a depolarized membrane resting potential and an increased firing frequency. Thus, PRV-152 is a useful tool for anatomical studies but possibly less suited for electrophysiological questions. The use as a viral vector for electrophysiological experiments is restricted to first order infected neurons and the time window for experiments is short.

# Chemical heterogeneity of extracellular matrix in the mice MNTB

Maren Blosa[1], Gudrun Seeger[1], Gert Brückner[1], Rudolf Rübsamen[2], Thomas Arendt[1], Markus Morawski[1]

[1]Paul Flechsig Institute for Brain Research, Department for Molecular and Cellular Mechanisms of Neurodegeneration, Jahnallee 59, 04109 Leipzig, Germany

[2]Institute of Biology II, Department of Zoology and Neurobiology, Talstr. 33, 04103 Leipzig, Germany

The medial nucleus of the trapezoid body (MNTB) is the vital structure for sound localization in the auditory brainstem. Every principal cell of the MNTB is contacted by an excitatory giant axosomatic terminal ending called the calyx of Held. It is known that mammalian MNTB show prominent perineuronal nets (PNs), but the precise chemical composition in mice is not well-investigated.

PNs are a special kind of the extracellular matrix (ECM) in the central nervous system. They are associated with heterogenous subpopulations of neurons and occur in different forms of appearance. To date, the function of PNs is largely unknown. There is evidence that PNs are involved in stabilization of synaptic contacts, influence the ion homeostasis and have a potential protective function in neurodegenerative diseases.

With immunohistochemical and electronmicroscopical techniques we studied the chemical organization, structural appearance and cellular interaction of PNs in the mice MNTB. We could show that PNs of the principal cells of MNTB consists of proteoglycans differentially decorated with chondroitin side chains, tenascin, link protein and hyaluronan. Furthermore, we could demonstrate that the glycinergic principal cells which are innervated by glutamatergic and GABAergic synapses are enwrapped by a PN composed of a multitude of ECM proteins. Additionally, these neurons express calcium binding proteins and the voltage gated potassium channel subunit Kv3.1b, an established marker of fast-spiking neurons. Our data supports the hypothesis that PNs may influence the electric activity of neurons.

# Circuitry analysis of the central nucleus of the Inferior Colliculus of Mongolian gerbils

Lina Yassin[1], Felix Felmy[1]

[1]Ludwig Maximilians Universität, Dept. Biol. II, Div. of Neurobiology, Martinsried-Planegg, Germany

The Inferior Colliculus (IC), a major auditory nucleus in the midbrain, receives inputs from many brainstem auditory nuclei as well as higher brain areas. The central nucleus of the IC (cIC) is proposed to consist of distinct fibrodendritic laminea organized tonotopically, however little is known about the functional inputs and the intracollicular connectivity.

We performed whole-cell voltage-clamp recording from neurons of acute brain slices of Mongolian gerbils at postnatal day 15-17 at 34°C. Evoked responses were elicited using bipolar-stimulating electrode positioned in the lateral lemniscuses (LL). Evoked excitatory post -synaptic currents (eEPSC) were recorded in the presence of GABA and glycine receptors antagonists while evoked inhibitory post-synaptic currents (eIPSC) were recorded in the presence of AMPA and NMDA receptors antagonists.

Our results revealed two distinct neuronal populations, with different response profiles. Phasic -type (P-type) neurons responded with short latencies (<1.5 ms) and were localized in the dorosmedial region of the cIC. Recurrent–type (R- type) neurons responded with longer latencies and were localized ventrolaterally. Detailed analysis showed that the response time course (activation window) was almost tow fold greater for the R-type neurons compared to P-type neurons. This resulted in a two fold increase in synaptic charge. Furthermore, both frequency and amplitude of the spontaneous EPSC (sEPSC) of R-type neurons were significantly greater. The profiles of inhibitory currents were also different in these two populations. Surprisingly, both the latency and activation window of eIPSC of R-type neurons were tow fold greater. R-type neurons also exhibited greater sIPSC amplitude, but similar frequency compared to P-type neurons.

Taken together, our data shows spatially divergent populations with matching inhibition and excitation profiles. The existence of subnetwork or functional lamiae may shed more light on the specific function these regions may play in auditory processing.

# Coding of complex objects in the auditory midbrain of awake and behaving bats

Uwe Firzlaff[1], Lutz Wiegrebe[2], Susanne Hoffmann[2]

[2] Division of Neurobiology, Department Biology II, Ludwig-Maximilians-Universität Munich, Großhaderner Str. 2-4, D-82152 Martinsried-Planegg, Germany.

Echo imaging is an active sensory process by which microchiropteran bats are able to identify objects by the comparison of the emitted calls and the reflected echoes. In a natural situation, bats continuously adjust temporal and spectral parameters of their calls according to the changing requirements of the specific task. The processing of echo acoustic information by auditory neurons must therefore also be a dynamic process in which information about the emitted call must be included. However, with few exceptions neural coding of complex object has mainly been investigated in bats which were either anesthetized or not actively vocalizing.
In the current experiments we recorded neural activity with chronically implanted electrodes in the inferior colliculus (IC) of awake bats actively performing in a psychophysical discrimination task. The bats were trained to discriminate the degree of temporal envelope fluctuations ('echo roughness') of complex echoes of their own emissions. Neural activity following each emission onset in a fixed time window of 18 ms was analysed for 'smooth' and 'rough' echoes and compared for trials with wrong or correct decisions.
The results reveal that: 1) neural activity in the IC increased with increasing echo roughness thus corroborating the basic results obtained from anesthetised bats [1, 2]. Response rates evoked by smooth and rough echoes differed significantly for higher roughness differences and reflected the psychophysical performance of the bat. 2) In trials in which the bats could not discriminate the roughness of two echoes correctly, response-rate differences were less significant compared to correct trials. Thus, neural activity in the IC reflected the behavioural performance of the bats.

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# Comparison of appetitive and aversive reinforcement in an auditory discrimination task in mice

Angela Kolodziej[1,2], Wolfram Wetzel[1], Jürgen Goldschmidt[1], Frank W. Ohl[1,2]

[1]Leibniz-Institute for Neurobiology, Magdeburg, Germany
[2]Otto-von-Guericke-University, Magdeburg, Germany

Behavior is influenced by both appetitive and aversive stimuli. Here we investigate the influence of the polarity of the reinforcement on learning-induced mechanisms. To this aim we had previously developed a behavioral paradigm that allows the application of reward (electrical stimulation of the medial forebrain bundle) and punishment (foot shock) in the same experimental design in Mongolian gerbils (Ilango et al., Neurosci 2010) and in C57 BL/6 mice (Kolodziej et al., 8th Göttingen Meeting of the German Neuroscience Society). The animals were trained in a single response paradigm (shuttle-box) to respond to a change in auditory input (background: 8-4kHz, unconditioned stimulus: 4-8kHz). We use this new model to analyze the proteome of four brain regions (Prefrontal cortex, hippocampus, striatum, auditory cortex) after appetitive or aversive learning. In addition, we investigate the influence of the reinforcement polarity on learning correlates in the sensory processing using electrophysiological recording.

# Context-dependence of STRF-characteristics of primary auditory cortical neurons

Jan-Philipp Diepenbrock[1], Max F.K. Happel[1,2], Arne F. Meyer[3], Jörn Anemüller[3], Frank W. Ohl[1,2]

[1]Leibniz Institut for Neurobiology, Neuroprostheses, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Otto-von-Guericke University, Magdeburg, Germany
[3]Carl-von-Ossietzky University, Oldenburg, Germany

The spectro-temporal receptive field (STRF) is a well-established way of describing response properties of neurons in sensory systems.

Traditionally, it was used to characterize single neuronal response properties and predict a neuron's response to various stimuli. While this approach has performed well in predicting responses to stimuli similar to the ones used for estimating the STRF, problems regularly occur with predictions of responses to stimuli from more dissimilar classes. This is partially due to the fact that (higher) non-linearities are not taken into account, but also because certain requirements for application of the underlying theory are not fulfilled.

In this study, single and multi unit data of neurons in primary auditory cortex of anaesthetized Mongolian Gerbil (Meriones unguiculatus) was used to further investigate this context dependence of the STRF.

Therefore, neuronal responses have been recorded, using several stimulus sets of frequency modulated (FM) tones. The sets of FM tones have been designed such that they sample a large portion of the input space to yield a proper description of the response field while allowing various context conditions.

To this aim, we varied the ration of rising and falling FM-Tones to investigate neuronal responses to standard and deviant stimuli. Responses of neurons in new contexts as deviant events might show non-linearities or system states, which then could be described more precisely. Here we report results from STRF estimations using single and multi-unit activity in comparison to STRF estimation based on the local field potential (LFP).

# Development and function of voltage-gated calcium channels in the lateral superior olive of the Mongolian gerbil

Marc Christopher Ford[1], Veronica Egger[2], Benedikt Grothe[1], Ursula Koch[1]

[1]LMU Munich, Division of Neurobiology, Department Biology II, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany[2]LMU Munich, Dept. of Cellular Physiology, Institute of Physiology, Pettenkoferstr. 12, 80336 München

Changes in intracellular calcium concentration mediated by voltage -gated calcium channels (VGCCs) can contribute to the specific firing pattern of neurons and are also involved in plastic changes of neural circuits. The lateral superior olive (LSO) is one of the first stations in the ascending auditory pathway that process interaural differences. High- frequency LSO neurons process interaural level differences whereas low-frequency LSO neurons contribute to interaural time difference processing. They perform these computations by integrating excitatory inputs driven by the ipsilateral ear with inhibitory inputs driven by the contralateral ear.
Recent work indicates that VGCCs are necessary to model the typical in vivo response pattern of LSO neurons. Zacksenhouse and colleagues (JNeurophysiol 79:3098, 1998) suggested that calcium may mediate two aspects of LSO response patterns, (1) serial dependence of inter-spike intervals and (2) the chopping response seen in some LSO cells. Experimental data indicates the presence of VGCCs in the LSO before hearing onset (Kullmann et al., EurJNeurosci 15:1093, 2002), however, it is not known whether VGCC expression is maintained after hearing onset and, if so, whether VGCCs are expressed differentially in the high and low frequency limbs of the LSO.
To study the postnatal development of VGCCs in the LSO of Mongolian gerbils (Meriones unguiculatus), we performed whole-cell voltage-clamp recordings from neurons located in the high- and low-frequency limb of the LSO at three points in time during postnatal development: a few days before hearing onset (P9/10), some days after hearing onset (P14/15), and in mature-like neurons (P17/18). Ba2+ was used as a charge carrier to avoid Ca2+-dependent inactivation of calcium channels and Ba2+ -currents were pharmacologically isolated.
We found that at P9/10 Ba2+-currents consisted of a low- and a high-voltage-activated component in the high- as well as in the low-frequency limb of the LSO. After hearing onset, at P14/15 the low-voltage-activated component was not present anymore and the high-voltage-activated component has increased in amplitude. Between P14/15 and P17/18 Ba2+-currents developed differentially in the high- and low-frequency limbs of the LSO. In the high-frequency limb high-voltage-activated Ba2+-currents increased further in amplitude, whereas in the low-frequency limb the amplitude declined.
Using two- photon calcium imaging in a brain slice preparation we found that VGCCs were concentrated on dendrites, even at their very distal endings. Calcium transients could be evoked in the entire cell by single action potentials or action potential trains triggered by somatic current injections.
In current-clamp experiments pharmacological blockade of VGCCs resulted in an increase in input resistance whereas spiking -induced calcium influx through VGCCs decreased input resistance of LSO neurons and hyperpolarized the membrane potential.
These findings suggest that expression of VGCCs is maintained after hearing onset and that calcium influx through VGCCs might shape the integrative properties of LSO neurons.

# Development of synaptic inputs to the dorsal nucleus of the lateral lemniscus of Mongolian gerbils

Julian Johannes Ammer[1], Benedikt Grothe[1,3], Felix Felmy[1]

[1]Ludwigs-Maximilian University, Division of Neurobiology, Munich
[2]Graduate School of Systemic Neurosciences (GSN[LMU]), Munich
[3]Bernstein Center for Computational Neuroscience Munich

As a part of the binaural auditory pathway, the dorsal nucleus of the lateral lemniscus (DNLL) receives excitatory and inhibitory inputs from lower auditory brainstem nuclei and sends inhibitory GABAergic projections to the inferior colliculus and the contralateral DNLL. To better understand possible postsynaptic integration mechanisms and their development within the DNLL, it is essential to have information about the intrinsic membrane properties and synaptic inputs. The goal of this study was thus to comprehensively describe the developmental changes in basic membrane characteristics and in the kinetics of the major synaptic inputs to the DNLL.

For this purpose, we performed on-cell and whole-cell current- and voltage-clamp recordings from DNLL neurons in brain slices of Mongolian gerbils of four postnatal (P) age groups (P9-10, P13, P17 and P23-26) at 35°C. To characterise the synaptic input kinetics, synaptic currents were evoked with a concentric bipolar electrode and isolated pharmacologically. GABAergic currents were stimulated via the commissure of Probst and glutamatergic inputs were evoked by stimulating the fibres of the lateral lemniscus ventral to the DNLL.

The developmental changes we observed in basic membrane characteristics and action potential (AP) generation properties all point towards an enhanced capability of fast and precise signal integration in these neurons. Accordingly, the decay time constants of synaptic GABA, AMPA and NMDA currents decrease significantly with age. The comparison of evoked synaptic currents with miniature events recorded in the same neurons indicates that the synaptic release becomes more phasic during development. Furthermore, the NMDA/AMPA ratio decreases, indicating a reduction of the synaptic NMDA component over age. However, on-cell recordings of APs evoked by fibre stimulation followed by whole-cell voltage-clamp recordings or combined with the blockade of NMDA receptors revealed that the remaining NMDA receptor mediated current can still contribute to AP generation in older animals.

Taken together, the acceleration of synaptic inputs, the speeding in basic membrane parameters and the increased capability of fast AP generation with development suggest that relaying inhibition from the lower auditory nuclei to the midbrain with high temporal precision might be crucial for physiologically relevant auditory information processing. The finding that NMDA receptor mediated current can cause the generation of additional APs at all ages suggests that it affects the postsynaptic signal integration even in mature DNLL neurons by amplifying the excitatory input and thus prolonging the time frame for AP generation.

# Direct electrical effects on the macaque's auditory cortex induced by activation of the dopaminergic ventral mesencephalon

Judith Mylius[1], Alexander G. Gorkin[2], Mikhail Babanin[1], Michael Brosch[1]

[1]Leibniz Institute for Neurobiology, Special Lab of Primate Neurobiology, Brenneckestrasse 6, 39118 Magdeburg, Germany
[2]Institute of Psychology, Russian Academy of Sciences, Yaroslavskaya Street 13, 129366 Moscow, Russian Federation

During the last decades the mesocortical dopaminergic system was shown to be involved in working memory, learning, plasticity, reward, addiction, mental disorders and, therefore, has become of keen interest for many researchers. A vast number of studies focused on mesofrontal pathways, whereas recently other cortical areas have become of increasing interest. Previous studies in the auditory cortex of rodents indicated that the dopaminergic system is involved in memory consolidation and learning-induced plasticity. However, the data shown in such studies usually reflect the effects of dopaminergic activity on behavioural performance or acoustic responses only (e.g. receptive field plasticity). The direct electrical influence of the mesencephalic dopamine system on the auditory cortex has, however, been remained elusive.

Here we investigated the direct effects of electrical stimulation of the dopaminergic ventral mesencephalon on the auditory cortex in macaques. We recorded local slow-wave field potentials and multiunit activity from the auditory cortex in the cynomolgus monkey after electrical stimulation of the ventral mesencephalon.

Electrical stimulation of a large area in the midbrain was able to evoke similar responses in the auditory, the overlaying parietal and subjacent temporal cortex. The electrically evoked field potentials (EEP) revealed surprisingly stereotypic wave forms, consisting of three to four waves, cycling at approximately 4-10Hz and might, therefore, potentially be ubiquitous in the neocortex. Systemic application of the dopamine D1-like receptor antagonist Sch23390 affected the EEP waveform in a region dependent manner. For stimulation electrodes positioned in more ventral sections of the mesencephalon in areas, corresponding to the ventral tegmental area (VTA) for example, revealed an inversion or latency shift of the EEP waveform, whereas positions in more dorsolateral parts, e.g. of the substantia nigra (SN), revealed nearly a complete vanishing of the EEPs. No effects of the pharmaceutical Sch23390 were observed on acoustically evoked potentials (AEP) in the auditory cortex. Multiunit activity revealed three distinct response types: (1) an early excitation (~40ms), followed by inhibition, (2) an excitation-only at ~75ms or (3) an excitation-only at ~110ms.

These data suggest that electrical stimulation of the ventral mesencephalon can evoke firing in the auditory cortex and therefore can have driving and not only modulatory effects on the neuronal activity in the auditory cortex, in addition to the long-term effects observed in previous studies.

Furthermore our data confirm that the VTA is not the only source of mesocortical dopaminergic innervation. Instead it seems, confirming earlier studies, that all mesencephalic dopamine sources (A8, A9, A10) contribute to widespread cortical innervations in a distinct manner, explaining the relatively huge area in the midbrain, where the here described stereotypic waveform of the EEPs could be evoked. The distinct effects of Sch23390 depending on the positioning of the stimulation electrodes are possibly due to additional impacts on intermediary polysynaptic relay stations or the result of the functional interaction of specific neurotransmitters. VTA neurons e.g. are known to use beside dopamine, gamma-aminobutyric acid and glutamate as neurotransmitter, whereas the latter can also been co-released with dopamine.

# Distribution of calcium-binding proteins in the auditory cortex and medial geniculate body in adult and juvenile short-tailed fruit bats

Julia Heyd[1], Marianne Vater[1]

[1]University of Potsdam, Institute of Biochemistry and Biology, Unit of Zoology, Karl-Liebknecht-Str. 24-25, 14476 Potsdam, Germany

The frugivorous short- tailed fruit bat ( *Carollia perspicillata*) uses brief, multiharmonic frequency -modulated echolocation signals for spatial orientation and target approach.
The hypertrophied auditory cortex consists of six fields among which are the tonotopically organized primary auditory cortex (AI) and two chronotopically organized high frequency (HF) fields which analyze the target distance through delay-sensitive cortical neurons.
This study investigates the distribution of calcium-binding proteins (CaBPs) in the auditory cortex and the medial geniculate body (MGB) of newborn and adult short- tailed fruit bats. Double-immunofluorescence-labeling techniques were employed using antibodies directed against parvalbumin (PV), calbindin (CB) and calretinin (CR). In mammals, these CaBPs are known to be expressed by non- overlapping subpopulations of GABAergic interneurons in the adult neocortex and to be differentially expressed in subdivisions of the MGB.
In the auditory cortex of adult short- tailed fruit bats, PV -immunoreactive ( -ir) neurons and strongly labeled neuropil were abundant in layers II to VI. In contrast, CR -ir neurons were extremely rare throughout all cortical layers, but the neuropil was intensely labeled, especially in layers III and IV. The few heavily labeled CB-ir cells were most common in the infragranular layers. Some of these neurons colocalized PV-immunoreactivity. The most intensely CB -labeled neuropil was found in layers III and IV. This basic distribution pattern of CaBP-immunoreactivity did not differ between AI and the HF fields.
Complementary distribution patterns of CaBP-immunolabeling were found in the MGB of adult bats. All main divisions of the MGB contained strongly labeled neuropil and neurons in CR - and CB-immunochemistry. Only the suprageniculate nucleus (Sg) was weakly labeled. The PV-immunoreactivity showed the opposite distribution. Only the large neurons in the Sg displayed strong PV-immunostaining, whereas in the remaining subdivisions PV-ir neurons were lacking. No double-labeling of PV-ir somata with CB- or CB-antibodies was present.

In newborn bats, an almost adult-like distribution pattern of these CaBPs was observed both in the auditory cortex and the MGB. These results indicate that auditory forebrain structures in short- tailed fruit bats are relatively mature at birth. This differs from findings in the insectivorous mustached bat (*Pteronotus parnellii*), which belongs to a related family within the superfamily of Noctilionoidea, in which the distribution of CaBPs in the auditory thalamus and cortex dynamically changes during and beyond the first postnatal month.

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# Effect of harmonicity on the detection of a signal in a complex masker and on spatial release from masking

Astrid Klinge[1], Rainer Beutelmann[1], Georg Martin Klump[1]

[1]Carl von Ossietzky University Oldenburg, Animal physiology and behaviour group, IBU, Carl-von-Ossietzky Str. 9-11, 26129 Oldenburg, Germany

In an acoustically complex environment, the perception of a behaviourally important target sound is often compromised by interfering masking stimuli. The amount of masking depends not only on the spectral and temporal characteristics of masker and target such as harmonicity, spectral content, modulation patterns, and common onset but also on their relative spatial location. In the natural environment these cues rarely occur in isolation. Therefore, a combination of cues needs to be studied to evaluate the ability of the auditory system to detect a behaviourally relevant sound in a masking environment.

A free-field simultaneous masking experiment providing a combination of harmonicity and spatial cues was conducted in humans. A pure tone target had to be detected in a masker that was a harmonic or inharmonic complex or a noise burst. One form of masking is the energetic masking that arises due to an overlap of acoustic energy of the target and the masker in an auditory filter. However, non-energetic effects also have an influence on the amount of masking. For example, additional masking occurs when there is a high stimulus uncertainty ("informational masking"). On the contrary, grouping cues can improve the detection of a target in a masker. The detectability of a target in a masker can also be considerably improved by separating both spatially. The spatial release from masking (SRM) is defined as the difference between the masked threshold in the co-located configuration (masker and target from the same direction) and the masked threshold in the spatially separated configuration (masker and target coming from different directions).

Thresholds were determined for combinations of two target frequencies (1 and 8 kHz), two spatial configurations (co-located, masker and target spatially separated by 90 degrees azimuth), and five different masker types (four complex stimuli, one noise masker). The results were compared to masked thresholds that were predicted by a combined binaural and energy detector model assuming that only energetic masking occurs. The amount of masking significantly depended on the masker type. An inharmonic relation between a harmonic complex masker and a mistuned target improved the masked thresholds for both 1 and 8 kHz targets compared to thresholds estimated for purely energetic masking. A harmonic relation between target and a harmonic complex masker had no effect on the amount of masking in the 1 kHz condition but masked thresholds were improved in the 8 kHz condition. An inharmonic masker with a constant frequency composition throughout a session resulted in thresholds similar to those obtained with the harmonic complex masker. An inharmonic complex masker with a frequency composition varying randomly throughout a session resulted in an elevated threshold in the 1 kHz condition compared to the predicted energetic masking suggesting informational masking. Spatial release from masking was found in all masker types as predicted by the energetic masking model for both frequency ranges, but it was only significant for the 8 kHz targets.

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# Effects of cortical cooling on single unit responses in auditory thalamus of awake marmosets

Marcus Jeschke[1], Frank W. Ohl[2,3], Xiaoqin Wang[1]

[1] Laboratory of Auditory Neurophysiology, Department of Biomedical Engineering, Johns Hopkins University, Baltimore, Maryland, USA
[2] BioFuture Res. Group, Leibniz Inst. for Neurobiology, Magdeburg, Germany
[3] Inst. for Biol., Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

A number of anatomical studies have shown that auditory cortex sends massive feedback connections to the auditory thalamus. Based on their microstructural appearance most of these connections have been believed to have a modulatory role on thalamic processing. How this suggested modulation manifests itself has not been fully explored. Few experiments have investigated the nature of corticothalamic modulation in awake animals. We used a small cooling probe to inactivate the auditory cortex of awake common marmosets (*Callithrix jacchus*) while recording single-unit activity in the auditory thalamus. In the present study, we investigated the effects of corticofugal feedback on the processing of time-varying sounds and spatial slectivity in auditory thalamus.
The maximum stimulus evoked firing rate was mostly reduced as a result of cooling the auditory cortex. Spatial receptive fields of thalamic neurons generally became narrower when auditory cortex was inactivated by cooling. Best azimuths mainly stayed within the same hemifield while best elevations tended to shift towards 45 degree elevation. Using temporally modulated stimuli we observed reduced synchronization in the thalamus especially at lower modulation frequencies, coinciding with the frequency range in which cortical neurons synchronize their firing to temporally modulated stimuli. In addition, cooling- induced changes in synchronization of thalamic neurons appeared to occur largely independent of firing rate changes. Our findings suggest that the processing of spatial locations as well as temporal modulations in auditory thalamus is regulated by the corticofugal feedback.

# Effects of hearing aids use on sound processing in primary auditory cortex of Mongolian gerbils

Konstantin Tziridis[1], Sönke Ahlf[1], Holger Schulze[1]

[1]University of Erlangen-Nuremberg, Experimental Otolaryngology, Waldstrasse 1, 91054 Erlangen, Germany

Damage of the cochlea caused by acoustic trauma, ongoing noise exposure, injuries or simply age (presbyacusis) results in hearing impairments. In humans, these are usually treated with different types of hearing aids that are adapted to the individual hearing impairment, e.g. by amplification and dynamic range compression of relevant frequencies. Unfortunately, these adjustments often do not lead to "normal" sound perception – even with advanced devices – and often hearing aids are refused by the users. As perception of sound is determined by its cortical processing and representation we here examine the effects of hearing aid use on sound processing in primary auditory cortex of Mongolian gerbils.

Single and multi-units responses to pure tones were recorded in the left auditory cortex of gerbils with and without two different commercial hearing devices (HD), a simple one-channel system and an advanced six-channel device. In healthy, normal hearing animals, HD use led to a strong increase in firing rate and a shift in the best frequency reflecting the spectral transfer function of the HD. This increased firing rate was expected as it resembles response changes of cortical auditory neurons with monotonic rate-intensity-functions. In contrast, in hearing impaired animals (acoustic trauma at 2 kHz, 115 dB SPL, 75 min) one-channel HD use led to a reversed effect in ca. 80% of the neurons: neuronal discharge rate did not significantly increase the or even decreased in response to pure tones at day 7 after the trauma and a shift of the best frequency was observed in less than 10% of the recorded neurons.

These results point to a reduced dynamic range of auditory cortical neurons in the hearing impaired animals. This may explain the problems reported by many patients with one-channel HDs; especially the diminished dynamic range of loudness perception. This in turn may explain the problems with speech intelligibility reported by hearing impaired subjects in noisy environments.

We now have started to use six-channel HDs and will compare the effects found with the one channel system with the neuronal responses obtained from animals supplied mono- and binaural with the more advanced HDs.

# Effects of motion history on motion-onset auditory evoked potentials

Ramona Grzeschik[1], Martin Böckmann-Barthel[2], Roland Mühler[2], Michael B. Hoffmann[1]

[1]Otto-von-Guericke-University of Magdeburg, Department of Ophthalmology, Visual Processing Laboratory, Leipziger Straße 44, 39120 Magdeburg, Germany

[2]Otto-von-Guericke-University of Magdeburg, Department of Experimental Audiology and Medical Physics, Leipziger Straße 44, 39120 Magdeburg, Germany

Purpose: Adaptation with visual motion greatly affects visual evoked potentials to visual motion-onset [1]. Here we tested whether adaptation with auditory motion affects potentials evoked by auditory motion-onset. Methods: Auditory evoked potentials (AEPs) were recorded from 33 EEG channels in 18 subjects to the motion onset of a sound (white noise) virtually moving in the horizontal plane at a speed of 60 deg/s from straight ahead (0 deg) to the left (-30 deg) or right (+30 deg), presented in randomized order. AEPs for baseline- and adaptation-blocks were compared in a balanced design. In each trial (1500 ms duration) onset of a 500 ms epoch of auditory motion was presented after a 1000 ms epoch of a central stationary sound. For the baseline blocks no additional motion history was presented. The adaptation blocks were preceded by a 1 minute block of adaptation, consisting of an adaptor sound source moving 60 times from +30° to -30° or, in a different block, in the opposite direction. This preceding adaptation was topped up regularly after six stimulus trials by another nine unidirectional repetitions of the adaptor sound. This paradigm opens the possibility to assess whether motion adaptation affects the motion onset AEPs and whether it does so in a direction specific manner. Results: Typical motion-onset AEPs were obtained for the baseline condition, namely a fronto-central response complex dominated by a negative and a positive component, the so-called change-N1 and change-P2 after around 175 and 260 ms, respectively. For the adaptation condition this complex was shifted significantly into the positive range, indicating that adaptation abolished a negativity within a time window of approximately 150 to 260 ms. This shift was more pronounced for adaptation and test stimuli moving in the same than in the opposite direction. Conclusion: Motion-onset AEPs critically depend on history [2]. Consequently, adaptation has to be taken into account as a potential confound in the design of motion-AEP studies. On the other hand, adaptation might be of assistance for the isolation of AEP correlates of veridical motion processing and to reveal the neuronal substrate of direction specific processes of auditory motion perception.

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# Electrophysiological characterization of an fMRI-identified voice-preferring region

Catherine Perrodin[1], Christoph Kayser[1], Nikos K. Logothetis[1,2], Christopher I. Petkov[1,3]

[1]Max Planck Institute for Biological Cybernetics, Physiology of Cognitive Processes, Spemannstrasse 38, 72076 Tuebingen, Germany
[2]Imaging Science and Biomedical Engineering, University of Manchester, Manchester, UK
[3]Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, UK

A region of 'voice' clusters has recently been identified in the macaque auditory cortex with functional magnetic-resonance imaging (fMRI). These clusters show a strong fMRI activity preference for the voice of conspecific individuals and appear to functionally correspond to those from the known human voice region. In the visual system fMRI has been used to guide electrophysiological recordings from neurons in the monkey brain that were shown to be extremely selective for faces, so-called 'face' cells. We investigated whether fMRI-guided electrophysiology would reveal comparable levels of selectivity in one of the monkey voice clusters, and how the functional properties of those 'voice' cells compares to those of their visual counterparts.

During fMRI acquisition and electrophysiological recordings, three categories of 12 sounds were used for stimulation: macaque vocalizations (MVocs), other animal vocalizations (AVocs), and natural sounds (NSnds). The sound categories were comparable in their low-level acoustical features, having been selected for this from a large set of sounds. We first used the stimuli during fMRI, as we have previously done, to identify clusters with a strong activity preference for MVocs. Then electrophysiological responses to the auditory stimuli were recorded from the anterior voice cluster in two awake macaques (total of 186 responsive single- and multi-units). A significant majority of the neurons (45%, X2-test: $p = 0.0013$) responded better to MVocs than to any of the other two complex natural sound categories. The area's preference for MVocs was also present in the population spiking and local-field potential response, consistent with the fMRI results. Adapting the frequently employed criterion used to define 'face' cells as responding at least two-fold stronger to faces than to other objects, 25% of the neurons recorded could be classified as 'voice' cells. Finally, we evaluated the response selectivity to individual stimuli within the MVocs, and found that units in the voice area responded to an average of 27% of the MVocs stimuli.

Our results suggest that a strong fMRI activity preference need not result from a large proportion of highly selective neurons, and describe a population of neurons with a preference for voices over other complex natural sounds. The proportion of identified 'voice' cells is comparable to what the majority of studies on 'face' cells report. However, 'voice' cells seem to be more selective for individual voices than 'face' cells, which have been shown to respond to ~62% of the face stimuli. This divergence in functional properties between 'voice' and 'face' cells may reflect evolutionary differences that have affected voice- and face- specialization in primate brains. Namely, the visual system appears to have specialized during vertebrate evolution to represent canonical facial features (e.g., two eyes, a nose and a mouth). By contrast, the auditory system could have had less opportunity to specialize for canonical auditory features, given that many animals modify the acoustics of their vocalizations to be distinct from those of other animals and to circumvent environmental noise.

# Factors controlling the input-output relation of spherical bushy cells in the gerbil cochlear nucleus

Thomas Kuenzel[1], J. Gerard. G. Borst[1], Marcel van der Heijden[1]

[1]Department of Neuroscience, Erasmus MC Rotterdam
[2]Institut für Biologie II, RWTH Aachen

*Motivation:* Despite the presence of large endbulb inputs, the spherical bushy cells (SBC) of the rostral anteroventral cochlear nucleus do not function as simple auditory relays. Instead, failures of transmission occur frequently in vivo. We wanted to understand how cell-inherent factors and properties of excitatory and inhibitory inputs converging on SBC contribute to failures of transmission and how failures of transmission impact the coding function of SBC in the auditory pathway.
*Method:* We used juxtacellular recordings in anesthetized, adult gerbils in combination with closed-field sound stimulation. The good signal-to-noise ratio of these recordings allowed us to address cell-physiological questions in the intact animal by event-based analysis of the complex waveforms from the SBCs.
*Results:* We identified two main causes for failures in spontaneous events. First, the Endbulb of Held synapse operated close to action potential threshold and did not show evidence for short-term depression. This suggests that the Endbulb of Held synapse had a much lower release probability in vivo than expected from previous in vitro studies, and that many failures resulted from stochastically occurring weak synaptic events. Second, the refractory period of the postsynaptic membrane increased the threshold of the postsynaptic membrane at small inter-spike intervals. During sound stimulation, many more failures occurred. A non-interval-dependent increase in the minimum size of the EPSP needed to trigger an AP was identified as the cause of these failures. Simulations suggested that weak, delayed, non phase-locked, hyperpolarizing inhibition could sufficiently explain the observed effects. By using the threshold increase as a metric for inhibitory strength we could characterize the properties of the inhibitory inputs: frequency tuning was similar to excitatory inputs, but thresholds were generally higher.
Remaining successful events in inhibited conditions were better phase-locked than the totality of inputs, causing an increase in temporal precision from input to output at the level of the SBC, despite the fact that AP generation close to threshold introduced additional temporal jitter.
*Interpretation:* We thus conclude that through the cell-inherent and stimulus related mechanisms that we identified, the SBC can dynamically adapt their sensitivity and temporal coding. At low stimulus levels, SBC are sensitive detectors and also code the stimulus level in their output rate. At higher levels, SBC output becomes a precise spike-timing code of the stimulus temporal structure, but at the cost of strongly reduced output rates.

# Functional microcircuitry of spectral integration and perceptual relevance of recurrent corticothalamic loops in primary auditory cortex

Max Happel[1], Marcus Jeschke[1], Juliane Handschuh[1,2], Mathias Deliano[1], Frank W. Ohl[1,2]

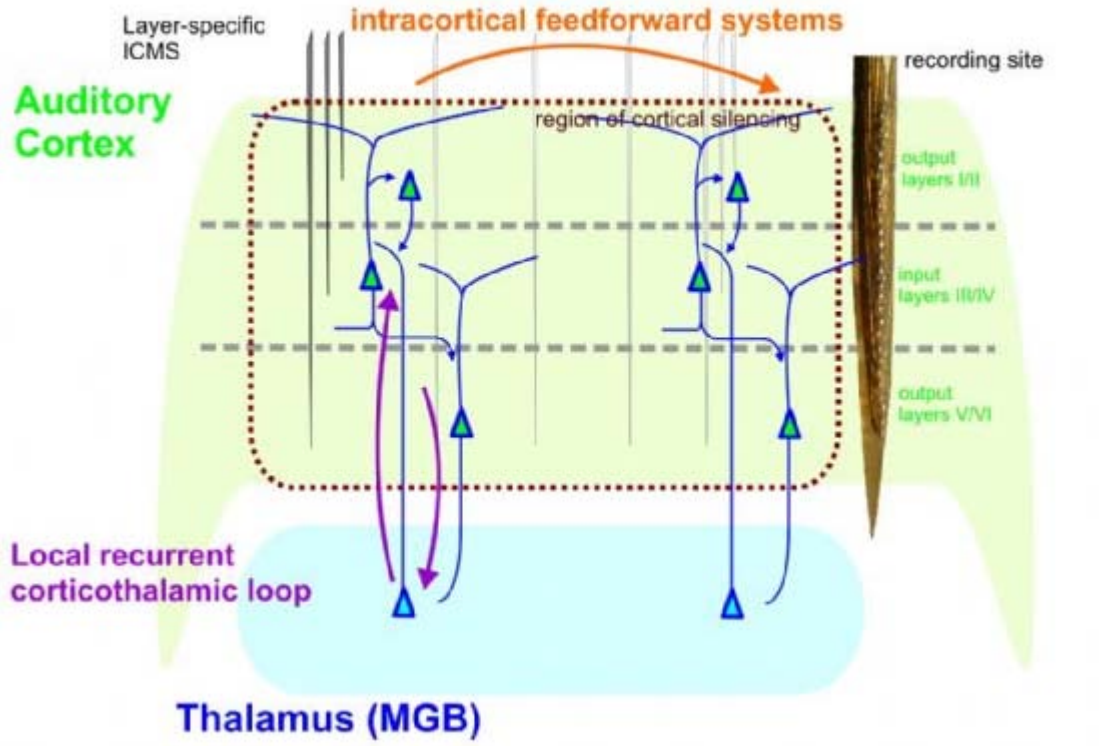
[1]Leibniz Institute for Neurobiology, Auditory learning and Speech, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Otto-v.-Guericke University, Magdeburg, Germany

Local processing of feedforward input in sensory cortex strongly depends on different short- and long-range intracortical pathways, as well as on corticofugal pathways. Though various functions have been attributed to these pathways, their specific roles for representation of physical stimulus features, as well as perception are still under debate. By a combination of methods, including current-source-density (CSD) analysis, pharmacological deactivation, and layer-specific intracortical microstimulation (ICMS) in primary auditory cortex of Mongolian gerbils, as well as by behavioral signal detection analysis, we dissociated different functional roles of the aforementioned pathways in spectral integration and perception in general.

We found a temporally highly precise integration of thalamocortical inputs and intracortical horizontal inputs when the stimulation frequency was in close spectral neighborhood of the best frequency, where the overlap between both inputs is maximal. Local horizontal connections provide both, directly feedforward excitatory and modulatory input from adjacent cortical sites, which determine how concurrent afferent inputs are integrated (Happel et al., 2010, J.Neurosci.). Further, we differentially activated the aforementioned subsystems using layer-specific ICMS. Analysis of detection performance showed that behaviorally interpretable percepts were caused by driving recurrent corticothalamic feedback loops, whereas wide-spread activations across cortical columns per se had no perceptual impact.

Our data suggest a conceptual framework of information flow in primary auditory cortex based on temporally precise interactions of afferent inputs and different short- and long-range intracortical networks and a local recurrent excitatory loop in the cortico-thalamocortical circuitry, which serves as a re-entrant loop of input information, which provided neural signals suitable for a “read-out” by horizontal cortical processing.

Experimental design of layer-specific ICMS and CSD-recordings



# Implications of stimulus-level and inter-stimulus-interval on adaptation in the barn owl's auditory midbrain

Roland Ferger[1], Martin Singheiser[1], Mark von Campenhausen[1], Hermann Wagner[1]

[1]Institute for Biology II, RWTH Aachen University, Department of Zoology and Animal Physiology, Mies-van-der-Rohe-Straße 15, 52056 Aachen, Germany

Neurons in the auditory system typically respond with a higher rate to the onset of a stimulus than during ongoing stimulation. This decline in their initially high response rate to a steady-state at later phases of the stimulus presentation is termed spike-frequency adaptation. The underlying mechanism is different from adaptation-of-excitation, where the capacity of a neuron to respond to subsequent stimuli is reduced, when the first stimulus was excitatory itself. Adaptation has been observed at almost all levels of the auditory system and is linked with effects like the "dynamic-range problem" or novelty detection.

In this study we were interested whether adaptation occurs in monaural responses of the narrowband neurons in the central nucleus of the inferior colliculus (ICC) of anaesthetized barn owls. We first determined the best frequency (BF) of a neuron using tones of varying frequency with 100 ms duration and 5 ms cosine-squared on and off ramps. Secondly, a monaural rate level function (RLF) was recorded at the neuron's BF. We then tested adaptation at BF by presenting two consecutive stimuli. The first stimulus was presented at a given level (10%, 30%, 50%, 70%, 90% of the saturating responses of the RLF); the second stimulus was presented either at varying levels of the second stimulus (2ndlevel tuning, ISI = 0 ms, level varying in 5 dB steps from 0 to 25 dB louder) or at varying inter-stimulus intervals (ISI, ISI tuning, ISI varying from 25 ms to 1600 ms, equal levels). We quantified adaptation by several measures: first, the response ratio (the quotient of the response to the second stimulus divided by the response to the first stimulus); second the adaptation ratio (quotient of the response rate at steady state divided by the peak response in the peri-stimulus-time histogram (PSTH)) and third the time constants t of the decline of the response in the PSTH.

The responses ratio in the 2nd level tuning clearly depended on the level of the saturating responses in the RLF. At levels louder than 70% of the RLF, the response of the second stimulus was always smaller than the first response and no compensation could be achieved by increasing the level of the 2nd stimulus. At lower intensities (10-30% of the saturating response in the RLF), an increase in level of 3 to 8 dB of the second stimulus resulted in equal response rates to both stimuli. In the ISI tuning, the effect was less pronounced and after an ISI of about 800 ms, no significant differences in response rate to the two stimuli could be observed for the five intensities of the RLF. The comparison of the adaptation ratios in both the 2nd level and ISI tuning revealed no significant differences between the first or second stimulus. For the time constants t we found no correlation with BF neither in the binaural nor in the monaural stimulation. However, in the 2nd level tuning, time constants of the response to the second stimulus increased in comparison to the first stimulus with decreasing level of the saturating response (70-10% of the RLF). This effect was significant especially at higher intensities of the second stimulus (15 to 25 dB louder than the first stimulus). No such observation could be made in the ISI tuning, where t seemed to be independent of stimulus level and ISI.

# Interaction (collision) of acoustic and direct electric cortical stimulation of gerbil primary auditory cortex AI

Achim Engelhorn[1], Matthias Deliano[1], Frank W. Ohl[1, 2]

[1]Leibniz Institute for Neurobiology, BioFuture Research Group, Brenneckestraße 6, 39118 Magdeburg, Germany
[2]Otto von Guericke University, Faculty of Natural Sciences, Magdeburg, Germany

Simultaneous acoustic (pure tones) and direct electrical (biphasic, charge-balanced current pulses) stimulation of gerbil primary auditory cortex AI was investigated as a model of how physiological processing in the cortex is changed by artificial, direct electrical stimulation (acoustic-electric collision).
Two stimulation electrodes (S1, S2) were implanted tangentially in layer IV with a distance representing a frequency difference of 2-3 octaves in the AI tonotopic map. For the recording of local field potential (LFP) depth-profiles, a shaft-multielectrode (23 channels, 65µ interelectrode distance) was implanted radially in the vicinity of the caudal stimulation electrode. Best frequencies of the caudal and rostral stimulation electrode site were taken as best (

# Interactions revealed by functional receptor and synapses distributions in medial superior olive neurons of the adult Mongolian gerbil

Kiri Anne Couchman[1], Benedikt Grothe[1,3], Felix Felmy[1]

[1]Ludwigs-Maximilian University, Division of Neurobiology, Munich
[2]Graduate School of Systemic Neurosciences ($GSN^{LMU}$), Munich
[3]Bernstein Center for Computational Neuroscience Munich

In mammals, the location of low frequency sounds in the horizontal plane is initially processed in the medial superior olive (MSO) by a neuronal coincidence mechanism. These neuronal coincidence detectors are highly sensitive to the timing of binaural excitatory and inhibitory inputs. There is anatomical evidence that these input types are segregated on MSO neurons. However, to date there has been no functional investigation of location or pharmacological description of synaptic inputs in mature animals. Here, we investigated the functional distribution of receptors and their synaptic inputs, and explore some functional consequences of these distributions in MSO neurons from matured, 30 day old Mongolian gerbils.

The distribution of pharmacologically isolated AMPA, NMDA and GABAA receptors (Rs) was determined using single-photon UV-laser uncaging of MNI-glutamate and CNB-GABA combined with whole-cell voltage-clamp recordings. For glycine (Gly) Rs, minimal picospritzer pressure-application was used. Synaptic input to these receptors was localised to cellular compartments using local pressure-application of a 40 mM $K^+$ solution. Further experiments utilised electrical fiber stimulation and uncaging techniques. Functional receptor and synapse distributions were recorded at room temperature; other experiments were completed at ~35°C.

We find that AMPARs were evenly distributed on MSO dendrites, matching their synaptic input. GlyRs were mainly somatically restricted, along with their synaptic input, though a substantial current was still present on distal dendrites. We also report the functional maintenance of NMDARs and GABAARs on mature MSO neurons. However, it was not possible to stimulate synaptic input to GABAARs, suggesting a role for these receptors in volume transmission. A consistent synaptic input to NMDARs was also difficult to elicit, with a minimal NMDAR component present in excitatory current from only 10/18 cells. In search of a functional role for these predominantly extra-synaptic NMDARs, we uncover an interaction with synaptically released glycine at the MSO soma.

These results suggest that a role for NMDA and GABAA signalling may be maintained even at mature stages in the MSO. These additional conductances may play a role in activity-dependant gain control or homeostatic mechanisms. Furthermore, they introduce the possibility for interactions between excitatory and inhibitory transmission systems at the level of the neuronal membrane, dynamically modulating coincidence detection at this nucleus.

# Layer-specific intrinsic properties of pyramidal neurons and interneurons in the auditory cortex of mice

Andreas Abraham[1], Hartmut Niekisch[1], Florian Hetsch[1], Marianne Vater[1]

[1]University of Potsdam, Institute for Biochemistry and Biology, Department of Zoology, Karl-Liebknecht-Str. 24-25, Haus 26, 14476 Potsdam, Germany

In the neocortex, GABAergic inhibition opposes excitatory transmission, in both the spatial and temporal domain. Hence, inhibitory cortical interneurons serve to coordinate and synchronize the activity of larger populations of neurons during oscillatory and transient brain states. In the auditory cortex, interneurons play a pivotal role in the analysis and extraction of auditory information, including shaping of frequency tuning, possibly by feed-forward and feedback inhibition onto pyramidal neurones. Such inhibitory processes can be simple but effective means of noise suppression, but also mediate selection among competing inputs, and possibly even implement complex computations. Simplified, within a cortical column, layers III and IV receive the major lemniscal thalamic input of auditory information, initiating a flow of information up in the supragranular layer II (and upper parts of layer III) where lateral projection form horizontal connections to other cortical columns. Moreover, the processed information is send down to the infragranular layers V and VI which provide subcortical projection, and feedback projection to the thalamus.

Despite of their important function, the information about the intrinsic properties, the morphology and the distribution of auditory cortical interneurons is limited. Here, we used the whole-cell patch clamp technique to compare some of the intrinsic properties of pyramidal neurons and interneurons in layers II-VI of the mice (postnatal age > 14 days) auditory cortex. A set of 25 electrophysiological parameters was tested, and dependent on the measured parameter a total number of 15-52 pyramidal neurons and 5-17 interneurons were included in the analysis. For morphological investigations, some cells were labelled with fluorescence markers and analyzed with confocal microscopy.

First, we could show a pronounced layer-specific variability of intrinsic properties in interneurons but not in pyramidal neurons. Second, in layer II current clamp analysis revealed a significantly more depolarized resting membrane potential, higher input resistance, lower conductance, lower activation threshold, smaller action potentials (AP), shorter AP width, a more pronounced sag, and smaller neuronal capacitance in interneurons compared to pyramidal neurons. Voltage clamp analysis was employed to evaluate the time- and voltage-dependent kinetics of two hyperpolarization-activated currents, i.e. the fast inward rectifier (Kir) and the more slowly activating IH, consisting of a fast (IH-fast) and slow (IH-slow) component. Compared to pyramidal neurons, interneurons exhibit significantly smaller Kir currents, slower time constants for IH-fast and IH-slow, and a smaller total current. In layers III/IV, interneurons showed a significantly more depolarized resting membrane potential, smaller APs, shorter AP width, smaller neuronal capacitance, slower time constants for IH-fast, and higher densities for Kir, IH-slow and total current when compared to pyramidal neurons. In layers V/VI interneurons showed smaller APs, shorter AP width, and an almost significant smaller neuronal capacitance compared to pyramidal neurons.

In conclusion, the distinct differences of intrinsic parameters between interneurons and pyramidal neurons in auditory supra- and infragranular layers are probably governed by different expression of inwardly rectifying conductance's (Kir and IH) and may contribute to different stages of information processing in different parts of a cortical column.

# Layer-specific processing of wriggling calls in the auditory cortical fields of mother mice

Diana Beatrix Geissler[1], Günter Ehret[1]

[1]University of Ulm, Institute of Neurobiology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Spectral properties and the recognition of the acoustical Gestalt of complex communication calls (wriggling calls) based on a temporal property are represented in auditory cortical fields of mother mice (Mus musculus, NMRI strain)1. The role of the auditory cortical layers in this context is largely unknown. Here we study, how the auditory cortical layers are involved in wriggling call processing towards recognition.

Mouse pup wriggling calls, produced while struggling for their mother's nipples in a nursing situation, release pup-caring behavior in the mothers. The harmonically structured calls cover a frequency range between about 2 and 20 kHz and have an average duration of 120 ms. The simultaneous presence of three frequencies at 3.8, 7.6 and 11.4 kHz for 100 ms is necessary and sufficient for wriggling call recognition, i.e., for the release of maternal behavior2. An advanced or delayed beginning of the fundamental frequency by 50 ms reduced call recognition to very low rates in the mothers. We stimulated with a call model releasing maternal behavior either at a high rate (efficient call model leading to recognition) or low rate (inefficient call model leading to perception without recognition)3. Neural activation was shown via c-Fos immunocytochemistry in the primary auditory cortical fields (primary auditory field AI, anterior auditory field AAF), and higher-order fields (second auditory field AII, dorsoposterior field DP). Fos-positive cells were counted in serial frontal sections of the auditory cortex AC of both hemispheres and were attributed to the cortical layers 2/3, 4 and 5/6. To assign immunoreactive cells to the AC and its fields, the left AC of each animal was electrophysiologically mapped before the behavioral test of maternal behavior and c-Fos immunocytochemistry.

Here we show, that labeling within the auditory cortical fields indicate a non-homogeneous distribution of Fos-positive cells across the cortical layers. (1) In all fields, Fos-positive cells occurred at significantly lower numbers in layer 4 compared to layers 2/3 and 5/6. (2) In AI and AAF of both hemispheres the stimulation frequencies 3.8, 7.6 and 11.4 kHz of both call models led to three local peaks with increased numbers of Fos-positive cells compared to the labeling in the inter-peak intervals1. We found that the frequency-related peaks in both primary fields were mainly due to a significant increase of Fos-positive cells in layers 2/3 compared to layers 5/6. (3) In AII, stimulation with the efficient call model led to a significant lower amount of Fos-positive cells than stimulation with the inefficient call model1. In the left and right hemisphere, both call models led to significantly higher numbers of Fos-positive cells in layers 5/6 compared to layers 2/3. (4) DP showed a left-hemisphere advantage in the number of Fos-positive cells in mothers perceiving the efficient call model1. This left-hemisphere advantage is due to increased labeling both in layers 2/3 and 5/6.

We conclude that the biological meaning of complex communication calls in mother mice is seen only in a generally different activation within the auditory cortical fields but not in a differential activation of layers.

1 Geissler & Ehret, 2004, EJN 19:1027-40; 2 Ehret & Riecke, 2002, PNAS 99: 479-82; 3 Geissler & Ehret, 2002, PNAS 99: 9021-25.

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# Layer-specific pup call processing in auditory cortical fields during the mouse estrous cycle

Christina Schmid[1], Günter Ehret[1]

[1]University Ulm, Institute of Neurobiology, Albert-Einstein-Allee 11, D-89081 Ulm, Germany

Information processing through the cortical layers in sensory, motor and association areas of the mammalian neocortex may follow a general basic scheme (e.g. Silberberg et al, 2002, TINS 25:227-30). It is largely unknown how the cortical layers transform in a nonlinear and adaptable way the thalamic input to an integrated and complex neuronal response for the generation of adequate behavior (e.g. Atencio et al, 2009, PNAS 106:21894-99). Here we demonstrate that estrous cycle dependent behavioral variations of emotional auditory perception and responding in female mice are reflected in the specific activation of certain layers in fields of the auditory cortex.
Pup-naïve adult females in a nursing/warming position on mouse pups respond to series of harmonically structured low-frequency "wriggling calls" of the pups with maternal behavior. By changing acoustical parameters, wriggling call models can be efficient (biologically significant) or inefficient (biologically insignificant) releasers of maternal care. In behavioral tests, only females in diestrus/proestrus discriminate efficient form inefficient call models (Ehret & Schmid 2009, Physiol Behav 96: 428-33). For our acoustic stimulation here, we used an efficient and an inefficient call model as releaser of maternal care in females in the estrous phases diestrus/proestrus, estrus or metestrus. Neuronal activation was quantified via c-Fos immunocytochemistry by counting Fos -positive cells in frontal sections of the auditory cortex of both hemispheres (Geissler & Ehret, 2004, EJN 19:1027-40) and separately for the cortical fields and layers 2/3, 4 and 5/6.
Among our results are the following: (1) Activation in the layers of the primary auditory cortical fields (AI, AAF) is not estrous cycle dependent. (2) The second auditory cortical field (AII) shows a left-hemisphere advantage in activation for inefficient call models caused by the strong activation of layer 2/3 in diestrus/proestrus and metestrus and of layers 2/3, 4 and 5/6 in estrus. Significantly reduced numbers of Fos-positive cells occur in diestrus/proestrus in layers 2/3 and 5/6 in response to the efficient call model compared to the inefficient one. (3) The dorsoposterior field (DP) shows a left-hemisphere advantage in the number of Fos-positive cells specifically in layer 2/3 only in diestrus/proestrus animals perceiving the efficient call model.
Our results show for the first time an estrous cycle dependent and field specific activation of the auditory cortical layers in response to communication calls. Differences in the activation strength of layers 2/3 and 5/6 in AII reflect the identification ("what") of communication calls which is seen only in diestrus/proestrus. In layer 2/3 of DP, the left-hemisphere advantage of processing efficient call models by diestrous females corresponds to a left hemisphere advantage of general DP activation shown already for mothers (Geissler & Ehret, 2004, EJN 19:1027-40).

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# Localization of frequency modulated tones in barn owls

Lutz Kettler[1], Katrin Vonderschen[1,2], Hermann Wagner[1]

[1]RWTH Aachen Institute for Biology II, Department of Zoology and Animal Physiology, Mies-van-der-Rohe-Straße 15, 52056 Aachen, Germany [2]Department of Physiology, McGill University, Montreal, Canada

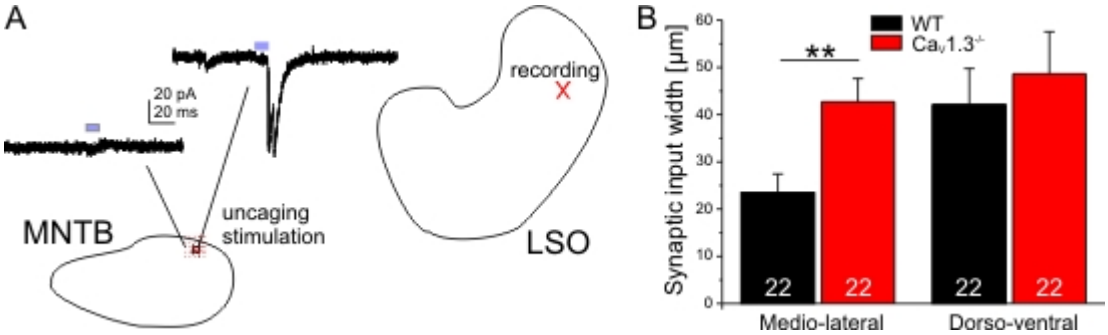
Barn owls (Tyto alba) localize sound sources in azimuth by detecting the onset and ongoing time differences between both ears (ITD). Due to the computation of sound signals in the midbrain, narrowband noise carries ambiguous information about the position of a sound source. Phantom sound sources appear at positions which differ from the real position by an angle that can be determined from the period at the signal's center frequency and a factor converting ITD into space. Broadband noise helps in disambiguating the signal.
Since frequency modulation is a common feature of complex sounds, we speculated that an unambiguous localization might also be possible with frequency modulated signals. To investigate this issue, we trained two barn owls to localize noise signals of a given center frequency and different bandwidths presented via headphones. Signals were presented with different ITDs. Alternatively, the owls were stimulated with linear frequency modulated tones (FM sweeps) which had the same center frequency as the constant-frequency noise and a modulation range corresponding to the bandwidth of the constant-frequency sounds. Modulation ranges from 1 – 5 kHz were used in the tests. The direction and amplitudes of head turns as well as the latencies of the head turns were analyzed.
We observed that the owls made localization errors as long as the bandwidth of the constant-frequency noise was below 4 kHz. Head turns to the real sound source as well as to phantom sources were recorded. Head-turning latencies were shorter with broad band noise stimuli than with narrow band signals.
Preliminary data with FM sweeps suggested a dependence of head-turning amplitude and latency on modulation range. The highest modulation ranges tested elicited the lowest numbers of head turns toward phantom sources. Furthermore, head-turning latencies decreased with increasing modulation range.
These findings imply that barn owls are able to localize FM-signals with a wide modulation range more precisely than pure tones and narrowband noise signals.

# Loss of Cav1.3 calcium channels leads to impaired development of a topographic inhibitory projection in the auditory brainstem

Jan Hirtz[1], Katrin Janz[1], Désirée Griesemer[1], Eckhard Friauf[1]

[1]University of Kaiserslautern, Animal Physiology Group, Department of Biology, Erwin Schrödinger-Straße 13, 67663 Kaiserslautern, Germany

The place principle of frequency analysis in the cochlea is preserved in a remarkably precise pattern along the auditory brainstem stations. The inhibitory projections from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) process information from high-to-low frequencies from medial-to-lateral. To assess the role of Cav1.3 in the activity-dependent development of this topographically organized circuit, we analyzed Cav1.3-/- mice (Platzer et al., 2000, Cell), in which the auditory brainstem nuclei are deprived from cochlea-driven activity. We previously described a reduction in volume throughout the auditory brainstem nuclei in Cav1.3-/- mice. Also, the Cav1.3-/- LSO has lost its typical U-shape, in which the topography is realized. In acute brain slices, focal photolysis of caged glutamate via a laser diode was used to stimulate MNTB neurons and combined with patch-clamp recordings of postsynaptic LSO neurons to characterize the topography in the MNTB-LSO projection (Fig. 1A). This method allows the determination of the MNTB area (input area) that converges to one single LSO neuron. It is known that the number of MNTB neurons projecting to one LSO neuron is reduced to less than half during the first two postnatal weeks in wild-type (WT) mice (Noh et al., 2010, Nat Neurosci). While we observed no difference in the dorso-ventral width of the input area between the genotypes at P10-12, the medio-lateral width was nearly twofold broader in Cav1.3-/- mice (Fig. 1B; 42±5 µm vs. 23±4 µm in WT). These results indicate an impaired refinement of the projections. The positions of all recorded LSO neurons correlated with the corresponding input area positions in WT and Cav1.3-/- mice, displaying the topography of the projections from medial-to-lateral. However, a small part of the lateral MNTB (~15%) in Cav1.3-/- mice projected to a large part of the LSO (~50%). Such a disproportion was not observed in WT. Stimulus conditions were unchanged in Cav1.3-/- mice, because the maximal distance between the uncaging spot and a patched MNTB neuron at which action potentials could be evoked (~10 µm) did not differ between genotypes. Dye tracing of single MNTB axons is currently performed to gain further insight into the projection patterns. Together, our results indicate an important role of Cav1.3 for the development of an inhibitory projection within the auditory brainstem.

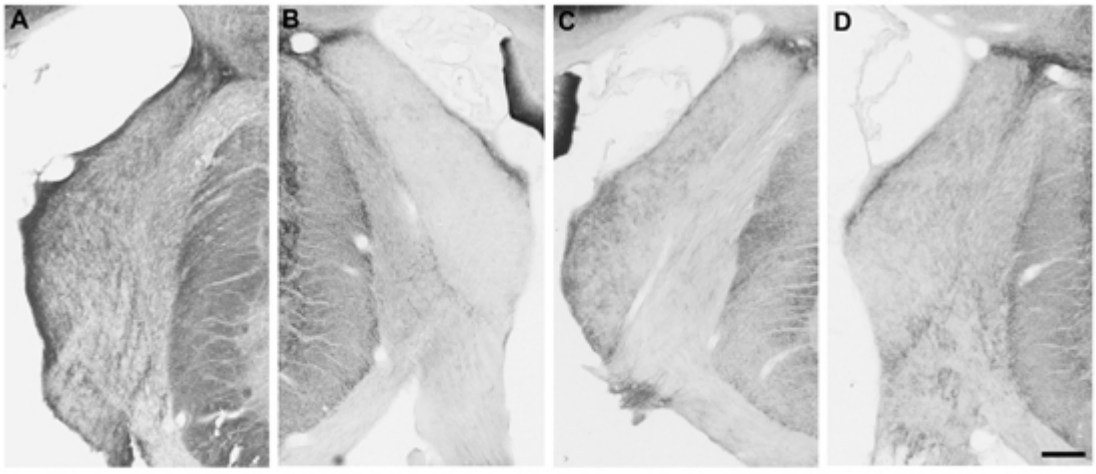


# MMP-2, but not MMP-9 expression pattern depends on the arrival of GAP-43 positive axons in the cochlear nucleus after cochlear ablation in rat.

Michaela Fredrich[1], Robert Benjamin Illing[1]

[1]Neurobiological Research Laboratory, University of Freiburg, Dept. of Otorhinolaryngology, Kilianstr.5, 79106 Freiburg, Germany

Lesion induced neuroplasticity, including axonal growth and synaptogenesis, involves dynamical changes of the extracellular matrix (ECM). The matrix metalloproteases MMP-2 and MMP-9, major ECM remodelers are changed in distribution and increased in amount in the anteroventral cochlear nucleus (AVCN) of the rat following deafferentation by cochlear ablation. While MMP-9 activation due to cochlear ablation is highest already one day after deafferentation (POD1), MMP-2 distribution and amount are changed not until POD3 but these changes are maximal at POD7. GAP-43 is a key marker of synaptogenesis in AVCN on POD7 after cochlear ablation. While MMP-9 holds no interdependence with GAP-43, double labeling of MMP-2 with GAP-43 showed a remarkable coincidence of MMP-2 accumulation and GAP-43 expression in time and space. In order to determine if the lesion-dependent emergence and redistribution of GAP-43 and MMP-2 are interdependent of each other, injections of kainic acid into the ventral nucleus of the trapezoid body (VNTB), killing the cells that deliver GAP-43 to AVCN after cochlear ablation, were done prior to cochlear ablation. If only part of VNTB neurons were destroyed, GAP-43 emerged in AVCN in a patchy pattern. Combining kainic acid injection followed by cochlear ablation, two major findings were obtained. First, the arrival of GAP-43 in AVCN following deafferentation by cochlear ablation has no effect on distribution and amount of MMP-9 in the same region. Neither in the early phase after cochlear ablation where the auditory nerve fibers and their synaptic terminals in the AVCN degenerate nor in the following phase of regeneration when massive synaptogenesis takes place did we find differences between cases with combination of kainic acid injection followed by cochlear ablation and cochlear ablation alone for MMP-9. Second, level and pattern of MMP-2 remained control-like despite a preceding ipsilateral cochlear ablation in case of complete destruction of VNTB neurons. If patches of GAP-43 expression due to an incomplete VNTB lesion were present, normal cochlear ablation-dependent changes of MMP-2 staining were encountered only in GAP-43 positive regions. In conclusion, then, we proved that GAP-43, imported from VNTB into AVCN to deliver synaptic replacements after sensory deafferentation, is the cause of changes in distribution and amount of MMP-2, but not of MMP-9.




**Extent of synaptogenesis in the anteroventral cochlear nucleus (AVCN) of the auditory brainstem of the adult rat following cochlear ablation or kainic acid injection combined with cochlear ablation.**
Seven days after cochlear ablation massive, homogeneously distributed expression of GAP-43 is observable in the AVCN (A), while there is no GAP-43 expression in the AVCN of adult control animals (B). If only part of the neurons of the VNTB were destroyed by kainic acid injection preceding cochlear ablation, GAP-43 showed up in a patchy pattern in the AVCN (C). Destruction of all VNTB neurons combined with cochlear ablation lead to a massive reduction in GAP-43 expression (D). Scale bar (A)-(D): 0,2mm

# Modelling asymmetry of ITD tuning curves in the AAR of the barn owl via frequency integration

Jessica Anita-Frida Lehmann[1], Philipp Tellers[2], Hermann Wagner[2], Hartmut Führ[1]

[1]RWTH Aachen, Lehrstuhl A für Mathematik RWTH Aachen, Templergraben 55, 52056 Aachen, Germany

[2]Institute of Biology II RWTH Aachen, 52056 Aachen, Germany

The Jeffress model explains how the owl compute ITDs using an arrangement of coincident detectors. Coincidence detector neurons, receive input from each ear and these path lengths vary from neuron to neuron. For each neuron, there is an ITD that offsets the difference in path length such that the inputs arrive in coincidence and the neuron fires maximally generating an arrangement called delay line.
In the barn owl, the sound-localization information is processed in two parallel pathways, the midbrain and the forebrain pathway.
The auditory arcopallium (AAr) in the forebrain as well as the inferior colliculus (ICx) in the midbrain receives afferent input from the lateral shell of the central nucleus of the inferior colluculus (ICcl). ITD response functions at different frequencies recorded extracellularly in-vivo, show that the representations in the forebrain pathway differs from the one in midbrain. In the AAr tuning curves are asymmetric with a steep slope toward larger contralateral times. In contrast, tuning curves of the ICx are symmetric. (Vonderschen, Wagner 2009)
In this study, we propose a mechanism in which this observed tuning behavior in the AAr and in the ICx arises from the afferent inputs arriving from the ICcl. The single ICcl unit is modeled as a leaky integral neuron which has a best frequency and a best ITD. The distribution of the best ITD of the afferent ICcl neurons differs for each pathway. In the midbrain pathway, the ICcl neurons afferent to a given ICx neuron have the same best ITD and varying best frequencies (frequency integration). In opposition, the ICcl neurons which project to the AAr have different best ITDs and frequencies.

# Modulation of auditory mismatch negativity using muscarinic drugs in awake rats

Fabienne Jung[1], Rosalyn Moran[2], Tetsuya Kumagai[1], Heike Endepols[1], Klaas Enno Stephan[2,3], Rudolf Graf[1], Marc Tittgemeyer[1]

[1]Max Planck Institut für neurologische Forschung, Gleueler Straße 50, 50931 Köln

[2] Wellcome Trust Centre for Neuroimaging, Institute of Neurology, University College London, London, UK

[3] Laboratory for Social and Neural Systems Research, Institute for Empirical Research in Economics, University of Zurich, Switzerland

The auditory mismatch negativity (MMN) is a component of evoked potentials in response to an odd stimulus in a sequence (oddball paradigm). While MMN has been recorded in various species (primates, cats, guinea pigs, rabbits), results of these studies in rats are inconsistent (Ruusuvirta et al., 1998; Lazar & Metherate, 2003, Astikainen et al., 2006; Tikhonravov et al., 2008).

MMN recordings are mostly performed in anesthetized rats even though it is known that narcotics influence central sensory processing. In the present study, we aimed to demonstrate evoked MMN in awake and unrestrained rats. Furthermore, we applied two drugs with opposite effects at the same receptor to manipulate neuronal adaption and thereby gain deeper understanding of mechanisms of MMN generation.

Stimulus-specific adaptation was suggested as a mechanism in MMN-generation and was investigated in single neurons in the cat auditory cortex (Ulanovsky et al., 2003; Nelken & Ulanovsky, 2007). Adaptation, the reduction in firing rate over prolonged firing, also called spike frequency adaptation (SFA), results from slow afterhyperpolarising potassium currents (sIAHP). These are calcium-dependent but voltage-independent and lead to a prolonged hyperpolarization of the neuron, thus reducing the frequency at which action potentials can be generated. Pharmacologically, SFA is strongly influenced by several neuromodulators, but particularly through muscarinic acetylcholine receptors. Therefore, we chose two muscarinic agents: 1) pilocarpine, a non-selective agonist of the muscarinic ACh receptor which reduces SFA and 2) scopolamine, an antagonist of the same receptor which increases SFA, to manipulate muscarinic receptor status and consequently change adaptation in auditory cortical neurons during acoustic stimulation. In addition to influencing SFA, muscarinic receptors also play an important role in modulating short-term plasticity of glutamatergic synapses (Gu, 2002).

We set up a telemetrical system that allowed wireless recording of EEG and auditory evoked potentials from the cortex. Two electrodes were implanted epidurally over the auditory cortex in both hemispheres of 4 Lister hooded rats. After successful recording of evoked potentials, we administered pilocarpine (3 and 6 mg/kg), scopolamine (1 and 2 mg/ kg) or vehicle intraperitoneally. 20 min after treatment recordings were performed.

MMN-like potentials in rats, displayed as the difference between potentials evoked by standard sounds and potentials evoked by deviant sounds, consisted of an early positive (~30msec) and a later negative wave (~100 ms).

Muscarinic agents changed MMN-like potentials in amplitude and latency. After scopolamine application, latency of the late negative difference wave decreased compared to MMN recorded after vehicle injection. The early positive peak was not altered. On the other hand, pilocarpine reduced the amplitude of both positive and negative wave, while it seemed to change their onset latency.

In summary, muscarinic drugs changed different components of MMN-like potentials, indicating that muscarinergic mechanisms, including neural adaptation and/or short-term plasticity of glutamatergic synapses, may be involved in MMN generation.

Ruusuvirta et al., Neurosci Lett. 1998
Lazar & Metherate, Hear Res. 2003
Astikainen et al., Neuroreport. 2006
Tikhonravov et al., Brain Res. 2008
Ulanovsky et al., Nat Neurosci. 2003
Nelken & Ulanovsky, J Psychophysiol 2007

Gu, Neuroscience 2002

# Multimodal thalamocortical connections of primary sensory cortices in the Mongolian gerbil

Julia Henschke[1], Henning Scheich[1], Eike Budinger[1]

[1]Leibniz Institut for Neurobiology, Dept. Auditory Learning and Speech, Brenneckestraße 6, 39118 Magdeburg, Germany

It has long been the prevailing view that primary sensory cortices like the primary auditory (A1), somatosensory (S1) and visual field (V1) are unimodal, i.e. are dominated by neurons responding only to their own sensory modality. However, very recent studies have shown there are also neurons in A1, S1 and V1 which respond to stimuli of other sensory modalities. This raises the question about the anatomical pathways how the multisensory information is forwarded to these cortical areas?
Here, we used the retrograde transport of two sensitive and fluorescent neuronal tracers, namely fluorescein-labelled dextranamine (FDA) and tetramethylrhodamine-labelled dextranamine (TMRDA) ,in order to examine the thalamo-cortical afferents of A1, S1 and V1 in the Mongolian gerbil (Meriones unguiculatus), a commonly used animal model in multisensory research (Budinger & Scheich, Hear Res 258:16-27, 2009). Following simultaneous injections of FDA and TMRDA into these areas (in combination: A1/S1, V1/S1, A1/V1) we found overlapping clusters of single FDA - and TMRDA -labelled as well as double -labelled cell bodies in several sensory thalamic nuclei. This indicates that there are individual thalamic nuclei as well as individual neurons, which project in a divergent manner to more than one primary sensory area. Overlapping clusters were found in several nuclei of the medial geniculate body (medial division, dorsal division, marginal zone and suprageniculate nucleus), ventral thalamus (ventral posterolateral, ventrolateral and ventral posteromedial nucleus), lateral thalamus (lateral posterior and laterodorsal nucleus) and posterior thalamic nuclear group. Double -labelled cells were found in the medial geniculate body (medial division, dorsal division and suprageniculate nucleus), posterior thalamic nuclear group and in the brachium of the inferior colliculus.
In summary, approx. 10% of the retrogradely labeled cells in the thalamus project not only to the primary sensory cortex of their own but also to cortices of other modalities. These thalamic afferents provide one possible anatomical basis for the integration of multisensory information at a very early cortical level.

# Neural Representation of Echoes in the Auditory Cortex of the Ferret

Sandra Tolnai[1], Neil C Rabinowitz[1], Ben DB Willmore[1], Ruth Y Litovsky[2], Andrew J King[1]

[1]University of Oxford, Department of Physiology, Anatomy, and Genetics, Parks Road, OX1 3PT Oxford, United Kingdom
[2]University of Wisconsin, Waisman Center,1500 Highland Ave., Madison, WI 53705, United States

Listening in complex, echoic environments is an everyday task whose neuronal basis is poorly understood. A simple way of simulating echoes is by presenting two sounds from different locations with a short delay between them. Human psychophysical studies have shown that, depending on the delay, the two sounds are perceived as (i) a single fused sound from an intermediate location, (ii) a single sound and its echo, or (iii) as two sounds. Neural responses of single units to such paired sounds have been studied at virtually all levels of the auditory pathway. However, how responses of a population of neurons relate to the actual perception of paired sounds is still poorly understood.

Here, we first investigated ferrets' localization performance in response to paired stimuli. We trained them in a free-field 2AFC task to discriminate the direction of a sound as coming either from the left or from the right. Stimuli were short paired noise bursts presented via the left and right speaker at different interstimulus delays (ISDs). Ferrets were rewarded for approaching the speaker of the leading noise burst in the pair. Localization accuracy of the leading sound peaked at low to intermediate ISDs, suggesting that the precedence effect is indeed present in ferrets. At longer ISDs, performance deteriorated, implying that the leading and lagging sounds may have both been perceived.

We then sought to understand the basis for the observed behavior by developing a population model based on the responses of cortical neurons. We recorded populations of single units in the auditory cortex of both hemispheres of the anesthetized ferret. Stimuli were presented in virtual acoustic space to resemble stimuli used in the behavioral experiment. In accordance with former studies, responses to the echo were suppressed for small delays and recovered as the delay between the two sounds was increased. The strength of suppression, however, depended on the location of the echo. In a subset of units – generally units with a strong preference for either hemifield – the echo responses were not suppressed at all. As a result, although individual units do not consistently represent the echo, its presence can be decoded from the population activity.

# Neuronal plasticity induced in auditory cortex of Mongolian gerbils with central tinnitus

Sönke Ahlf[1], Konstantin Tziridis[1], Holger Schulze[1]

[1]University of Erlangen-Nuremberg, Experimental Otolaryngology, Waldstr. 1, 91054 Erlangen, Germany

The tonotopic representation of sound frequency in the auditory cortex reflects the spectral analysis of the cochlea and is shaped along the auditory pathway by excitatory and inhibitory interactions along and between frequency channels, e.g. for spectral contrast enhancement. Malfunctional synaptic transmission of the sensory information between these neurons may lead to erroneous cortical activations and consequently erroneous auditory percepts like central tinnitus. Primary cause of such an impaired sound processing usually are damages of the sensory epithelium of the inner ear (e.g., after a noise trauma) that result in a lack of lateral inhibition along the auditory pathway which in turn lead to over-excitation of the frequency channels adjacent to the lesioned area. Finally, such over-excitation may induce plastic changes in the primary auditory cortex tonotopic organization that are reflected in a chronic central tinnitus (for review see Eggermont, 2003).
In our lab we aim at the development of a therapy for central tinnitus that reverses these neuro-plastic cortical changes. For this purpose we have established a behavioral and electrophysiological animal model, allowing us to investigate the neuronal mechanisms that result in the percept of a central tinnitus.
After an acoustic trauma (2kHz, 115dB SPL, 75min), the prepulse inhibition of the acoustic startle response was recorded in Mongolian gerbils (Meriones unguiculatus) using, a gap-noise paradigm to identify a tinnitus percept in these animals. In circa 75% of the subjects, a significant decrease in the effect of the gap in reducing the startle response was found for frequencies above the trauma frequency.
The electrophysiological characterization of neuronal responses to pure tones in the left auditory cortices of these animals showed, first, an over-excitation of neurons in the frequency ranges up to one octave adjacent to the trauma frequency, which fits well to the established hypothesis of central tinnitus development. Second, we found significant changes of tonotopic map topography, i.e. an over-representation of frequency ranges adjacent to the trauma frequency. Finally, across the whole population of neurons in auditory cortex we found increased neuronal sum activity to frequency ranges up to 3 octaves above the trauma frequency which fits our behavioral data.
All together, we have now access to a behavioral and electrophysiological animal model of a central tinnitus in our lab. It enables us to investigate the neuronal mechanisms underlying the development of central tinnitus and gives us the possibility to test new therapeutic strategies for the treatment for this disease.

Ref: Eggermont, JJ (2003) Central tinnitus. Auris, Nasus, Larynx, 30: S7-S12
This work was supported by the Interdisciplinary Center for Clinical Research (IZKF, Project E7) at the University Hospital of the University of Erlangen-Nuremberg, Germany

# Neuronal response properties to tones and complex communication sounds in secondary field (AII) of the awake mouse auditory cortex during the estrous cycle

Michaela Glowina[1], Günter Ehret[1], Simone Kurt[1]

[1]University Ulm, Institute of Neurobiology, Albert-Einstein-Allee 11, 89069 Ulm, Germany

The cyclic changes of blood estrogen levels during the reproductive cycles in mammals including humans modulate sound perception and acoustic response behavior (Parlee 1983; Ehret and Schmid 2009). It is unclear where in the brain this estrogen level-related perceptual plasticity is expressed in respective modulations of neural activity. Here we show that the response properties of neurons in the secondary field (AII) of the mouse auditory cortex change in several parameters during the estrous cycle. We recorded multi-unit responses from the left-side AII in unanesthetized females (*Mus musculus*, outbreed hybrids of feral mice and NMRI-mice) to tone bursts and models with or without biological significance of mouse pup wriggling calls (Ehret and Schmid 2009).
Among our results are the following: (1) In general, neurons were broadly frequency tuned (Q40 values between 0-3) and most of them responded preferably to frequencies higher than about 11 kHz so that approximately 30% responded to wriggling call models (frequency range 3.8 – 11.4 kHz). Approximately 12% of the neurons showed tonic responses lasting longer than the duration of the pure tones (200 ms). (2) Estrous effects were seen (a) in response latencies to tones (70 db SPL) with longest latencies in proestrus, (b) in tonic versus phasic responses to tones with about 50% tonic or phasic responses in diestrus and tonic responses dominating in the other estrous phases, (c) in the occurrence of on versus on/off responses with about 50% on or on/off responses in diestrus and on/off responses dominating in the other estrous phases, (d) in the response rates to call models with higher rates in estrus/metestrus than in diestrus/proestrus.
In diestrus, estrogen levels increase to a maximum in proestrus and then rapidly decrease in estrus to very low levels in metestrus. So, in a phase of rapid decrease or low level of estrogen, AII neurons responded with higher rates to call models than in phases of increasing or high levels of estrogen. Since females discriminate between call models of biological significance only in the diestrus/proestrus phases (Ehret and Schmid 2009), call discrimination and preference of call models is accompanied by low activation (low response rates) in AII.

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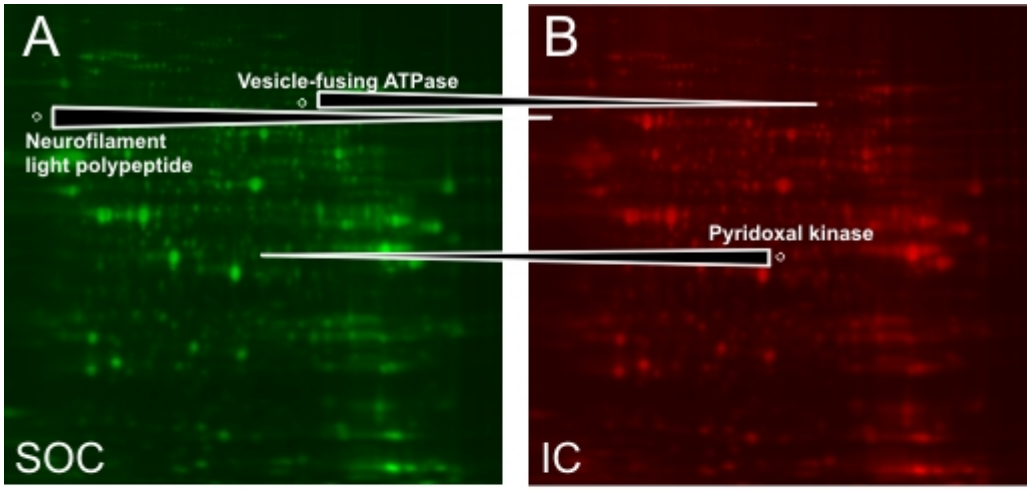
# Neuroproteomics in the rat auditory brainstem: identifying region-typical protein profiles

Christian Moritz[1], Eckhard Friauf[1]

[1]Animal Physiology Group, Department of Biology, University of Kaiserslautern

Brain regions are characterized by typical anatomical, morphological, physiological, and biochemical properties, which are basically determined by the peculiarity and amount of their proteins. Comparative profiling and quantification of these proteins across distinct brain regions is therefore of substantial interest. The central auditory system is composed of several specialized regions. Among those are the cochlear nuclear complex (CN), the superior olivary complex (SOC), and the inferior colliculus (IC). Each region is specialized in processing basic acoustic features. The present study aimed at revealing the proteomic background of these individual characteristics. Regarding protein profiles of adult rats, we hypothesized strong differences between the three auditory regions versus the rest of the brain, and also between the auditory regions themselves, although to a lower degree. We used fluorescence-based two-dimensional difference gel electrophoresis (2-D DIGE) and matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-MS) to map and quantify the soluble proteins and to identify those showing significant level differences. From each of the four regions analyzed (CN, SOC, IC, rest of the brain = "Rest" in the following), five biological replicates, resulting in 20 distinct samples, were loaded on ten DIGE gels (Fig. 1A,B). A total of 1864 distinct protein spots were found, and 644 of them displayed significant level differences between regions. With the application of stringent criteria, their analysis revealed highest similarities between CN and SOC, where only 2% (39 spots) showed significant differences (Fig. 1C). Most differences arose in the comparisons CN vs. Rest (24%) and SOC vs. Rest (23%). Interestingly, the IC differed from the Rest in only 5% of the spots, whereas by 10% from the other two auditory regions. Thus, based on the protein profiles, the three auditory regions could be separated into the medullary and the midbrain category. All proteins that were significantly higher abundant in one brain region compared to the three others were defined as region-typical. For example, Vesicle-fusing ATPase, a protein required in synaptic vesicle cycling, was identified as one out of 13 SOC-typical proteins (Fig. 1D). Out of five CN-typical and four IC-typical proteins, Calretinin and Pyridoxal kinase, respectively, were regarded as candidate proteins of particular interest. These and others are validated by Western blots. Furthermore, their localization *in situ* is assessed by immunohistochemistry. When considering complete biological processes, a high content (>40%) of the proteins typical for the three auditory regions are involved in energy metabolism, whereas none of the Rest-typical proteins belonged to this group. This confirms the enhanced neuronal activity in the auditory brainstem. Taken together, our study is the most exhaustive proteomic analysis in the central auditory system so far. Out of 644 spots with differential levels, we selected more than 20 region-typical proteins which are suitable to analyze their role within anatomical, morphological, physiological, or biochemical contexts.

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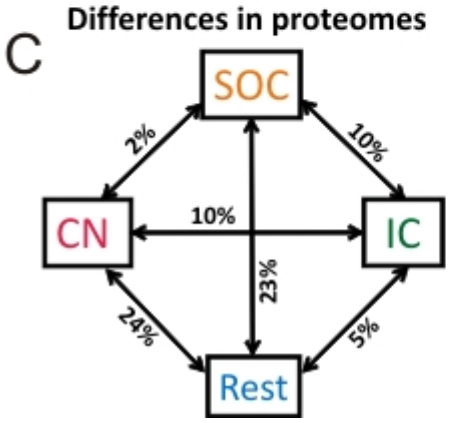


D **SOC-typical proteins** (5/13)

| Protein | Faktor |
|---|---|
| Gelsolin | 4.1 |
| Vesicle-fusing ATPase | 2.8 |
| Serotransferrin | 2.4 |
| Glutaredoxin-3 | 2.1 |
| Acetyl-CoA acetyltransferase | 2.1 |

# Origin of the neurophonic: Linear summation of the monaural responses predicts the binaural response in the nucleus laminaris of the barn owl

Paula Tuulia Kuokkanen[1], Catherine E. Carr[3], Hermann Wagner[4], Richard Kempter[1,2]

[1]Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Berlin, Germany
[2]Bernstein Center for Computational Neuroscience Berlin, Germany
[3]Department of Biology, University of Maryland, College Park, Maryland
[4]Institute for Biology II, RWTH Aachen, Germany

The neurophonic potential is a frequency-following extracellular potential that can be recorded e.g. in the network formed by the nucleus magnocellularis (NM) and the nucleus laminaris (NL) in the brainstem of the barn owl. The neurophonic has a temporal precision below 100 microseconds.

Putative generators of the neurophonic are the activity of afferent axons from NM, synaptic activation onto NL neurons, and spikes of NL neurons. The source of the neurophonic has not been identified yet – a high number of independent sources (> 300) is needed for its the generation [1]; spike sorting is not possible.

We hypothesize that the input to NL, i.e. NM axons and their synapses, are the origin of the high-frequency (> 3 kHz) component of the neurophonic whereas the output of NL, i.e. the spikes of the NL neurons may contribute to the weaker low-frequency (0.5-2 kHz) component. To test this hypothesis, we analyzed monaural and binaural *in-vivo* responses to acoustic stimulation with tones. The figure shows the power spectral density (PSD) of a response to a 4.8 kHz stimulus.

A prediction of our hypothesis is that a linear combination of two monaural responses matches the binaural response. We analyzed several signatures of the responses, some showing tuning of the interaural time difference (ITD) such as the peak of the PSD, and others that turned out to be uncorrelated to ITD such as the noise level in the PSD. All signatures were highly correlated between the linear prediction and the measured binaural response. In particular, it was not necessary to consider the nonlinear responses of NL coincidence detector neurons, whose firing rate and synchrony strongly depend on ITD. Our results support the hypothesis that the input to NL is the origin of the high-frequency component of the neurophonic.

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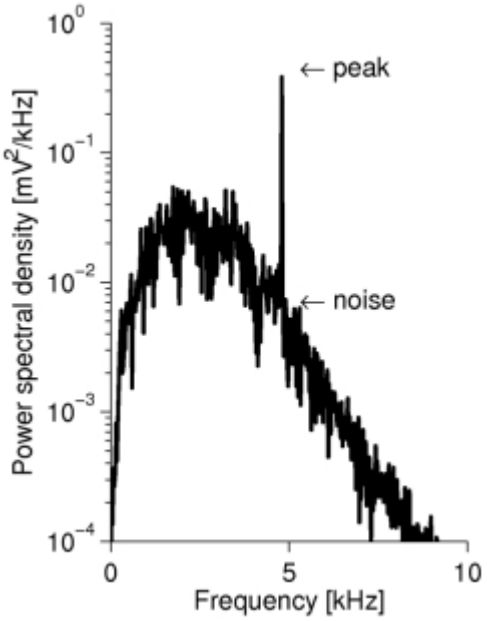
$10^0$
$10^{-1}$
$10^{-2}$
$10^{-3}$
$10^{-4}$
Power spectral density [mV$^2$/kHz]
← peak
← noise
0
5
10
Frequency [kHz]

# Physiology of the femoral chordotonal organ of adult *Drosophila melanogaster*

Catherine Lefevre[1], Reinhard Lakes-Harlan[1]

[1]University Giessen, Institute for Animal Physiology, Integrative Sensory Biology, Wartweg 95, 35392 Giessen, Germany

Chordotonal organs are mechanoreceptors composed of scolopidial units. They are located in between different joints of the insect body to detect position and movement (Field and Matheson 1998). In the legs of adult *Drosophila* the femoral chordotonal organ is a large sensory organ consisting of three groups. One large group is associated with the cuticular surface of the distal femur, whereas the other two groups are associated with femoral muscles (Shanbhag *et al.* 1992). The femoral chordotonal organ develops and differentiates during metamorphosis (Lakes and Pollack, 1990). Physiologically, a passive flexion of the tibial -femoral joint activates the femoral chordotonal organ and this in turn causes a resistance reflex (Reddy *et al.* 1997).
In our study the physiology and anatomy of the femoral chordotonal organ was investigated. Therefore the forelegs of female adult *Drosophila* were stimulated with sinusoidal movements of frequencies ranging from 0.2 Hz to 500 Hz. The responses were extracellularly recorded with sharpened tungsten electrodes from the leg nerve. Analyses of phase histograms indicated phase coupled responses in a frequency range from 0.2 Hz to about 40 Hz. At 1Hz the phase coupling was around 90°, with increasing degrees at increasing frequencies. Further physiological and anatomical investigations will show whether different receptor units are involved in this response. The data can be used for investigations on the biological functions of the sense organ and on sensory transduction processes.

# Postnatal development of delay-sensitive neurons in the auditory cortex of the Short-tailed Fruit Bat

Cornelia Voss[1], Manfred Kössl[1]

[1]Institut für Zellbiologie und Neurowissenschaften, AK Neurobiologie und Biosensorik, Siesmayerstraße 70A, 60323 Frankfurt, Germany

The postnatal development of delay-sensitive neurons and their organisation in the auditory cortex (AC) was studied the Short-tailed Fruit Bat. *Carollia perspicillata* uses frequency-modulated (FM) echolocation signals for general orientation and navigation. In adults, target-range is encoded by delay-sensitive neurons (FM-FM neurons) that respond to specific pulse-echo delays and as in specialized insectivorous bats (CF-FM bats) these neurons are organized chronotopically with shortest characteristic delays (CD) represented most rostrally.
In the present study, the properties of FM-FM-neurons were examined during ontogenesis. During ongoing postnatal maturation, three key developmental changes of the FM-FM neurons and their cortical organization can be observed. (1) In newborn bats, 21% of auditory neurons in the dorsal auditory cortex are already delay-tuned. The percentage of FM-FM neurons increases in the first postnatal week to 56%. In the third postnatal week, it increases abruptly to 84% according to the developing flight capability of the animals. After the first postnatal week, the CD are distributed in the same way as in adult animals, which means that these animals can already detect the same distances as adults on the cortical level. (2) During the development from newborn to adults, the sensitivity at the CD increases by approximately 20 dB. (3) In newborn bats, the FM-FM neurons were chronotopically arranged in the dorsal AC. A consistent correlation between CD and rostro-caudal position is present across all estimated age groups. This study demonstrates that cortical time processing areas and the chronotopic organization are established prenatally. The cortical time processing mechanism are already installed at the time of birth, although the bats do not require it for the following two to three weeks until they start to fly and use active echolocation signals for orientation.
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# Rats *w/ or w/o* tinnitus unravel a tinnitus specific trait

Lukas Rüttiger[1], Wibke Singer[1], Annalisa Zuccotti[1], Masahiro Matsumoto[2], Marlies Knipper[1]

[1]University of Tübingen, Tübingen Hearing Research Centre, Elfriede Aulhorn Str. 5, 72076 Tübingen, Germany
[2]Department of Otolaryngology, Head and Neck Surgery, Graduate School of Medicine, Kyoto University, Kyoto, Japan

Tinnitus is a prevalent audiologic complaint characterized by auditory perception without an external physical source. About 10% of the European population are subjected to chronic, persistent tinnitus that dramatically affects quality of life. The pathogeneses of Tinnitus is one of the most challenging clinical problems (Eggermont, 2007); nevertheless, the molecular basis of tinnitus is still unknown. In many cases, tinnitus can be linked to a damage within the peripheral hearing system (Sindhusake et al., 2004) even when a hearing impairment cannot (yet) be assessed by standard clinical audiometry (Shiomi et al., 1997). Considering the likelihood of hearing impairment in subjects with tinnitus, why does hearing loss *not always* lead to tinnitus? One way to answer this question is to examine tinnitus specific features in a standardized animal model, comparing animals with tinnitus and animals without tinnitus, both suffering from hearing loss after equal exposure to traumatizing noise. In the present study we compared equally acoustically exposed rats with similar hearing impairment, that were behaviorally selected (Rüttiger et al., 2003) in groups with and without tinnitus. In a comprehensive study, we were looking for tinnitus specific changes including the cochlea, the auditory pathway and the auditory cortex. Results suggest a novel tinnitus specific trait.

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# Response properties of neurons in auditory cortical fields of awake mice (*Mus musculus*)

Simone Kurt[1], Bettina Joachimsthaler[1], Michaela Glowina[1], Frank Miller[1], Anja L. Dorrn[1], Günter Ehret[1]

[1]University Ulm, Institute of Neurobiology, Albert-Einstein-Allee 11, 89069 Ulm, Germany

Response properties of neurons in fields of the auditory cortex (AC) are well described for anesthetized house mice (Stiebler et al., 1997). As demonstrated for other species, effects of anesthesia may play a critical role in generating response properties which are effective in situations of sound communication. Here we present multi-unit electrophysiological mapping data from auditory cortical fields of adult, awake, head-fixed mice (outbreed hybrids (*Mus musculus*) of feral mice and NMRI-mice). We characterize neuronal pure tone responses from the left auditory cortex (AC) and discriminate the fields within the AC as described by Stiebler et al. (1997), namely primary fields (primary auditory field, AI, and anterior auditory field, AAF), and higher-order fields (second auditory field, AII, and dorsoposterior field, DP). Among our results are the following: (1) Most neurons in all fields had high spontaneous rates (about 10 – 40 spikes/s); (2) response latencies varied between about 8 – 20 ms with longest latencies in AII; (3) sharpness of frequency tuning expressed as Q40 values varied between about 0.5 – 7 with sharply tuned neurons occurring mostly in AII and DP; (4)especially neurons in the fields AII and DP showed phasic-tonic or tonic responses whereas phasic responses were mainly present in primary fields. These response characteristics of neurons in the AC of the awake mouse contrast with what is known from many recordings in anesthetized mammals.

# Song pattern recognition of selectively perturbated signals in grasshoppers

Stefanie Krämer[1], Bernhard Ronacher[1]

[1]Humboldt Universität zu Berlin, Department of Biology, Behavioural Physiology Group, Invalidenstraße 43, 10115 Berlin, Germany

The acridid grasshopper species *Chorthippus biguttulus* employs acoustic communication not only for mate localization and recognition; moreover it is a central prerequisite for species isolation. In this bidirectional communication system males produce stereotypic species- and sex-specific calling songs. They will trigger response songs in a receptive female, provided that she is attracted by the detected signals. Subsequently, songs are emitted alternately by both sexes, enabling the male to phonotacically approach the female. A characteristic temporal pattern of amplitude modulations, i.e. a sequence of syllables and pauses is crucial for song recognition. Earlier studies with model songs have shown that in *C. biguttulus* a certain ratio between the durations of syllables and intermittent pauses corresponds to a species and sex specific cue (von Helversen 1997 J Comp Physiol A 180:373). Just like in other acoustic communication systems the temporal structure of the signals is masked and distorted during its transfer from sender to receiver in the biotope. Degrading factors amongst others are atmospheric turbulences and additional sound sources accounting for amplitude fluctuations. Here we investigated the influence of amplitude perturbations, differing in modulation depth and occurring at different times in a grasshopper's song. In behavioural experiments on females we systematically screened subunits in a song, testing at what position recognition is particularly susceptible to degradation. Unexpectedly, envelope perturbations at the beginning of a syllable did not lead to a decline in female response, whereas interferences in middle or ending parts led to a considerable decrease in song attractiveness. The behavioural experiments will be compared to the results of electrophysiological experiments using the same stimuli.

# Spatial resolution of bat sonar

Lutz Wiegrebe[1], Cornelia Geberl[1]

Ludwig-Maximilians-Universität München, Division of Neurobiology, Dept. Biologie II, Grosshaderner Str. 2, 82152 Martinsried, Germany

Ultrasonic echo imaging enables bats to identify three-dimensional objects in space as well as to orient through complex environments in total darkness. When a bat ensonifies a natural echo-acoustic scene, many echoes will impinge almost simultaneously from many different directions onto the bats' ears. Thus, a bat hunting a flying insect in front of vegetation is faced with the problem of discriminating between the echoes reflected by the insect and the echoes reflected by the surrounding vegetation. The current study aims to investigate the bats' capability to spatially resolve single reflectors from this multitude of echoes. The spatial resolution of bat sonar is investigated in a series of formal psychophysical phantom-target experiments: In a six-channel, two-alternative, forced-choice setup, the echolocating bat Phyllostomus discolor is trained to detect a rewarded phantom-target surrounded by masking phantom targets. The recruitment of multiple maskers allows quantifying sonar spatial receptive fields perceptually and comparing these with the directionality of the sonar beam, the directionality of the bats' outer ears, and electrophysiologically measured spatio-temporal receptive fields. The results show, that unlike other mammals tested so far, echolocating bats can exploit the strong directionality of their outer ears together with the directionality of their sonar beam to focus there auditory attention to a relatively narrow point in space.

# Spatio-temporal features of stimulus-related activity in the inferior colliculus

Dominika Lyzwa[1,2], Dmitry Bibichkov[1,2,3], Hugh H. Lim[2,4], J. Michael Herrmann[1,2,5]

[1]Max Planck Institute for Dynamics and Self-Organization, Nonlinear Dynamics, Bunsenstrasse 10, 37073 Göttingen, Germany
[2] Bernstein Focus Neurotechnology, Göttingen, Germany;
[3]Max Planck Institute for Biophysical Chemistry, Department of Membrane Biophysics, Göttingen, Germany;
[4]Hannover Medical School, Department of Otolaryngology, Hannover, Germany;
[5]University of Edinburgh, Institute of Perception, Action and Behaviour, Edinburgh, U. K.

The inferior colliculus (IC) is an important stage in the auditory processing pathway. Understanding its encoding mechanism is currently specifically relevant since the IC has become a target for auditory prosthesis (Auditory Midbrain Implant - AMI). Knowledge of the spatio -temporal encoding taking place in the IC would enable development of an algorithm for mapping auditory signals onto 3d electric stimulation patterns. This could help to improve the acoustic perception in AMI-patients.

We analyzed multi-unit activity recorded from the IC in cats during acoustic stimulation with pure tones of varying frequency and intensity. The data were studied using principal and independent component analysis. The recordings were classified into stimulus categories using linear discriminant analysis.

The first components, contributing most to the variance of the data and characterizing most significant neural activity were sufficient to verify the tonotopic arrangement of the IC.
The first components are relatively simple time courses and differ systematically for various tone frequencies.
Separability of neural responses into stimulus classes depended critically on the filter range used for preprocessing. While low- frequency bands of the response carried information about the stimulus parameters, frequency bands above 1 kHz did not improve the classification.
Furthermore, the separability varied strongly for different time windows within the time course of the neural response. For a time window starting 5-10 ms after stimulus onset the classification performance was optimal, indicating the short latency of neural response in the IC. Already an interval of 0.5 ms taken from this time window was enough to allow for discrimination between neural responses to different tone frequencies.
The latency of the neural response was longer for high stimulus tones than for low ones, e.g. on average 5 ms for tone frequency of 1 kHz and 7.5 ms for tone frequency of 24 kHz.
The latency of the neural response and the classification latency were indistinguishable which implies that the onset properties of the neurons are critical for the classification.
We found that the classification performance was also affected by the position of the recording site along the tonotopic axis in the IC, and was best in layers most responsive to middle stimulus-frequencies.
Low frequencies were relatively well separated in all recording sites.

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# Steroid Hormones as Modulators of Audio-Motor Integration in the Midbrain of Anuran Amphibians

Christof Legler[1], Stefan Huggenberger[1], Wolfgang Walkowiak[1]

[1]Zoologisches Institut, Universität zu Köln, Biozentrum, Zülpicher Straße 47 b, 50674 Köln, Germany

Acoustic communication is the primary mediator of synchronized reproductive behaviour in nearly all anuran amphibians during the breeding season. The behaviourally most relevant acoustic signals are the male's advertisement calls, which elicit phonotaxis in females and vocal response in other males to defend territories. The onset of acoustic communication strongly depends on the inner state of the animals. For appropriate behavioural reactions to conspecific calls the interaction between the auditory system and motor networks is essential. Yet, little is known about the neurocellular mechanisms underlying hormonal effects on sensory and motor systems. In the auditory midbrain a sensory motor interface has been identified in the torus semicircularis (TS, the homolog of the mammalian inferior colliculus)[1]. The TS acts as an audio motor integrator where, e.g. slightest lesioning disrupt phonotactic behaviour in females. The neurons converge ascending and descending auditory inputs and project extensively to premotor and motor areas. The TS network is tuned by GABAergic neurons from the subnuclei of the TS, the nucleus laminaris (NL) and nucleus magnocellularis (NM)[2-4]. Interestingly, neurons in the NL were found to have oestrogen and testosterone receptors[5].

Taken together this leads to the assumption, that sex steroids may regulate functional plasticity of the auditory system by modulating the audio motor interface neurons in the TS. In our study we focus on the modulation of synaptic functions by steroids in neurons of the NL. Putative targets for the sex steroid estradiol to modulate synaptic function in a rapid manner are $GABA_A$-receptors.

We started to characterise synaptic input to the NL from the ascending auditory pathway in whole cell voltage and current clamp recordings from labelled neurons in parasagittal brain slices. We observe higher excitability of neurons in the NL after applying $GABA_A$-receptor antagonists Baclofen and Gabazine. This effect is compared to activity of the same neuron after wash-in of estradiol. In addition to the electrophysiological studies we examine the labelled TS neurons concerning morphological characteristics by confocal microscopy.

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# Stimulus specific adaptation to FMs in the awake rat auditory cortex

Carsten Klein[1], Wolfger von der Behrens[2], Bernhard H. Gaese[3]

[1,3]Goethe University, Cell Biology and Neuroscience, Siesmayerstr. 70, 60323 Frankfurt a. M., Germany
[2]University of Zurich, Institute of Pharmacology and Toxicology, Rämistrasse 100, CH-8091 Zurich, Switzerland

One of the main tasks our brain is confronted with is the structuring of the sensory environment. Therefore it is important to detect changes in the surroundings that might indicate novel events of presumed behavioral relevance, and to separate known from unknown stimuli. A first hint at how and where this could happen in the auditory domain was given by the phenomenon of mismatch negativity (MMN), observed in event related potentials in humans. When an infrequent deviant stimulus was presented in a series of repetitive standard stimuli, a negative wave occurred ~200 ms after deviant onset. The neural mechanisms generating MMN, however, remain unclear.

Stimulus specific adaptation (SSA) which was first described at the single cell level in primary auditory cortical areas, was recently suggested to be the neural substrate of MMN (Nelken et al, J. Psychophysiol, 2007). As for MMN, a deviant tone in SSA evokes an increase in neuronal firing rate compared to the adapted response to the standard. While SSA was best described in cortical areas (von der Behrens et al., J. Neurosci, 2009), it was also found in the inferior colliculus (Malmierca et al., J. Neurosci, 2009) and the medial geniculate body (Anderson et al., J. Neurosci., 2009).

Almost all these studies on SSA so far used pure tones to investigate adaptation effects depending on tone frequency. As we were interested in the encoding of more complex stimuli, we tested if frequency modulated tones (FMs) could also induce SSA in the auditory cortex of the awake rat. Logarithmically upward and downward modulated FM tones, as well as pure tones were used as test stimuli in an oddball paradigm.

Consistent with previous findings, significant SSA to pure tones was found in the initial stimulus-evoked response and in the first negative deflection of the local field potentials (LFPs). While FM tones did not evoke such a stereotypical response pattern in the spike data or in the LFPs, clear SSA was found in the main component of the FM-evoked response. Adaptation to FM tones was significantly smaller than to pure tones and was strongly dependent on the parameter of deviance. Only FM stimuli differing in modulation direction but not in modulation speed induced SSA. Investigating the adaptation process in more detail revealed, that depending on the neuron, only upward or only downward-modulated FM-tones could induce SSA. Using wavelet-based clustering of LFP waveforms we determined different classes of responses. We found six different clusters that, surprisingly, did not differ in adaptation behavior. In addition, there was no correlation between SSA to pure tones and SSA to FMs for individual neurons, implying that SSA is not a general property of all cells. Taken together, we found that complex tones such as FM tones can create adapting responses in the context of SSA. This might only occur if the parameter of deviance is of general importance for sensory processing.

# Stimulus-specific adaptation in the gerbil primary auditory thalamus is the result of a fast frequency-specific habituation and is regulated by the corticofugal system

Peter Bäuerle[1], Wolfger von der Behrens [2], Manfred Kössl[3], Bernhard H. Gaese[4]

[1 3 4]Institute for Cell Biology and Neuroscience, Goethe University, Department of Biological Sciences, Siesmayerstr. 70A, 60323 Frankfurt, Germany

[2]Institute of Pharmacology and Toxicology, University Hospital Zurich, Section Functional Imaging and Neurovascular Coupling, Rämistrasse 100, 8091 Zurich, Switzerland

It is a common feature of neuronal information processing that repetitive stimulation results in reduced neuronal activation. Stimulus-specific adaptation (SSA) describes a particular situation, where neurons show a preferential response to rare, unexpected acoustic stimuli among frequently occurring, steady background signals and is often seen as a potential mechanism to detect novel events. SSA is a now well established phenomenon found in neuronal responses at different levels along the mammalian auditory pathway. However, neurons with strong SSA that are suitable for novelty detection are in fact only a fraction of the neurons exhibiting adaptation at all. Neurons in some auditory structures such as the ventral division of the auditory thalamus (vMGB) are in general only adapting to a minor degree.

We investigated SSA at the level of the vMGB with an oddball paradigm involving repetitive stimulation (rate 2 Hz) with pure tones of two frequencies (1 oct. separation) in which low probability deviants (10%) were interspersed among frequent standard stimuli. Neuronal activity was recorded in anesthetized gerbils from up to 12 sites simultaneously using a multi-channel approach. Spike rates from the first 30ms of pure tone responses were analyzed and compared to control conditions (equal probability of standard and deviant).

Our data demonstrate that neurons in the gerbil vMGB show not very strong but significant SSA compared to the control condition (two-sample Kolmogorov-Smirnov test, $p < 0.001$). The main determinant of this adaptation process is a frequency-specific fast habituation of responses to the frequently presented standard stimuli. This habituation is already established after the first few standard tone repetitions. We therefore introduced a 'roving frequency paradigm' to investigate adaptation effects in more detail. In this paradigm stimulus trains of 5 identical pure tones (rate 2 Hz) were presented followed by a different stimulus train separated by a frequency step (upwards or downwards) of at least one octave. In the context of SSA, the first stimulus of each train was considered as a 'deviant stimulus' (new) while the last one (fifth) was considered being a common 'standard stimulus'. Our data not only suggest a non-uniform distribution of SSA within a given frequency response area, but also indicates a possible topographic arrangement of adaptation effects across simultaneously recorded units.

It is well known that the auditory cortex adjusts and improves signal processing at subcortical stages via descending projections to the thalamus and the midbrain. This includes frequency-specific facilitation and inhibition acting on different time scales from short-term changes to long-term plasticity. This makes it rather likely that the strong effects of SSA in the auditory cortex are relayed to the vMGB directly or via corticofugal projections to the inferior colliculus and the N. reticularis where, again, SSA has already been described. We tested this hypothesis at the network level by inactivating the auditory cortex with Muscimol. As expected, we found a strong reduction of adaptation effects during auditory cortical inactivation, thereby indicating the importance of cortical activity for auditory adaptation in general.

# Synaptoporin and TIP39 in the auditory brainstem - partners in processing multimodal influences?

Denise Linsmayer[1], Ulla Stier[1], Julia Braun[1], Stefan Reuss[1]

[1]University Medical Center, Johannes Gutenberg-University Mainz, Institute of Microanatomy and Neurobiology, Langenbeckstraße 1, 55131 Mainz, Germany

Our studies on the distribution of the synaptic vesicle protein synaptoporin in the rodent brain demonstrated that it is highly expressed in the periolivary area (PA) of the auditory brainstem. This region receives multimodal, extra-auditory input that is thought to originate partly in the medial paralemniscal nucleus (MPL). Recent studies showed that the majority of neurons in this nucleus is characterized by immunoreactivity to tuberoinfundibular peptide 39 (TIP39; Dobolyi et al., Prog. Neurobiol. 90:29-59, 2010).
Our study sought to clarify whether synaptoporin and TIP39 are in related structures in the periolivary region and whether these peptides may originate in the MPL. We therefore used various combinations of anterograde neuronal tracing and double-immunofluorescence labelling of synaptoporin and TIP39.
The anterograde neuronal tracer Phaseolus vulgaris-Leucoagglutinin (Pha-L) was targeted to the MPL. For double labelling, sections were first immunolabelled for TIP39 by using tyramide amplification and Cy3-visualisation. Sections were then incubated with the synaptoporin antibody and marked with Cy2-conjugated secondary antibody.
Upon anterograde tracing, many Pha-L-immunofluorescent fibers and putative terminals were observed in sections of the periolivary region. Double incubations showed that synaptoporin- as well as TIP39-immunoreactivity were present in the same area. These peptides were not colocalized but often seen in close spatial relationship.
Our data suggest that in auditory regions such as the periolivary area, two aspects of neuronal input are characterized by TIP39- or synaptoporin-terminals and that these inputs may interact locally in processing multimodal information to the auditory brainstem.

# The Cricket Auditory System Responds to Bilateral Phase-Shifts

Kelly Marie Seagraves[1], Bryony Shelton[1], Catherine Zhang[1], Tim Bayley[1], Stefan Schoeneich[1], Berthold Hedwig[1]

[1]University of Cambridge, Department of Zoology, Downing Street, Cambridge CB2 3EJ, United Kingdom

The cricket's auditory system is a highly directional pressure difference receiver (Schöeneich and Hedwig, 2010) tuned to the carrier frequency of the male calling song (Michelsen, 1998). The directional sensitivity of the system seems to depend on phase shifts within the auditory trachea that connects the left and right hearing organs. We tested whether experimentally induced phase shifts between a left and a right sound signal will lead to changes in the phonotactic behavior, the response pattern of the tympanic membrane, and auditory afferents.
In our first experiements we were interested in the steering response of freely walking female *Gryllus bimaculatus* to phase differences in the male calling song. The same calling song was played simultaneously from speakers at both the left and right sides of the cricket, at 75 dB SPL. However, on one side the sound pattern was shifted in phase by 90°, corresponding to a temporal shift of about 50 microseconds. Carrier frequencies in the range of 4.0-5.4 kHz were used for the male calling song, and a trackball system was used to quantitatively analyse phonotactic behavior (Hedwig and Poulet, 2005). Between 4.0- 4.7 kHz the crickets clearly steered towards the side that was lagging in phase, at 4.7-4.8 kHz the crickets showed no clear steering behavior, and at higher frequencies the animals steered towards the side that was leading in phase. The sensitivity of this steering response was then tested for constant carrier frequencies by altering the phase difference in steps of 22.5°. Some animals responded to a phase difference as small as 22.5°. A similar sensitivity to phase shifts was found in the tympanic membrane oscillations, which were measured with a laser vibrometer. We are now anaylsing the activity of the auditory nerve in response to the same phase shifted stimulus paradigm to determine if the afferent response to phase shifts will match the phonotactic behavior. As a whole, this set of experiments will provide further understanding on how crickets process the phase shifts that they experience in a natural environment to achieve their highly directional phonotactic behaviour.

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Michelsen A (1998) The tuned cricket. *News Physiol. Sci.* 13: 32-38.

Schöneich S and Hedwig B (2010) Hyperacute directional hearing and phonotactic steering in the cricket (*Gryllus bimaculatus*, deGeer), *PLoS-ONE*, accepted

# The rise and fall of an experimental paradigm: orienting asymmetries and lateralized processing of sounds

Julia Fischer[1], Ricarda I. Schubotz[2], Christoph Teufel[3]

[1]German Primate Center and University of Göttingen, Cognitive Ethology Lab, Kellnerweg 4, 37077 Göttingen, Germany

[2] Max-Planck-Institute for Neurological Research, Motor Cognition Group, Köln, Germany

[3] Behavioural and Clinical Neuroscience Institute, Brain Mapping Unit, Department of Psychiatry, University of Cambridge, Cambridge, England

Lateralized processing of speech is a well studied phenomenon in humans. Both anatomical and neurophysiological studies support the view that nonhuman primates and other animal species also reveal hemispheric differences in areas involved in sound processing. In recent years, an increasing number of studies on a range of taxa have employed an orienting paradigm to investigate lateralized acoustic processing. In this paradigm, sounds are played directly from behind and the direction of turn is recorded. This assay rests on the assumption that a hemispheric asymmetry in processing is coupled to an orienting bias towards the contralateral side. We presented speech stimuli as well as artificial sounds to 224 right-handed human subjects shopping in supermarkets in Germany and in the UK. To verify the lateralized processing of the speech stimuli, we additionally assessed the brain activation in response to presentation of the different stimuli using functional magnetic resonance imaging (fMRI). In the naturalistic behavioural experiments, there was no difference in orienting behaviour in relation to the stimulus material (speech, artificial sounds). Contrary to our predictions, subjects revealed a significant left bias, irrespective of the sound category. This left bias was slightly but not significantly stronger in German subjects. The fMRI experiments confirmed that the speech stimuli evoked a significant left lateralized activation in BA44 compared to the artificial sounds. These findings suggest that in adult humans, orienting biases are not necessarily coupled with lateralized processing of acoustic stimuli (Fischer et al., 2009). Furthermore, a systematic comparison of the results of studies employing the orienting-asymmetry paradigm in different species, ranging from harpy eagles to vervet monkeys, yielded largely inconsistent results (Teufel et al., 2010). Some of the findings of the orienting asymmetry paradigm even contradict results obtained with more established and direct measures of lateralized acoustic processing. In addition to the lack of empirical support for the orienting paradigm, there are also problems with the underlying assumptions: the neck muscles that are responsible for the orienting response are controlled by a ventromedial neural pathway, which projects ipsilaterally in the brain. In other words, an orienting response to the right would be controlled ipsilaterally, that is, by the right hemisphere—not the left hemisphere. We conclude that the empirical inconsistencies produced by the orienting-asymmetry paradigm, and the lack of sufficient evidence supporting the paradigm's underlying assumptions, warrant serious caution when interpreting results obtained by the method. Non-trivial interpretations of orienting-asymmetry results will require a much better understanding of how lateralized brain functions interact with overt behaviors.

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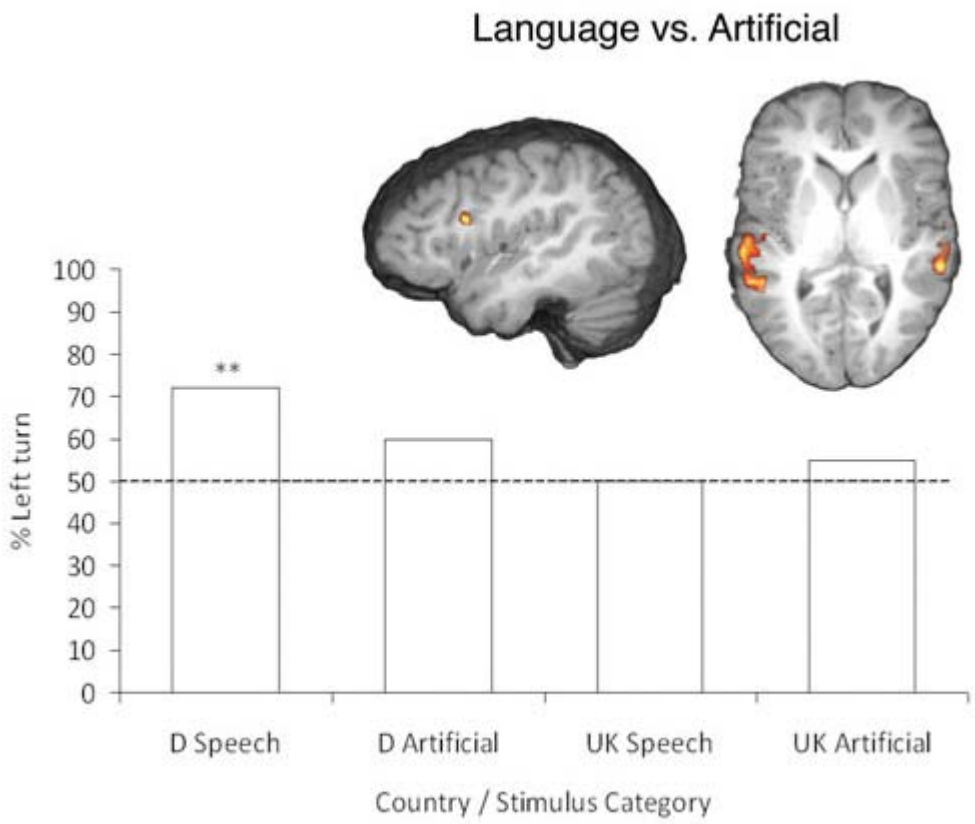
Language vs. Artificial
100
90
80
70
60
50
40
30
20
10
0
% Left turn
**
D Speech
D Artificial
UK Speech
UK Artificial
Country / Stimulus Category

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# Acid-sensing in the mouse vomeronasal organ

Annika Cichy[1], Jennifer Spehr[1], Marc Spehr[1]

[1]RWTH Aachen, Institute Biology II, Chemosensation, Worringerweg 1, 52074 Aachen, Germany

The mouse olfactory system is organized in at least four subsystems. Among those, the vomeronasal organ (VNO) plays an important role in the detection of pheromones and other social signals. However, most of the mechanisms underlying signal detection in the VNO remain unknown.
Here, we investigate acid-sensing mechanisms in vomeronasal sensory neurons in acute tissue slices of the mouse VNO by a pharmaco- electrophysiological approach. Using both current-clamp and voltage-clamp whole-cell recordings from optically identified vomeronasal neurons, we show here that acidic media of different pH values dose-dependently induce inward currents in voltage-clamp recordings. The same stimuli elicit robust action potential firing in current-clamp measurements. The pharmacological profile of the underlying ionic conductances indicates the possible contribution of multiple acid-sensing ion channels.
On-going molecular and biochemical studies as well as electrophysiological recordings will provide insight into the functional role of acid-sensing in the mouse vomeronasal organ.

# Age-related changes in the total number of olfactory microglomeruli in the mushroom bodies of the carpenter ant *Camponotus floridanus*

Carolin Sabine Bedel[1], Claudia Groh[1], Christina Kelber[1], Wolfgang Rössler[1]

[1]University of Würzburg, Biozentrum, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany[1]

Odors play an essential role for regulation of social interactions and colony organization in the carpenter ant *Camponotus floridanus*. The olfactory pathway of *C. floridanus* is well investigated (Zube et al. 2008 J Comp Neurol 506: 425). Projection neurons (PNs) leave the antennal lobe, the first olfactory center via a set of antennal lobe-protocerebral tracts (APT): a medial (m), lateral (l) and three mediolateral (ml) APTs. Whereas the ml-APTs exclusively project to the lateral horn (LH), the m- and the counterclockwise l-APT innervate both the mushroom bodies (MBs) as well as the LH. The MBs are regarded as sensory integration centers associated with learning and memory. M- and l-APT PNs differ in their target regions in the olfactory compartment of the MB-calyx lip (m- and l-APT region). We investigated how volumetric changes in the m- and l-APT region of the MB calyx relate to changes in the density and total number of microglomeruli (MG, characteristic synaptic complexes) in the MB calyx.

*C. floridanus* undergoes an age-related polyethism. To analyze age-related influences on structural plasticity of calycal MG, we established an immunolabeling-protocol for whole-mount preparations. Using confocal laser-scanning microscopy we quantified MG numbers in the m- and l-APT compartments of the MB-calyx lip by combining 2D- and 3D-image-analyses to determine both the volumes and MG densities. We compared three methods to estimate the total number of MG: calculation of optical-slice thickness, analyses of the number of MG in a defined volume (squared box-method), and measurements of MG-profiles.

The results show that the m-APT region contains a higher density and number of MG compared to the l-APT region, although the m-APT region volume is smaller (14% of the total lip volume in 13-15 day old ants, and 17% in 48-50 day old ants). The age-related increase in the total volume of the lip region was 19%. Using the squared box-method for extrapolation of the total number of MG in the different age-groups we found an increase in the total number of MG of 79% in the m-APT region compared to 36% in the l-APT region, and 45% for the total lip region. We conclude that age-related structural plasticity of MG is higher in the m-APT compared to the l-APT region of the MB-calyx lip.

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# Analysis of cAMP signaling in olfactory signal transduction in *Drosophila* larvae

Ulrike Pech[1], Atefeh Pooryasin[1], Andre Fiala[1]

[1]Department of Molecular Neurobiology of Behavior, Johann-Friedrich-Blumenbach-Institute for Zoology and Anthropology, Georg-August-University of Goettingen

The ability of organisms to sense their environment requires a molecular machinery which transduces sensory information into a neural signal. The mechanism of olfactory signal transduction in insects is currently under debate.
Olfactory signaling in *Drosophila melanogaster* larvae depends on the coexpression of a specific receptor and the non-specific Or83b coreceptor. Despite the knowledge of Or83b functioning as ion channel, it is not clear how the receptor proteins work together. Recent studies suggested the generation of a receptor potential via a second messenger independent pathway [1]. In another model the involvement of the second messenger cAMP is proposed. In this model Or83b forms a cAMP gated channel [2]. We have tested this model in *Drosophila* larvae.
*Drosophila* larvae express Or83b in all of their 21 olfactory sensory neurons and show a robust positive chemotaxis to different kinds of odors. We approached the question whether olfactory transduction is cAMP mediated by monitoring the behavior of transgenic larvae. Using the Gal4/UAS System we expressed the blue light sensitive adenylate cyclase PacÎ± [3] in olfactory sensory neurons; thereby increasing the cAMP level in the olfactory sensory neurons via light stimulation. As has been shown previously [4], we observed that a positive chemotaxis towards odors can be mimicked by light stimulation. To determine the role of Or83b in *Drosophila* olfactory transduction, we used PacÎ± to raise the cAMP level in olfactory neurons of larvae which lack Or83b. Our data show that in absence of the endogenous Or83b receptor, the light-induced behavioral attraction response is largely abolished.
To control for the proper functioning of the olfactory sensory neurons, we activated larval olfactory neurons using channelrhodopsin 2. We observed light-induced positive taxis behavior with or without Or83b.
The data indicate that cAMP is involved in *Drosophila* olfactory signaling and that Or83b is a target of cAMP. We therefore investigate possible functional implications of cAMP within the larval *Drosophila* olfactory signal transduction system.

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# Anatomical characterization of intrinsic neurons in olfactory and visual compartments of the mushroom-body calyx in the honeybee, *Apis mellifera*.

Sabrina Rippl[1], Jan Kropf[1], Wolfgang Rössler[1]

[1]Zoologie II (Universität Würzburg), AG Rössler, Am Hubland, 97074 Würzburg, Germany

Honeybee workers undergo an age-related polyethism and experience substantial changes in sensory input during behavioral transitions related to division of labor. These changes in sensory input and differences in performed tasks require a large degree of behavioral and neuronal plasticity. The mushroom bodies (MBs) represent higher sensory integration centers for olfactory and visual input and are associated with learning and memory. Consequentially, the MB calyces undergo substantial synaptic reorganization in the course of polyethic behavioral transitions. Olfactory projection neurons (PNs) from the antennal lobe innervate the lip and basal ring of the MB calyx, where they form large synaptic complexes (microglomeruli, MG), with dendritic spines from Kenyon cells (KC), the intrinsic MB neurons. One PN synapses on several KCs, and one KC receives synaptic input from several projection neurons, leading to divergence as well as convergence. The MB-calyx lip can be subdivided into two regions, each receiving input from the either the lateral or the medial antennal-lobe protocerebral tract (APT). The goal of this study is to characterize how the KC dendritic morphology and synaptic connectivity relates to the two subregions within the MB calyx lip, and to what extent different types of KCs in olfactory and visual compartments of the MB calyx change their dendritic branching patterns with major behavioral transitions. To analyze the dendritic morphology of individual or small groups of KCs, we used electroporation techniques. Glass-micropipettes were placed in different layers of the vertical lobe, close to the axonal endings of KC subpopulations. Current pulses were used to perforate cell membranes close to the electrode tip and to apply fluorescent markers that are taken up by individual KCs. The dye was allowed to be transported along the neurites, and subsequently brains were dissected, processed and finally analyzed under the confocal microscope as whole mounts or in thick sections. Image stacks were further processed with 3D software to obtain reconstructions of stained KCs. Furthermore, double stainings of PNs from the lateral or medial APT and KCs are performed to investigate the synaptic relationships with the two tracts of the dual olfactory pathway.

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# Biological function of odorant binding proteins in *Tribolium castaneum*

Stefan Dippel[1], Martin Kollmann[2], Joachim Schachtner[2], Stefan Schütz[3], Ernst A. Wimmer[1]

[1]Georg-August-Universität Göttingen, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Abteilung Entwicklungsbiologie, Justus-von-Liebig-Weg 11, 37077 Göttingen, Germany

[2]Philipps-Universität Marburg, Fachbereich Biologie, Abteilung Neurobiologie/Ethologie, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

[3]Georg-August-Universität Göttingen, Büsgen-Institut, Abteilung Forstzoologie und Waldschutz, Büsgenweg 3, 37077 Göttingen, Germany

In this project we combine transgenetic, reverse genetic, electrophysiological, chemical ecological, neurobiochemical and neuroanatomical approaches to study the correlation of odorants to odorant binding proteins (OBP) to odorant receptors (OR). The focus of
the project will lay on the biological function of OBPs which is still largely unknown, despite their necessity for olfaction. Especially the interaction of OBPs with ORs will be of key interest. Moreover, the established tools will also be applied to study postmetamorphic plasticity of the central olfactory pathway including the first central odour processing centers, the antennal lobes, and higher processing centers, the mushroom bodies.

# Brain architecture of *Nebalia* cf. *herbstii* (Crustacea, Leptostraca)

Matthes Kenning[1], Steffen Harzsch[1]

[1]Ernst Moritz Arndt Universität, Zoologisches Institut und Museum, Cytologie und Evolutionsbiologie, Johann-Sebastian-Bach Straße 11/12, 17487 Greifswald, Germany

Leptostraca, as the only extant subgroup in the malacostracan taxon Phyllocarida, are considered to be the most primitive members of the Malacostraca and sister-group of the Eumalacostraca. They are exclusively marine and, with few exceptions, benthic crustaceans found from the Arctic to Antarctica, from shallow water to abyssal depths. Within the Leptostraca the genus *Nebalia* contains approx. 23 of the 40 hitherto known species. In many crustacean groups outside the Decapoda our knowledge of the olfactory senses and integrating centers in the brain is quite limited. This is especially true for the more basal representatives of the Malacostraca. Hence, the present work focuses on the characterization of the general architecture of the central nervous system with regard to the central olfactory processing area of *Nebalia* cf. *herbstii* using immuno -histochemistry and fluorescence - and electron microscopy. Previous morphological and ethological studies suggest that terrestrial crustaceans, e.g. the hermit crabs *Coenobita clypeatus* and *Birgus latro*, possess an elaborate sense of aerial olfaction with corresponding adaptations in sensory equipment and brain areas. Due to the phylogenetic key position of the Leptostraca, data on their brain anatomy will help to reconstruct the architecture of the olfactory system in the ground pattern of Malacostraca .

# Ca2+ signals in genetically labeled GnRH receptor neurons in mouse brain slices

Christian Schauer[1], Oliver Mai[2], Iris N. Götze[1], Shuping Wen[2], Ulrich Boehm[2], Trese Leinders-Zufall[1]

[1]University of Saarland, School of Medicine, Department of Physiology, D-66421 Homburg, Germany
[2]Institute for Neural Signal Transduction, Center for Molecular Neurobiology, D-20251 Hamburg, Germany

An essential obstacle in neuroendocrinology is to understand how specific hormone-producing neurons are regulated by afferent pathways and how they impinge on downstream target cells to elicit behavioral and endocrine responses. A small subset of basal forebrain neurons that produce and secrete gonadotropin-releasing hormone (GnRH) controls reproductive physiology and behavior in mammals. To ensure reproductive success, the GnRH neuronal network has to process and integrate various cues. Likewise, it has to ensure that it selectively affects specific target cells in response to these cues.
Olfactory sensory signals follow distinct neural pathways dependent on the subtype of olfactory neuron. Some signals are relayed to the hypothalamus or the periaquaductal gray, which are implicated in mediating behavioral effects and neuroendocrine alterations triggered by pheromones. Pheromones have been implied to regulate GnRH neurons. We have therefore started to investigate the physiological properties of the subsequent target neurons containing the GnRH receptor (GnRHR).
To overcome a critical gap in neuroendocrinology we first had to develop a method to monitor both spatially and temporally stimulus-induced activity of individual GnRHR neurons in mouse brain slices. Our approach is based on a combination of confocal microscopy imaging of Ca2+ signals, the use of tissue slices to preserve the cytoarchitecture, and genetically identifiable GnRHR neurons.
To investigate the physiological properties of GnRHR neurons, we first recorded Ca2+ signals in brain slices using a confocal microsope. GnRHR neurons showed robust GnRH-induced Ca2+ elevations, however these signals differed in their waveform depending stimulus strength and area. Our studies set the stage to analyze how GnRH signaling effects on reproductive behavior and physiology are elicited in the mouse brain.

This work was supported by a Deutsche Forschungsgemeinschaft (DFG) grant to U.B. (BO1743/2) and a DFG Schwerpunktprogramm 1392 grant 'Integrative analysis of olfaction' to U.B. (BO1743/4) and to T.L.-Z. (Le2581/1). T.L.-Z. is a Lichtenberg Professor of the Volkswagen Foundation.

# Centrifugal olfactory information to the honeybee antennal lobe

Missanga Flôr van de Sand[1], C. Giovanni Galizia[1], Cyrille C. Girardin[1]

[1]University of Konstanz, Department of Neurobiology, Universitätstrasse 10, 78457 Konstanz, Germany

In the honeybee (*Apis mellifera*) information about odors is relayed from the antennae to the antennal lobes, the primary olfactory area in the brain (comparable to the olfactory bulb in vertebrates). The antennal lobe consists of a dense network of local neurons that interact with the incoming receptor neurons and the outgoing projection neurons. Higher areas such as the mushroom bodies and the lateral protocerebrum are innervated by these projection neurons receiving information from the antennal lobe. Is there a feedback connection from these higher brain areas into the antennal lobe?

In a combined physiological and pharmacological approach, we silenced two higher brain areas (mushroom bodies and lateral protocerebrum), while recording projection neuron responses in the antennal lobe using calcium imaging. Inactivation was induced using pressure injections of the local anesthetic lidocaine. We compared responses in projection neurons to odor stimulation before and after inactivation of higher brain areas.

Silencing the mushroom body output area (a-lobes) produced an increase of the sustained part of the response to odors in the projection neurons. These results suggest the existence of an inhibitory feedback loop from the mushroom bodies to the antennal lobe, either directly or via intercalated neurons.

Silencing the lateral protocerebrum led to a strong increase in the overall odor response. Interestingly this effect initiated only with a delay of about 30 minutes after drug application. Further investigations will reveal the mechanisms underlying this delayed increase of odor response. For example, lidocaine injections might affect memory pathways.

Our results suggest that secondary odor processing and possibly odor memory in the mushroom bodies and/or the lateral protocerebrum may influence primary odor processing in the antennal lobes. This mechanism could be involved in the integration of information from other sensory modalities or internal states into the primary sensory processing.

# Chemoperception in the human skin

Daniela Busse[1], Anna Christina Sondersorg[1], Hanns Hatt[1], Heike Benecke[1]

[1]Ruhr-University Bochum, Department of Cellphysiology, Bochum, Germany

Previous work indicates that the trigeminal nerve may contribute to odor perception. It was shown that high concentrations of almost all odors provoke typical trigeminal sensations *in vivo*, but only certain odors activate trigeminal neurons in cell culture. This leads to the suggestion that trigeminal perception may require a transfer of sensory information via crosstalk with additional cell types of the peripheral innervation area. The epidermis is extensively innervated by trigeminal fibres that form free nerve endings in close proximity to skin cells. Keratinocytes, the major cell type of the epidermis, express a variety of different sensory receptors that enable them to react to multiple environmental stimuli. Moreover, it was shown that keratinocytes release mediators upon thermal and mechanical stimulation that in turn activate trigeminal nerve endings and thus contribute to information processing in the skin.

This study is dedicated to unravel the molecular and cellular mechanisms underlying chemoperception in the human skin. For this purpose, physiological effects of odorant exposure were analyzed in primary keratinocytes using the calcium imaging technique. Several substances could be identified that induce calcium transients which were further characterized by pharmacological approaches. Moreover, expression analysis identified odorant receptors which might contribute to primary signal perception in keratinocytes.

Detailled investigation on the ability of keratinocytes to detect and convey chemical stimuli is important to understand how environmental influences are communicated within the skin.

# Chemosensory receptors of *Lepismachilis y-signata* (Insecta: Archaeognatha)

Christine Mißbach[1], Ewald Grosse-Wilde[1], Bill S. Hansson[1]

[1]Max Planck Institute for Chemical Ecology, Evolutionary Neuroethology, Hans-Knöll-Straße 8, 07745 Jena, Germany

The primary olfactory organs of insects are the antennae and maxillary palps. Both are covered with distinct types of olfactory sensilla. These sensilla house olfactory sensory neurons (OSNs) that send their axons into the antennal lobe, the first olfactory processing center in the insect brain. In the dendritic membrane of OSNs olfactory receptors (ORs) are located. ORs are multitransmembrane domain proteins unrelated to nematode or vertebrate olfactory receptors. Insect ORs function as heteromultimer composed of at least one ligand specific receptor and at least one OR83b coreceptor. It was hypothesized that ORs developed from gustatory receptors (GRs), another arthropod chemoreceptor family. Neither OR83b nor ORs were found in the genome of the crustacean *Daphnia pulex*, however, the related gustatory receptors were identified. Based on this study and the proposed relationship between insects and crustaceans we hypothesize that OR83b and ORs of the insect type emerged within insect evolution. To address this hypothesis we have chosen a very ancient insect group, the Archaeognatha, for our investigations. According to the fossil record Archaeognatha evolved about 390 million years ago. Morphological as well as molecular data support a sister group relationship between this insect taxon and the remaining insects.
Most recently olfactory glomeruli were described in *Machilis germanica*, a machilid archaognathan. We could show that a glomerular antennal lobe is also present in the jumping bristletail *Lepismachilis y-signata*. We analyzed transcriptomic sequences of *L. y-signata* to identify putative chemoreceptors with particular focus on ORs and OR83b.

# Colony recognition in social insects as a new model for quality coding of multi-component odors

Andreas Simon Brandstaetter[1], Wolfgang Rössler[1], Christoph Johannes Kleineidam[1,3]

[1]University of Würzburg, Biozentrum, Behavioral Physiology and Sociobiology (Zoologie II), Am Hubland, D-97074 Würzburg, Germany
[2]CNRS (UPR 9034), Evolution, Génome and Spéciation, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France
[3]University of Konstanz, Department of Biology, Universitätsstraße 10, D-78464 Konstanz, Germany

Cooperation within social groups is beneficial to defend resources against rivaling groups, and for an aggressive defense, foes need to be recognized accurately. Ants discriminate colony members (nestmates) from foreign workers (non-nestmates) by complex, multi-component colony odors. For discrimination, colony odors have to be classified by the nervous system as nestmate or non-nestmate specific, yet the neuronal basis of colony recognition remains elusive hitherto. We investigated the neuronal representation of nestmate and non-nestmate colony odor in the olfactory system of *Camponotus floridanus*: i) in olfactory receptor neurons (ORNs) of the antenna using electroantennography and ii) in the antennal lobe (AL), the first olfactory neuropil, using calcium imaging. Workers perceived nestmate and non-nestmate colony odors, since both stimuli elicited neuronal responses at peripheral (ORNs) and AL level. This finding invalidates the previously proposed sensory filter hypothesis, which suggested that ORNs are adapted to nestmate colony odor and only non-nestmate information is relayed to the brain.

Odors elicit spatial patterns of activity in the AL and previous studies indicated that these activity patterns reflect how an odor is perceived by an animal (odor quality). We measured colony odor-specific spatial activity patterns in the AL. Surprisingly, upon repeated stimulation the activity pattern was variable, and as variable as the activity patterns elicited by different colony odors. We conclude that the spatial activity patterns alone do not provide sufficient information about the colony odor to discriminate friends and foes. Our result illustrates the enormous challenge for the nervous system to classify multi-component odors and indicates that other neuronal parameters besides spatial activity patterns are important for coding of odor quality in insects.

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# Comparative brain morphology in representatives of the Diplopoda with a focus on the central olfactory pathway

Florian Seefluth[1], Andy Sombke[1], Steffen Harzsch[1]

[1]Universität Greifswald, Zoologisches Institut & Museum, Cytologie und Evolutionsbiologie, Johann Sebastian Bach-Str. 11/12, 174898 Greifswald, Germany

Besides hexapods and crustaceans, the myriapods are the third arthropod taxon within the Mandibulata. The position of the Myriapoda within the Euarthropoda is still in debate. In the traditional system of Heymons (1901), the Myriapoda were the sistergroup to the Hexapoda. Recently, this hypothesis was challenged by two controversial alternatives: the Myriochelata concept (Chelicerata and Myriapoda) and the Tetraconata concept (Hexapoda and Crustacea). Following the latter concept, the Myriapoda represent the sistergroup to the Tetraconata (Regier et al. 2010). In the last decade, comparative neuroanatomical studies have provided a fresh view on arthropod relationships so that an analysis of Myriapoda brain architecture may contribute to our understanding of arthropod brain evolution ("Neurophylogeny" Harzsch 2006). However, comparative studies on the myriapod nervous system are few in number. Besides recent anatomical studies on the Chilopoda (Sombke et al. 2010), brain architecture in this taxon has not been studied in any detail. In order to gain more insights into the brain morphology of myriapods, we focus on the central nervous system of diplopods using immunohistochemical methods combined with fluorescence and laser scanning microscopy, scanning electron microscopy, anterograd backfilling, serial-thin section and 3D-reconstructions. Thus, several questions can be asked: how are the olfactory neuropils organized and does the brain architecture corresponds to other mandibulate taxa? In a neurophylogenetic approach, our diplopod data will be used for reconstructing arthropod phylogeny and for understanding the evolution of arthropod olfactory systems.

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# Distinct populations of bitter taste receptor cells in mice

Sandra Hübner[1], Sabine Frenzel[1], Anja Voigt[1,2], Masataka Narukawa[1], Kristina Loßow[1], Ulrich Boehm[2], and Wolfgang Meyerhof[1]

[1]Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbrücke, Germany

[2]Institute for Neural Signal Transduction, Center for Molecular Neurobiology Hamburg, Germany

Mammals use the sense of taste to evaluate the nutritional value of food and avoid ingesting potentially harmful substances. Each of the five taste qualities sweet, umami, sour, salty, and bitter fulfils a specific subtask. In general, bitter taste is associated with avoidance behavior and believed to prevent organisms from ingesting poisonous substances. However, bitter compounds can also contribute to the enjoyment of food and beverages and some even elicit beneficial health effects. Thus, a discrimination of different bitter compounds by the taste system would be advantageous but is unproven.

Bitter taste perception is initiated in the mouth in special morphological structures called taste buds which contain segregated populations of receptor cells, each dedicated to detect stimuli of only one taste quality. Bitter receptor cells express G protein -coupled receptors of the Tas2r family that recognize the countless bitter substances. However, the extent of coexpression of the ~35 Tas2rs in the same cells is unknown.

To elucidate the extent of Tas2r coexpression in bitter taste cells, we investigated genetically manipulated mice. First, we generated mice in which the *Tas2r131* gene has been knocked out. In these mice we analyzed the expression of 14 different Tas2rs by RT-PCR and *in situ* hybridization of taste papillae. The data revealed that the loss of the *Tas2r131* gene did not affect the expression of other *Tas2r* genes.

Next, we generated mice with genetically ablated Tas2r131 expressing cells by crossing Tas2r131$^{Cre}$ with R26:lacZbpA$^{flox}$DTA mice and examined the expression of the same set of 14 Tas2rs as above in the progeny. As expected, we found that Tas2r131 mRNA was undetectable in the experimental mice clearly demonstrating that they lack all Tas2r131 expressing cells. Similarly, expression of 3 other *Tas2r* genes was extinguished indicating that they were always coexpressed with the *Tas2r131* gene in the same cells. In marked contrast, we observed detectable, although diminished expression of 4 *Tas2r* genes suggesting that these are expressed in both, Tas2r131 expressing cells and in cells not expressing Tas2r131. Finally, expression levels of 6 of the 14 *Tas2r* genes was unaltered in the experimental animals demonstrating that these 6 genes were only expressed in cells that do not express Tas2r131.

Together, our data clearly demonstrate the existence of specific subpopulations of bitter taste receptor cells characterized by the expression of distinct sets of Tas2rs. The data also indicate that the selection of Tas2rs to be coexpressed in the same bitter receptor cells is not stochastic but governed by gene regulatory mechanisms.

To examine functional consequences that the distinct populations of bitter cells may have, we are searching for cognate bitter compounds for the 14 Tas2rs and performing single cell imaging of taste cells, nerve recordings as well as brief access behavioral tests with various bitter compounds. The expected results will assess the discriminatory power of bitter taste.

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# Ectopically Expressed Olfactory Receptors

Markus Osterloh[1], Elena Guschina[1], Hanns Hatt[1]

[1]Ruhr-University Bochum, Department of Cell Physiology, Universitätsstr. 150, 44801 Bochum, Germany

G-Protein coupled receptors (GPCRs) are membrane receptors which conduct signals from the cell exterior to the inside. Olfactory receptors (ORs) comprise the largest group of GPCRs and are responsible for the recognition of different odorant molecules. Despite the original "dogma" that ORs are only expressed in the olfactory epithelium, it has been shown in recent years that this is not the case. Olfactory receptors are widely expressed among different tissues throughout the body in various organisms. In contrast to their wide expression, the physiological functionality of ectopically expressed human ORs is known for only a few ORs e.g. OR1D2 (chemotaxis in sperm) or OR51E2 (inhibition of proliferation in prostate cancer cells). Human skeletal muscle cells as well as human heart cells also express a number of ORs which has been shown by microarray analysis and RT-PCR. Publications on the murine skeletal muscle and the rat heart have shown a physiological relevance of certain ORs in these tissues leading to the assumption that they might play a role in humans as well. We could show so far that human primary skeletal muscle cells express certain ORs and respond to odor stimulation with an intracellular calcium increase. This calcium rise is specific to defined odors and also dependent on the developmental stage of the cells.

# Effect of TRP agonists upon human KCNK channels

Leopoldo Raúl Beltrán[1], Madeline Ferreira[1], Günter Gisselmann[1], Hanns Hatt[1]

[1]Ruhr-University Bochum, Department of cell Physiology, Universitätsstraße 150 , 44801 Bochum, Germany

KCNK channels (also known as K2P, two pore domain potassium channels) are considered to be the main channels responsible for the leak potassium current. A current that maintains the membrane resting potential and also exerts control over excitability, this by shaping the duration, frequency and amplitude of action potentials. Increased K+ leak current stabilizes the cell at hyperpolarized voltages below the firing threshold, whereas leak suppression permits or facilitates depolarization and excitation. In this manner KCNK channels play a role in such diverse processes as metabolic regulation, apoptosis, thermoception, and chemoperception.

Human KCNK channels are reported to be broadly expressed in the central and peripheral nervous system (Medhurst et al. 2001). Previous studies have shown that substances reported as prickling like ¦Á-hydroxy sanshool (Bautista et al. 2008) or as pungent like capsaicin (Beltr¨¢n, unpublished data) apart from their known effect on TRP channels, also present an effect on mouse KCNK channels.

In order to further study the role of human KCNK channels in chemoperception, with especial focus on trigeminal chemoperception, we performed a series of screenings with very well characterized thermo-TRP agonist e.g. capsaicin and 2-APB on hKCNKs. For this purpose we used *Xenopus laevis* oocytes injected with cRNA coding for 5 human KCNK channels (hKCNK2, hKCNK4, hKCNK9, hKCNK10 and hKCNK18).

Here we show that the human acid-sensitive channel hKCNK9 (hTASK3) is a target for many well known TRP agonist, mainly to plant-derived phenol-containing compounds, known to act as activators for TRPV3 (Xu et al. 2006), but also to TRPV1 agonists like capsaicin and piperine (Caterina et al. 1997 and McNamara et al. 2007). The concentration at which these compound act on hKCNK9 do not differ much form the ones required for an effect on TRP channels. Another compound that we found also to be highly effective was 2-APB. This worked as an activator for all the members of the TREK subfamily of hKCNK channels (hKCNK2, hKCNK4 and hKCNK10). Our results suggest a synergism at the primary level of chemoperception between the TRP and KCNK families in a scale broader as previously thought.

# Effects of brief sensory experience on the sensitivity of male moths to chemical cues: “general sensitization” or “selective attention”?

Sebastian Antonio Minoli[1], Violaine Colson[1], Virginie Party[1], Michel Renou[1], Frederic Marion-Poll[1], Sylvia Anton[1]

[1]INRA, Unite Mixte de Recherches en Physiologie d'Insectes-Signalisation et communication, Centre de Recherches de Versailles-Route de St Cyr, 78026 Versailles cedex, France

The innate responses of animals to sensory cues from their environment can be modulated by experience. Modulation may concern responses to the same stimulus, to different stimuli within the same modality or might even have an effect across modalities. It was shown in Spodoptera littoralis that a brief pre-exposure to the female pheromone increases the sensitivity of males to the same odour (Anderson et al., 2003). Differently from associative learning paradigms, no reward is necessary to induce this sensitization (non-associative). Here we tested if a brief pre-exposure to biologically relevant stimuli can change the level of response to the same or other stimuli in males of S. littoralis (Lepidoptera: Noctuidae). Olfactory (pheromone and plant odours) and gustatory (sucrose and quinine) pre-exposure and subsequent behavioural tests were carried out to analyze possible intra- and cross-modal effects. Results show a general increase in the response of the males 24 hours after pre-exposure. This sensitization was shown to work within stimulus (i.e. animals pre-exposed to a compound increased their sensitivity to the same compound), within modality (i.e. animals pre-exposed to a stimulus are also more sensitive to another compound of the same modality), and even across modalities (i.e. animals pre-exposed to a gustatory stimulus increased their olfactory sensitivity and vice versa). However, a strong mechanical stimulus (vortex shaking) applied as a pre-exposure treatment did not modify the behaviour of S. littoralis males. Based on our results, we propose that the observed effects represent a form of general sensitization rather than a phenomenon of selective attention. We suggest the existence of a general switch from “low” to “high” sensitivity maintained for at least 24h. In this way, the sensory system might economize energy being less sensitive when no external cues are present, and becoming more efficient when they are exposed to a rich sensory environment.

Reference:
Anderson,P., Sadek,M.M. and Hansson,B.S. (2003) Pre-exposure Modulates Attraction to Sex Pheromone in a Moth. Chem. Senses, 28, 285-291.

# Electrophysiological investigation of mitral cell signaling properties in the mouse accessory olfactory bulb

Monika Gorin[1], Silke Hagendorf[1], Marc Spehr[1]

[1]RWTH Aachen, Institut für Biologie II, Chemosensorik, Worringer Weg 1, 52074 Aachen, Germany

The accessory olfactory bulb (AOB) represents the first relay station of information processing in the rodent accessory olfactory system. In the vomeronasal organ, social chemosignals activate sensory neurons that form synaptic contacts with AOB mitral/tufted cells which are the main excitatory projection neurons in the AOB. In turn, mitral cells receive modulatory input from different classes of inhibitory interneurons, such as granule and periglomerular cells. However, the role of mitral cells in social information coding and signal integration in the AOB is not fully understood.

Here, we investigate the biophysical properties of AOB mitral cells using a combination of electrophysiological, pharmacological and imaging approaches in acute mouse AOB tissue slices.

We show that the physiological properties of mitral cells enable the faithful transmission of primary sensory signals from vomeronasal neurons, modulation of these signals and their relay to higher brain centers.

These data will advance our understanding of the functional role of mitral cells in olfactory information coding and processing in the mammalian accessory olfactory system.

# Endocannabinoid action in the olfactory epithelium of *Xenopus laevis* tadpoles

Esther Breunig[1], Ivan Manzini[1,3], Fabiana Piscitelli[4], Benjamin Gutermann[1], Vincenzo Di Marzo[4], Dirk Czesnik[1], Detlev Schild[1,2,3]

[1]University of Göttingen, Department of Neurophysiology and Cellular Biophysics, Humboldtallee 23, 37073 Göttingen, Germany
[2]Deutsche Forschungsgemeinschaft Cluster of Excellence 171, 37073 Göttingen, Germany
[3]Deutsche Forschungsgemeinschaft Research Center for Molecular Physiology of the Brain, 37073 Göttingen, Germany
[4]Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, 80078 Pozzuoli, Naples, Italy

A recent study has shown, that the cannabinoid receptor 1 (CB1 receptor) is expressed on dendrites of olfactory receptor neurons in the olfactory epithelium of Xenopus laevis tadpoles. Pharmacological interference with this receptor results in altered olfactory responses, antagonists of the CB1 receptor reduce and delay odorant-induced $[Ca^{2+}]_i$ transients.

Since it has been suggested that the feeding state modulates the olfactory sensitivity, we speculated, that endocannabinoids might be involved in the underlying mechanisms. In this study we identified 2-AG as an endocannabinoid acting in the olfactory epithelium of larval Xenopus laevis. Supression of 2-AG production delayed and decreased odorant -induced responses. Two differential synthesis pathways exist in the olfactory epithelium: First, 2- AG is produced by diacylglycerol lipase ß in olfactory receptor neurons and acts in an autocrine way at CB1 receptors on their dendrites, second, 2- AG is produced by diacylglycerol lipase a in sustentacular supporting cells and acts in a paracrine way at CB1 receptors on the sensory cell dendrites. Synthesis of 2-AG in sustentacular supporting cells depends on hunger. Well fed animals exhibited low 2-AG concentrations in their olfactory epithelia. Vice versa, food -deprived animals had increased concentrations of 2-AG, which renders olfactory receptor neurons more sensitive. As a consequence, we could show that odorant detection thresholds are decreased in these animals.

In summary, the endocannabinoid system in the olfactory epithelium modulates olfactory sensitivity in a hungerdependent manner and may thus control food intake.

# Exploring the properties of TMEM16b in cilia of olfactory sensory neurons

Bastian Toetter[1], Sebastian Rasche[3], Sonja Oberland[1,2], Thomas Pelz[1,2], Eva M. Neuhaus[2]

[1]FU-Berlin, Fachbereich Biologie, Chemie, Pharmazie, Takustr. 3, 14195 Berlin, Germany.
[2]NeuroScience Research Center, Charité, Universitätsmedizin Berlin, 10117 Berlin, Germany.
[3]Department of Cell Physiology, Ruhr-Universitaet Bochum, Universitaetsstrasse 150, 44780 Bochum, Germany.

Calcium-activated chloride channels (CaCCs) play a fundamental role in various physiological processes, such as fluid secretion, regulation of neuronal activity and amplification of sensory stimuli. Although CaCCs are involved in many different processes their molecular identities remain elusive in many cases. Recently a novel protein family with ten members, referred to as TMEM16a-k or Anoctamin 1-10, attracted attention as a new family of potential CaCCs. Up to now at least two members, TMEM16a and TMEM16b were clearly identified as CaCCs by independent groups. We showed that TMEM16b is specifically expressed in the cilia of olfactory sensory neurons and is consequently a bona fide candidate for the long sought CaCC of the olfactory signal transduction cascade. Since there are virtually no molecular characteristics of TMEM16b known, except for the basic electrophysiological properties, we investigate potential protein-protein interactions. Here we lay emphasis on those interactions that may influence the localization of the protein in the cilia. Our aim is to better understand the trafficking mechanisms of TMEM16b and the regulation by different components of the ciliary proteom in olfactory sensory neurons.

# Expression of odorant binding proteins and olfactory receptors in the antenna of the malaria mosquito, *Anopheles gambiae*

Jürgen Krieger[1], Maike Forstner[1], Anna Schultze[1], Danuta Schymura[1]

[1]University of Hohenheim, Institute of Physiology, Garbenstr.30, 70599 Stuttgart, Germany

*Anopheles gambiae* females are major vectors for the transmission of malaria causally linked to the requirement of a blood meal to complete their gonadotrophic cycle. While female malaria mosquitoes rely on their sense of smell to find a blood host, as well as sugar sources and oviposition sites, the nectar feeding males mainly locate host plant odours. Both sexes detect and discriminate volatile odorants by means of olfactory receptor neurons (ORN) located in sensory structures, named olfactory sensilla. The majority of olfactory sensilla populate the antennae, whereas much smaller numbers are found on the maxillary palps. In the female antenna around 750 olfactory sensilla distribute over all 13 flagellomeres, while the male antenna carries around 250 "olfactory hairs" restricted to the distal two segments. Upon entering a sensillum through pores in the cuticle odorant molecules are supposed to be captured by odorant binding proteins, which solubilize the signal molecules in the aqueous sensillum lymph and transfer them towards olfactory receptors in the dendritic membrane of the ORNs. Large and diverse gene families encoding 57 candidate odorant binding proteins (AgOBPs) and 79 putative odorant receptors (AgORs) have been annotated from bioinformatic screens of the *A. gambiae* genome. However, the number and topographic distribution of the cells, which express the various AgOBPs and AgORs is largely unknown. In order to visualize the AgOBP- and AgOR-expressing cells in the antenna we have adapted a whole mount in situ hybridization method based on fluorescent riboprobes (WM-FISH). The WM-FISH results indicate that different AgOBPs and AgORs differ in the number and distribution of the expressing cells along the 13 flagellomeres of the female antenna and in the last two segments of the male antenna. Both sexes express the same AgOBP- and AgOR-types, but there is a significant sexual dimorphism concerning the number and topography of the expressing cells. This may suggest gender-specific differences in the ability to detect distinct odorants, specifically human host-derived volatiles.

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# Expression of the Immediate Early Gene egr1 (krox-24, zif 268, ngfi-a and zenk) as neuronal activity marker in zebrafish (Danio rerio)

Sigrid Kress[1], Mario Wullimann[1]

[1]Ludwig-Maximilians-University Munich Division of Neurobiology , Department Biology II , Großhaderner Straße 2 , D-82152 Planegg-Martinsried , Germany

Immediate Early Genes (IEGs) are a class of genes that are rapidly and transiently transcribed in response to neuronal activity. Therefore, their gene expression indicates that a neuron is activated by synaptic input and thus shows increased neuronal activity. IEG expression has been used as a marker for neuronal activity in the brain of various vertebrate species in different behavioural contexts from perception to cognition, for example in teleosts (Harvey-Girard, et al., 2010).

The sense of smell plays a crucial role in fish in predator avoidance, feeding, reproduction, migration and kin recognition. For this reason the current study investigated the potential role of egr1 as neuronal activity marker related to odor perception and in particular for kin recognition. Zebrafish kin recognition is based on phenotype matching of olfactory cues which is caused by imprinting on urinary odour during development (24h time window from 6 – 7 days post-fertilization, Gerlach and Lysiak, 2006; Gerlach, et al., 2008). Likely, zebrafish larvae use kin recognition for aggregating (shoaling) and adult females for inbreeding avoidance. Therefore, we developed an olfactory test procedure to determine brain activity triggered by kin recognition in zebrafish larvae and adult females. We wanted to show differences in brain activity between imprinted and non-imprinted larvae and also the basal adult female expression of egr1. Therefore, we first established the basal expression pattern of the IEG egr1 (krox-24, zif 268, ngfi -a and zenk) in imprinted and isolated zebrafish larvae between day 4 and 8 and in imprinted adult female zebrafish. The results showed an extensive activity of egr1 in both imprinted/non-imprinted larval and in adult female zebrafish forebrain, including the olfactory bulb, and midbrain, but not in the hindbrain (except for a new domain in the cerebellum in adult females). There was no apparent quantitative difference between imprinted and non-imprinted larvae.

In mice, egr1 mediates activity-dependent tyrosine hydroxylase (TH) expression in dopaminergic periglomerular neurons in the olfactory bulb (OB) (Akiba, et al., 2009), since odor deprivation with naris-occlusion revealed almost complete absence of egr1 and TH expression levels in these dopamine cells, while egr1 expression in the more numerous GABAergic cells is not affected. Therefore, double-labeling for TH and egr1 was performed in brains of adult female zebrafish that were exposed to male odor water or neutral water with the goal to determine a possible effect on the number of egr1/TH positive neurons in the OB. The results demonstrated that a double-label is the exception in the zebrafish brain but that in the OB egr1/TH co-localization does occur in periglomerular cells, as in the mouse. Further experiments are needed to show under which olfactory conditions, egr1/TH expression changes in the zebrafish OB.

# Expression of Voltage Gated Sodium Channels in Identified Granule Cells of the Mouse Olfactory Bulb

Daniel Nunes[1], Thomas Kuner[1]

[1]Institute for Anatomy and Medicine Cell Biology,University of Heidelberg, Heidelberg, Germany

The olfactory bulb (OB) generates a complex spatio-temporal pattern of input activity representing the first neuronal representation of odors. As in other sensory systems, lateral inhibition may provide an important mechanism to process spatio-temporal activity patterns for improved discrimination of similar sensory stimuli. In the OB, the synapse between mitral cells (MCs) and granule cells (GCs) is thought to mediate the process of lateral inhibition in three possible ways: recurrent inhibition, local lateral inhibition and global lateral inhibition. Recurrent inhbition may only occur upon weak stimulation of the GC, when GABA is only released at the synapse activated. Local lateral inhibition may involve the activation of nearby synapses, while global lateral inhibition causes inhibition of all mitral cells connected to a GC. The latter is thought to be mediated by action potentials spreading throughout the GC. Yet, while sodium-based action potentials have been shown to occur in GCs, their functional role on a cellular and behavioral level remains unclear.

Here, we identified and determined the distribution of voltage-gated sodium channel a-subunits in the mouse olfactory bulb, with a particular focus on GCs. Since there are nine different a-subunits described, which expression varies among cell type and tissue, we first used rtPCR and western blotting to determine which of those are present in the entire olfactory bulb. The rtPCR results showed that SCN1a (Nav1.1), SCN2a (Nav1.2), SCN3a (Nav1.3) and SCN8a (Nav1.6) are the main subunits expressed in the olfactory bulb. All of these subunits were detected in western blot, but Nav1.7 (encoded by SCN9a, not detected in rtPCR) was found to be present as protein. Immunohistochemistry performed in free-floating sections revealed that Nav1.7 is present at the terminals of the olfactory receptor neuron axons forming synapses in the glomerular layer of the OB.

To delineate which of these subunits are expressed in GCs and how they are spatially distributed, we used targeted 3D-immunohistochemistry. In short, by means of viral gene delivery, the GC layer was infected with AAV1/2 that induced over-expression of membrane bound GFP (mGFP) in GCs. The infected GCs were easily identified and the combination of the mGFP signal with the immuno-signal allowed to visualize unequivocally the distribution of sodium channel a-subunits within GCs. Further, the mGFP defined volume occupied by a GC was determined by tresholding the mGFP signal and it was subsequently used to excise only those immuno-signals residing within the GC. Using this approach, we found that mature GCs express only the Nav1.2 subunit. The immuno-signals revealed a clustered expression of Nav1.2 along the plasma membrane, in the cell body, dendrites and at the gemmules (spines of the GC with large spine heads, that are part of the dendro-dendritic synapse). A more detailed analysis showed that Nav1.2 is present all along the dendrites, with very dense clusters at denditric bifurcations and origins of gemmules. Furthermore, Nav1.2 clusters were regularly found in the gemmules, both at the neck of the gemmules and in the transition between neck and head.

This localization pattern supports the hypothesis that sodium-dependent action potentials may be triggered by local depolarizing events in gemmules and hence, may play a pivotal role in mediating global lateral inhibition.

# Formation and activation of OR37 glomeruli

Verena Bautze[1], Raphaela Bär[1], Heinz Breer[1], Jörg Strotmann[1]

[1]Universität Hohenheim, Institut für Physiologie, August-von-Hartmann-Str. 3, 70599 Stuttgart, Germany

In the mammalian olfactory system sensory neurons which express the same odorant receptor (OR) gene are usually broadly dispersed throughout the olfactory epithelium (OE) and send their axons to two distinct glomeruli in the olfactory bulb. In contrast, cells which express a member from the unique OR37 subfamily are concentrated in a small central patch in the OE and send their axons to only one glomerulus per bulb. The glomeruli formed by neurons expressing the different OR37 subfamily members are located in immediate vicinity to each other and although the receptors are highly similar in sequence, each glomerulus is precisely innervated by only one, receptor-specific population. However, this precision in glomerular targeting is achieved only postnatally, suggesting that odor-induced activity might be required for this process. To test this hypothesis, knock-out animals for a subunit of the olfactory cyclic nucleotide gated channel were analyzed. The formation of the subtype-specific glomeruli was not affected, however, a few additional glomeruli were now formed by OR37 expressing cells. Analyzing the distribution pattern of OR37 expressing cells revealed many cells ectopically positioned outside the patch, suggesting that odor induced activity is essential for the establishment of their correct spatial patterning in the OE.

A detailed molecular characterization of the OR37 receptors demonstrated that they are not only expressed by cells with an atypical topographic patterning, but display several other special features, as well. Only this group of ORs is characterized by a unique structural element, an insertion of six amino acids in the third extracellular loop. Comparative analyses showed that OR37 type receptors exist in diverse mammalian species and their coding sequences display an unexpected high degree of conservation; bioinformatic data revealed that these genes are under negative selective pressure, a feature that is quite unusual for OR genes. Together these data indicate that the OR37 receptors may be tuned to special ligands. As a novel approach to identify ligands for these receptors the fact was exploited that the axons of sensory neuron populations expressing a distinct OR37-subtype converge onto a receptor specific glomerulus. Former studies with rats and preliminary tests with mice indicated that molecules with long hydrocarbon chains activate ventrally positioned glomeruli. Using transgenic mice in which distinct OR37 subtypes are coexpressed with tau-lacz it was possible to visualize and identify the appropriate glomerulus. An activation was monitored through the expression of the activity marker c-fos in the periglomerular cells. Molecules with a chain length between 14 and 18 C-atoms and different functional groups were systematically analyzed. A set of molecules activating the OR37 glomeruli was identified.

# Gene expression patterns on antennal RNA of the leaf-cutting ant *Atta vollenweideri*.

Sarah Koch[1], Christoph J. Kleineidam[1], Ewald Grosse-Wilde[2]

[1]University of Konstanz, Neurobiology, Universitätsstr. 10, 78456 Konstanz, Germany
[2]MPI Chemical Ecology, Neuroethology, Hans-Knöll-Straße 8, 07745 Jena, Germany

Leaf-cutting ants live in highly organized colonies with a single queen and several million of workers. Division of labor within the worker caste is based on task allocation of workers that differ in size (alloethism). The worker alloethism is not only related to body size but also related to different neuroanatomical phenotypes. In our previous studies, we found three distinct phenotypes in workers that differ in the organization of the first olfactory neuropil, the antennal lobe (AL; 1). In addition, a pronounced sexual dimorphism was described in the olfactory system of the other two castes, the males and the queens (2). All 5 phenotypes differ in number (240-440) and size of glomeruli. In large workers, one glomerulus and in males three glomeruli are extremely large (macroglomeruli; MG).
The different AL phenotypes are the neuronal basis for distinct behavioral patterns and division of labor. As an example, the MG in workers processes information about the trail pheromone and workers with MG are more sensitive to the pheromone.
It has been reported for other insect species, including the hymenopteran Apis mellifera that the total number of glomeruli in the AL correlates with the number of expressed OR genes. Therefore, we assume that the AL polymorhpisms are reflected in the expression pattern of OR coding genes in the antenna. We analysed the totality of antennally expressed genes across castes (the antennal transcriptome) by random next-generation sequencing. From this data we identified over 200 putative OR coding genes and subsequently performed caste-specific microarrays to reveal the phenotypical polymorphism on a receptor level. Our aim is to identify the neuronal basis of the distinct odor-guided behaviors.

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# Identification of novel olfactory receptor interaction partners

Sonja Oberland[1], Thomas Pelz[1,2], Bastian Toetter[1,2], Eva M. Neuhaus[2]

[1]FU-Berlin, Fachbereich Biologie, Chemie, Pharmazie, Takustr. 3, 14195 Berlin, Germany.
[2]NeuroScience Research Center, Charité, Universitätsmedizin Berlin, 10117 Berlin, Germany.

Olfactory receptor genes represent the largest gene family in the mammalian genome. Although there are approximately 1200 functional genes in mouse, only few receptors are functionally characterized. One challenge is the expression of olfactory receptors in heterologous cells, which typically fails as they are retained in the endoplasmic reticulum or degraded. To find essential interaction partners supporting the accurate transport and functioning of olfactory receptors, we started searching highly expressed proteins in olfactory cilia. Therefore a proteomic analysis of cilia preparations was screened for candidate genes. Expression levels were further confirmed and quantified using real time PCR. Selected proteins were cloned into expression vectors to investigate their subcellular localization in heterologous cells and their putative influence on olfactory receptor localization or expression. Furthermore we analyze occurring protein-protein interactions using a BRET assay and validate their presence and functional implications in olfactory sensory neurons.

# Immunocytochemical describtion of serotonergic neurons in the central nervous system of Remipedia (Crustacea)

Torben Stemme[1], Steffen Harzsch[2], Gerd Bicker[1], Stefan Koenemann[3]

[1]University of Veterinary Medicine Hannover, Division of Cell Biology, Institute of Physiology, Bischofsholer Damm 15, D-30173 Hannover, Germany

[2]Ernst-Moritz-Arndt-University of Greifswald, Department of Cytology and Evolutionary Biology, Institute of Zoology, D-17487 Greifswald, Germany

[3]University of Siegen, Department of Biology and Didactics, D-57068 Siegen, Germany

Remipedia Yager, 1981 are blind subterranean Crustacea, which live exclusively in anchialine cave systems. Due to the almost inaccessible habitat and the late discovery, several aspects of the biology of these animals are still not understood. Although previous studies have provided a detailed anatomical description of the remipede brain, the neuroanatomy of the ventral nerve cord is still largely unknown. Here we examine the central nervous system of Remipedia and describe the distribution and morphology of serotonergic neurons.

Vibratome sections of three species (*Speleonectes tulumensis* Yager, 1987, *Godzilliognomus frondosus* Yager, 1989, *Cryptocorynetes haptodiscus* Yager, 1987) were labelled against serotonin and synapsin by immunofluorescence. Using confocal laser-scanning microscopy, serotonin was detected in all regions of the central nervous system. In the brain we could localize approximately 120 serotonergic neurons. Concerning the protocerebrum, serotonin-immunoreactivity was present in the neuropils of the central body, the protocerebral bridge and the lateral protocerebral sublobes. In the deuterocerebrum, the olfactory neuropils and the lateral antennal neuropils were innervated by fine serotonergic fibres. The olfactory neuropils are the most dominant neuropils in the remipede brain, comprising three sublobes. This olfactory hypertrophy results probably from the adaptation to the cave environment (troglomorphy) in order to compensate the loss of a visual sense.

Ganglia of the ventral nerve cord displayed a constant number and repetetive arrangement of serotonergic neurons. In two of three species, one serotonergic neuron was observed in the anterior region of each hemiganglion, while two serotonin-immunoreactive neurons were found posteriorly in all species. The neurites of these anterior and posterior neurons extended contralaterally. Furthermore, three serotonergic cell bodies were visible in the central region of the hemiganglia. All serotonergic neurons in the ventral nerve cord are monopolar, which means that only one axon grows out of each soma.

Comparisons regarding number, position and morphology of the serotonergic neurons in ground patterns of Arthropoda suggest close phylogenetic relationships between Remipedia and Malacostraca as well as Hexapoda. For example, Remipedia, Hexapoda and Malacostraca have monopolar serotonergic neurons, whereas Entomostraca have bipolar neurons. The fact that all serotonergic neurons in Remipedia and Hexapoda exhibit contralateral neurites seems to support a sister group relationship between these two taxa. By comparison, Malacostraca also possess ipsilateral axons. Moreover, serotonergic neurons in the central region of the hemiganglia could be detected only in Remipedia and Zygentoma (Hexapoda).

# Is the neuronal layering of the olfactory bulb sex-dependent?

Elke Weiler[1], Willi Bennegger[1, 2]

[1]Ruhr-Universität-Bochum, Neurophysiologie, Universitätsstr. 150, 44801 Bochum, Germany
[2]Maria-von-Linden-Schule, Heckentalstraße 86, 89518 Heidenheim, Germany

Neuronal layering is a characteristic structural feature, important for correct information processing. Due to its function, the layering is highly preserved with little variability and brain regions are differentiated according to the expansion of the layers, defining the area itself. The size of the whole brain however is diverse in most mammalian species between males and females. So we were interested, if all layers in a region are proportional equally effected from this expansion or if the composition shows differences between sexes.

Therefore we investigated a phylogenetically old and conserved structure, the olfactory bulb, for possible sex-dependent effects, in a species with a big difference in body size between sexes, the American mink color-variety "standard" (Neovison vison var. atratus) (adults; N = 26; 15 males, 11 females), where males have about twice the body weight (2072 ± 267 g) of females (1013 ± 108 g). The absolute brain weight in males (11.51 ± 0.73 g) also overwhelmes that in females (8.43 ± 0.35 g) by quite a bit, and also the olfactory bulb volumes are statistically highly significantly different (per bulb: males 152 ± 9.1 mm3; females 107 ± 4.1 mm3). Following histological processing, the olfactory bulb composition was analyzed with a morphometric system using weight/volume correction factors.

In the mink olfactory bulb of males as well as of females, the following layers can be distinguished: I) the most outer layer, the fila olfactoria layer (FOL) constituting the axons of the olfactory sensory cells, the olfactory information input, II) the glomerular layer (GLL), where the olfactory information is synaptically transmitted to the mitral cell apical dendrite within the glomeruli, surrounded by periglomerular interneurons, III) the external plexiform layer (EPL) which is a processing layer, comprising the external dendrites of the granule cells synapsing to the mitral cell secondary dendrites, IV) the mitral cell layer (MCL), the somata of the relais neurons, V) the internal plexiform layer (IPL), a connecting layer, where mitral cell axons and granule cell dendrites pass, VI) the granule cell layer (GCL) containing the granule cells, the major interneurons for information processing, VII) the stratum album (STR) containing centrifugal fibers, VIII) the subependymal layer (SUB) surrounding the ventricle and IX) the ventricle (VEN), which is less obvious in adults.

Although the portion of the olfactory bulbs on the whole brain is similar in males (2.74 ± 0.12%;) and females (2.64 ± 0.19%), the proportions of the layers on the composition of the bulb show statistically significant differences: In males, the olfactory fila comprise the major portion of all layers (26.9%) but only 17.7% in females, where the majors are granule cell (GCL 28.8%) and external plexiform layer (EPL 22.8%) overwhelming that in males (GCL 21.7%; EPL 19.2%) significantly. The mitral cell layer is significantly thicker in males (4.7%) than females (3.5%).

This indicates that males have a higher proportion on layers of connecting function, whereas in females the proportion on the processing neuronal layers is higher, suggesting a different function: a more simple but faster information conduction in males versus a more complex information processing in females. Thus, the proportion of the neuronal layers is sex-dependent.

# Activation of the trigeminal system by odorous substances – an in vivo and in vitro study

Matthias Luebbert[1], Markus Rothermel[3], Klaus Peter Hoffmann[1], Hanns Hatt[1]

[1]Ruhr University Bochum, Faculty for Biologie and Biotechnologie, Universitaetstr. 150, 44780 Bochum , Germany
[2]Ruhr University Research School
[3]The Brain Institude, University of Utah, Salt Lake City, UT 84108, USA

Mammalian chemosensation is predominantly mediated via the olfactory and the trigeminal system. Whereas the olfactory system mainly provides the sense of smell, the trigeminal system detects irritant and potentially harmful stimuli which produce unpleasant sensations. This detection occurs via free nerve fiber endings within the mucous membranes of the eyes, the nasal and the oral cavity, as well as the facial skin. The somata of all trigeminal sensory neurons are localized within the trigeminal ganglia (TG), at the basis of the skull. The functional organization and signal processing within the olfactory system is well understood. However, little is known about the organization and functional characteristics of the trigeminal system.

In order to investigate possible information processing at the level of the TG, we used voltage sensitive dye imaging to monitor neuronal activity at the level of the TG of anesthetized adult male whistar rats upon stimulation with different trigeminal stimuli. During the measurements, we used an olfactometer to apply different odorous substances like helional, citral, and vanillin directly to the animal`s nose. These substances elicited neuronal activity spreading nearly over the whole ganglion. However, application of the trigeminal stimuli EtOH and $CO_2$ led to a more complex activation pattern composed by an increase and a suppression of neuronal activation in different areas of the trigeminal ganglion. This might be a first hint that an early information processing occurs at the level of the TG.

In order to identify possible molecular players which contribute to the observed neuronal activation by odorants, we performed voltage-clamp recordings on dissociated trigeminal neurons of rats (P2-P5) under $Cs^+$ containing conditions. We identified two functional populations of trigeminal neurons. On one hand, application of helional and geraniol led to an increase in conductance, whereas the same substances inhibited background-currents within the second population.

Potential targets for interaction with the tested odorants could be members of the transient receptor potential family, including the transient receptor potential vanilloid 1 (TRPV1) which unselectively conducts currents carried by cations. TRPV1 serves as a polymodal receptor for noxious stimuli (e.g. vanilloids, heat, protons, and voltage). At room temperature, application of helional, geraniol, heliotropylacetone, and vanillin to CHO cells heterologously expressing rTRPV1 during voltage- clamp recordings elicited strongly outward rectifying currents. Prolonged application of helional (>1 min) reduced the response to a successive capsaicin application. When applied simultaneously with low concentrations of capsaicin, activation caused by helional and capsaicin behaved in an additive way. In contrast, we observed a slight inhibition of currents induced by higher capsaicin concentrations in the presence of helional. Elevating the temperature of the bath solution to 37°C significantly increased the amplitude of helional-induced currents.

Our data underline the importance of the transient receptor potential V1 during the perception of different odorous substances via the trigeminal system. Since TRPV1 expressed within the skin is involved in the development of itch, the understanding of a TRPV1 -modulation by helional and other odorous substances might be of great interest for the treatment of itch e.g. as a symptom of allergic contact dermatitis.

# Analysis of the Trigeminal Transcriptome by DNA-Array and Next Generation Sequencing Methods

Günter Gisselmann[1], Benjamin Schreiner[1], Nicole Schöbel[1], Ramona Lehmann[1], Markus Werner[1], Hanns Hatt[1]

[1]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstr. 150, 44780 Bochum, Germany

The trigeminal nerve is the largest of the cranial nerves. Its three branches converge on the trigeminal ganglion that contains the cell bodies of incoming sensory nerve fibers. The trigeminal ganglion is involved in detection or processing of sensory information like touch, pain and temperature information. In the recent years, several classes of membrane receptors and ion channels critical for trigeminal sensory perception have been described on a molecular level and many involved genes were identified. But so far, no attempts have been made to systematically describe the mammalian trigeminal transcriptome and compare it to gene expression in non-sensory tissue.

For transcriptom analysis, DNA-arrays are still the most common method used. Although it is a long established technique, continuous progress has been made by the introduction of new types like exon specific arrays. During the last few years, dynamic development of Next Generation Sequencing techniques in combination with rapidly dropping costs lead to a revolutionary extension of available experimental approaches in the field of transcriptome analysis. mRNA-seq is a paradigm-shifting technology because of its great sensitivity and dynamic range and its potential to analyze transcriptomes independently of existing genome annotations.

We used both, DNA-array and sequencing methods to analyze the trigeminal transcriptome from humans and mice. The present study mainly serves two goals: to describe the mammalian trigeminal transcriptome and to interpret distinct gene expression patterns in respect to trigeminal chemosensation.

In a dual experimental approach, we analyzed murine trigeminal (postnatal and adult) RNA expression on Affymetrix Exon 1.0 ST arrays. In a second experimental series, we characterized human and murine trigeminal gene expression by RNA -seq data generated by Illumina -Solexa Next Generation Sequencing. Both approaches allowed us to analyze expression profiles of known receptors and to identify potential new classes of genes involved in trigeminal (chemo)sensation.

# Do antennal lobe output neurons employ a latency code?

Tobias Rosenbaum[1], Martin F. Brill[1], Wolfgang Rössler[1], Martin P. Nawrot[2]

[1]University of Wuerzburg, Biozentrum, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Wuerzburg, Germany
[2]Freie Universitaet Berlin, Institut of Zoology, Dept. of Neurobiology, Neuroinformatics, Koenigin Luise Str. 1-3, 14195 Berlin, Germany

Honeybees perceive their environment to a large extent based on olfactory and visual stimuli. They possess a highly advanced olfactory system allowing sophisticated orientation, social communication, and learning using odor stimuli. The nature and temporal dynamics of the code employed by the honeybees' olfactory neuronal network is still under debate. Here we investigated whether populations of uniglomerular antennal lobe (AL) projection neurons (PNs) in the lateral and medial protocerebral tract (m- and l-APT) employ a spatio-temporal latency code to represent odor identity in a fast and reliable manner, as suggested by previous results from intracellular PN recordings (Krofczik, Menzel and Nawrot, 2008 *Front Comp Neurosci* 2:9).

The honeybee AL network constitutes the first level of olfactory processing. It receives input from about 60.000 olfactory receptor neurons. Circa 4.000 local interneurons provide the substrate for local computations within the AL. Approximately 800 uniglomerular PNs transmit the AL output code to the mushroom bodies and the lateral horn via two separated tracts, which resembles a special feature of Hymenoptera (reviewed in Galicia and Rössler, 2010 *Annu Rev Entomol* 55:399)

We analyzed odor representations in multiple single unit recordings from uniglomerular PNs that were performed simultaneously in the m- and l-APTs (see companion poster by *Brill et al.* this volume). Our results show that odor responses in individual units exhibit odor specific response latencies, both in the l- and in m-APTs. In simultaneously recorded units, a particular odor evokes an odor-specific spatio-temporal pattern of response latencies. With increasing concentration, the odor latencies tend to decrease but the temporal order of response onsets remains relatively stable.

We conclude that each odor evokes an odor-specific latency pattern across uniglomerular PNs. This constitutes a fast and possibly concentration invariant code of odor identity during the earliest phase of the odor response that might be translated into a spatio-temporal code at the level of the mushroom body Kenyon Cells (KC) that are believed to receive coincidental input from PNs of the two APTs.

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# Simultaneous recordings from multiple projection neurons in the dual olfactory pathway of the honeybee

Martin F. Brill[1], Isabelle Reus[1], Tobias Rosenbaum[1], Christoph J. Kleineidam[1,2], Wolfgang Rössler[1]

[1]University of Wuerzburg, Biozentrum, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Wuerzburg, Germany
[2]University of Konstanz, Department of Biology, Universitaetsstraße 10, 78457 Konstanz, Germany

Honeybees possess a highly elaborated olfactory system in order to orient in the environment, detect and remember floral odors, and to communicate using pheromonal odors for species-specific social interactions. Odors are received by olfactory receptor neurons (ORNs) located on the antennae. The axons of the ORNs project via the antennal nerve to functional units (olfactory glomeruli) of the primary olfactory neuropil, the antennal lobe (AL), which is the analog of the vertebrate olfactory bulb. In the AL, odor information is preprocessed by local interneurons (LNs) in a spatio-temporal manner. From the AL glomeruli, olfactory information is transferred by two separate uniglomerular projection neuron (PN) output-tracts, the medial and the lateral antennoprotocerebral tracts (m- and l-APT) to higher-order brain centers, the mushroom bodies (MB) and the lateral horn (LH). This dual olfactory pathway seems to be a unique feature in Hymenoptera (Kirschner et al. 2006, *J Comp Neurol* 499:933; Zube et al. 2008, *J Comp Neurol* 506:425; Galizia and Rössler, 2010 *Ann Rev Entomol* 55:399). It is assumed that the dual output tracts convey different aspects, such as quality, intensity or temporal structure of an olfactory stimulus.

Using simultaneous recordings with multiple wire electrodes (adapted from Okada et al. 2007, *J Neurosci* 27:11736) and a customized highly accurate multi-unit recording setup we analyzed electrophysiological properties of the two output streams by simultaneous recordings from multiple PNs in both APTs. For visualization of the exact position of the electrodes we developed a staining method that enables 3D-reconstructions to prove the accuracy of the recording sites.

First results from dual tract recordings revealed that PNs from both tracts respond to the same set of general odors and pheromones tested so far. Recordings from m-APT units showed a high specificity for odor identity and complex excitatory and likely inhibitory responses to odor stimulation. In contrast, recordings from l-APT units were less specific for odor quality. Considering the dual olfactory PN pathway as origin of coincidental input to the MB intrinsic neurons, the Kenyon Cells (KC), cross correlations from individual PN pairs were performed and analyzed. Furthermore, detailed rate-latency analyzes were conducted to investigate a potential latency code in PNs (see companion poster by *Rosenbaum et al.* this volume). Our results support parallel processing of different features from similar odors via the two APTs.

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# In vivo $Ca^{2+}$ imaging of juxtaglomerular neurons in the mouse olfactory bulb.

Yury Kovalchuk[1], Ryota Homma[2,3], Arthur Konnerth[1,3], Lawrence Cohen[2,3], Olga Garaschuk[3,4]

[1]Institute of Neuroscience, Technical University Munich, 80802 Munich, Germany
[2]Dept. of Physiology, Yale University, New Haven, CT, 06510, USA
[3]NeuroImaging Cluster, Marine Biological Laboratory, Woods Hole, MA, 02543, USA
[4]University Tuebingen, Institute of Physiology II, Keplerstr 15, 72074 Tuebingen, Germany

Juxtaglomerular neurons are the largest neuronal population of the main olfactory bulb. *In vitro* studies suggest that they play a key role in shaping or even gating the input from olfactory receptor neurons but their *in vivo* properties remain unclear. Here we assessed the *in vivo* function of juxtaglomerular neurons using a combination of two-photon $Ca^{2+}$ imaging and loose patch clamp recording technique. Dorsal area of the olfactory bulb was stained with a calcium indicator dye (Fura PE3 or Oregon Green BAPTA 1) using the multi-cell bolus loading technique and imaged by means of two-photon microscopy.

Pulses of odorants were presented to anesthetized (ketamine/xylazine) freely breathing animals through a custom-made flow-dilution olfactometer. Brief (2-s-long) odorant applications specifically activated distinct glomeruli in the olfactory bulb and evoked $Ca^{2+}$ transients in many individual juxtaglomerular neurons. Based on the waveform parameters of these transients we classified the neurons into three major classes: (i) ON neurons, that show an increase in the intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$) at the onset of the stimulus, (ii) OFF neurons, showing the increase in $[Ca^{2+}]_i$ at the offset of the odorant presentation and (iii) INHIBITED neurons, in which the odorant presentation caused an apparent decrease in $[Ca^{2+}]_i$. Out of 52 locations tested (8-51 responding cells per location) 85% of responding cell were ON cells, 12% INHIBITED cells and only 3% OFF cells. To get an insight into electrical activity underlying $Ca^{2+}$ signals in ON and INHIBITED cells, we conducted simultaneous two-photon imaging and loose-patch recordings from responding neurons. In ON cells an application of an odorant resulted in a rather homogeneous ~50% increase in the spiking rate per increase in odorant concentration by 0.7 log of saturated vapor, and was independent of the background activity of the neuron. In INHIBITED cells, an application of an odorant caused a decrease or even a complete blockade of cell firing. This was accompanied by a decrease in the $[Ca^{2+}]_i$. The INHIBITED neurons rapidly resumed firing after termination of odorant application.

Remarkably, neurons with ON and INHIBITED responses were closely clustered around glomeruli that exhibited the same kind of response. However, despite the clustering, neighboring neurons demonstrated very different odorant sensitivity, and rather narrow dynamic range, as revealed by odor presentation ranging from 0.1% to 9% of saturated vapor. On a population level, we found that an increase in odorant concentration was accompanied both with an increase in the amplitudes of odor-evoked $Ca^{2+}$ transients in individual cells and a recruitment of additional responding neurons.

In summary, this is the first comprehensive study of the functional *in vivo* properties of juxtaglomerular neurons. We show that individual juxtaglomerular neurons code for many perceptual characteristics of the olfactory stimulus including odor identity, odorant concentration, odorant onset and offset, and odorant accommodation. While some response features of juxtaglomerular neurons could be directly triggered by the activity of olfactory receptor neurons, others (e.g. INHIBITED responses) must be created by signal processing within the glomerular neuronal network.

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# Investigating the Olfactory System of Vitamin A Deficient Mice

Stefan Kurtenbach[1], Thomas Pelz[2], Hanns Hatt[1], Eva Maria Neuhaus[2]

[1]Ruhr University Bochum, Cellphysiology, Bochum, Germany
[2] NeuroScience Research Center, Charité, Berlin, Germany

In the olfactory epithelium (OE) neurogenesis takes place continuously throughout the whole lifespan, resulting in new neurons that grow axons to the brain where they form new connections to glomeruli in the olfactory bulb (OB). This fact makes the OE an interesting place to study neurogenesis e. g. to improve existing methods of transplantation of neuronal precursors. It is known that retinoids, derivates of Vitamin A, are involved in differentiation and survival of neurons. Retinoic acid (RA) is a derivate of Vitamin A and binds to retinoic acid receptors (RAR, RXR) which were shown to promote neuronal survival. Retinoic acid also enhances the recovery after olfactory nerve transection. We performed mass spectroscopy to unravel the membrane proteome from the OE of mice. Our data show high expression levels of a RA regulated receptors with unknown function. We investigated the expression levels of these receptors in mice fed a special diet containing no vitamin A, carotenoids or other precursors of RA (VitA - diet) for different time - spans. Our aim is to get a deeper understanding of the molecular effects of vitamin A deficiency in mice. We used immunohistochemistry to get insight into the morphology of the neurons and distribution of the proteins involved in the odor induced signal transduction cascade. Additionally we made quantitative PCR analysis to observe protein expression levels and performed electro olfactogram (EOG) measurements to investigate the capability of the OE to respond to odorous stimuli.

# Investigation on the the mechanisms of chemoperception in human skin

Anna Christina Sondersorg[1], Daniela Busse[1], Markus Rothermel[2], Hanns Hatt[1], Heike Benecke[1]

[1]Ruhr-University Bochum, Department of Cellphysiology, Bochum, Germany
[2]Department of Physiology; University of Utah, School of medicine, Salt Lake City, USA

The trigeminal nerve constitutes the fifth cranial nerve and provides tactile, proprioceptive and nociceptive afferences that innervate the facial skin, eyes, mouth and the nasal mucosa. Previous work including studies on anosmic patients indicates a functional contribution of the trigeminal nerve to odor detection *in vivo*. High concentrations of most odors typically provoke trigeminal sensations *in vivo*, but only certain odors elicit responses in mouse trigeminal monocultures. This leads to the suggestion that trigeminal perception may require a transfer of sensory information via crosstalk with additional cell types of the peripheral innervation area. The expression of several chemoreceptors such as olfactory receptor proteins and TRP channels as well as their ability to respond to various odors favors the idea that keratinocytes may participate in odor detection and subsequently transmit signals to neighboring trigeminal fibers in the epidermis.
This study is designed to a) identify odorants and other chemical substances that activate keratinocytes but fail to stimulate trigeminal neurons alone, b) to detect potential communications between skin keratinocytes and trigeminal neurons induced by these substances and c) to identify possible transmitter molecules.
Using the calcium imaging technique we screened substances with focus on odorants that reproducibly elicit calcium responses in keratinocytes, but not in trigeminal neurons. Sandalore and Sandranol, two derivates of sandalwood oil, as well as Histamine turned out to be good candidates. In contrast to monocultures we could detect increased numbers of calcium signals in trigeminal neurons in a co-culture approach induced by Sandalore and Histamine. These results support our hypothesis of an information transfer from keratinocytes to trigeminal neurons.

# Involvement of a G-protein in the detection of chemosensory signals and in the modification of aggressive behavior

Pablo Chamero[1], Vicky Katsoulidou[1], Philipp Hendrix[1], Bernd Bufe[1], Lutz Birnbaumer[2], Frank Zufall[1], Trese Leinders-Zufall[1]

[1]University of Saarland, Department of Physiology, 66424 Homburg, Germany
[2]Laboratory of Neurobiology, National Institute of Environmental Health Sciences, NIH, Research Triangle Park, NC 27709, USA

Several social behaviors in vertebrates are under the influence of the accessory olfactory system. The accessory olfactory system and in particular its sensing unit the vomeronasal organ (VNO) are generally considered to be specialized in the perception of chemical stimuli of social nature, including pheromone-like signals. An intriguing feature of the VNO is its segregation into two zones containing subpopulations of vomeronasal sensory neurons (VSNs) which differ in their expression of chemosensory receptors and G-protein subunits [either Gnai2 (Gi2) or Gnao1 (Go)]. It is unclear what role the two segregated neural pathways play to control complex social behaviors. Conditional gene targeting applying the Cre-loxP-mediated recombination system is increasingly used to evaluate the role of a gene of interest in a cell-type- or organ-specific manner. A mouse line carrying the tissue-specific disruption of the Go gene in olfactory sensory neurons offered the opportunity to evaluate the requirement of Go to mediate the detection of socially relevant chemosignals (pheromones) in the VNO and its ability to modify innate aggressive behavior. As a consequence of the deficiency, the number of VSNs that normally express Go decreased. VSN activation by some peptide and previously identified protein pheromones (1) is drastically reduced in the Go mutants indicating that this G-protein is necessary for the detection of these chemosignals. Detection of other ligands specific for Gi2-expressing VSNs is not affected. Display of both maternal and male territorial aggression is severely diminished. However, unlike mice with genetically ablated VNO function, the Go mutants display a normal mating partner choice and sex behavior. These findings indicate that Go is required in the olfactory system to detect protein and peptide pheromones and is necessary to generate aggressive behavior.
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(1) Chamero *et al*., Nature 450:899-902. 2007

# Ion channel properties of the Drosophila odorant receptor protein Or83b

Vardanush Sargsyan[1], Bill S. Hansson[1], Dieter Wicher[1]

[1]Max Planck Institute for Chemical Ecology, Department of Developmental Neuroethology , Hans-Knöll-St. 8, 07745 Jena, Germany

Olfactory receptors (Ors) expressed in the cell membranes of olfactory sensory neurons are the initial players in the signal transduction cascade. In insects, Ors belong to a family of G-protein-coupled-receptors (GPCRs) that lack any homology to other GPCRs and possess a distinct membrane topology with an intracellular N-terminus. Recent studies showed that insect Ors are heterodimers composed of an odor specific odorant receptor protein and a ubiquitously expressed chaperone protein Or83b. This indicates that insects utilize a unique signal transduction mechanism from that of vertebrates. Upon odorant stimulation, heterodimers lead to activation of nonspecific cation currents via an ionotropic and a metabotropic pathway (Wicher et al., Nature 452 (2008) 1007). Expression of Or83b alone leads to a functional ion channel that is unresponsive to odorants, but is directly activated by cyclic nucleotides.

The major objective of our study was to provide a deep understanding of OR83b function at the single channel level. To do this, we expressed recombinant Or83b in HEK293 cells and employed patch clamp recordings in excised patches. Our results demonstrate that upon cAMP application Or83b exhibited cAMP activated single channel activity. The dose-response curve revealed an $EC_{50}$ value of 0.7 nM for cAMP and a Hill coefficient of 0, 40. However, the cAMP mediated current was activated slowly and showed a delay of some seconds. Thus, Or83b channel gating differs from that of classical ion channels. Additionally, Or83b channels exhibited constitutive activity even in the absence of cyclic nucleotides. Taken together, our data suggest that cyclic nucleotides are important regulators of Or83b channel function.

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# Isomer-specificity in response to herbivore-induced plant volatiles in the antennal lobe of *Manduca sexta*

Anna Henning[1], S. Bisch-Knaden[1], A. Reinecke[1], S. Sachse[1], Bill S. Hansson[1]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knöll-Str. 8, D-07745 Jena

Volatile signals in plant-insect communication typically consist of compounds whose composition alters depending on physiological state of the plant. The ability to perceive, separate and classify volatile compounds is crucial for insects to generate behavioural responses and thus, survival. The insect olfactory system is remarkably similar to vertebrates. Olfactory sensory neurons located on the insect antenna project to the antennal lobe (AL), the first olfactory neuropil, which consists of spherical units called glomeruli. We used the herbivore-plant system of the wild tobacco *Nicotiana attenuata* and the tobacco hawkmoth *Manduca sexta* to examine the sensitivity and selectivity of the insect olfactory system to a group of herbivore-induced plant volatiles, so-called green leaf volatiles (GLV). *N. attenuata* feeding-damaged by *M. sexta* larvae results in a shift of the ratio of cis- to trans isomers of some GLVs thereby attracting a hemipteran predator. In order to test whether this shift is used by ovipositing females, we applied functional imaging to investigate the glomerular responses of the *M. sexta* AL to different ratios of cis-3- and trans-2-isomers of hexenol, hexenyl acetate and hexenyl butyrate. We found two isomer-specific glomeruli in the AL of *M. sexta* females that are either activated by cis -3-hexenyl acetate or its trans-2-isomer. For hexenol and hexenyl butyrate no isomer-specificity could be found. Furthermore, stimulation with different ratios of both acetate isomers resulted in distinguishable activation patterns. This isomer-specific spatial pattern may contribute to discrimination of plant physiological states by female *M. sexta* for oviposition.

# Mapping of waterborne odorants to subsystems of the olfactory system

Sebastian Gliem[1], Eugen Kludt[2], Detlev Schild[2,3], Ivan Manzini[2,3,4]

[1]PhD Neurosciences Program of the International Max Planck Research School, Göttingen, Germany

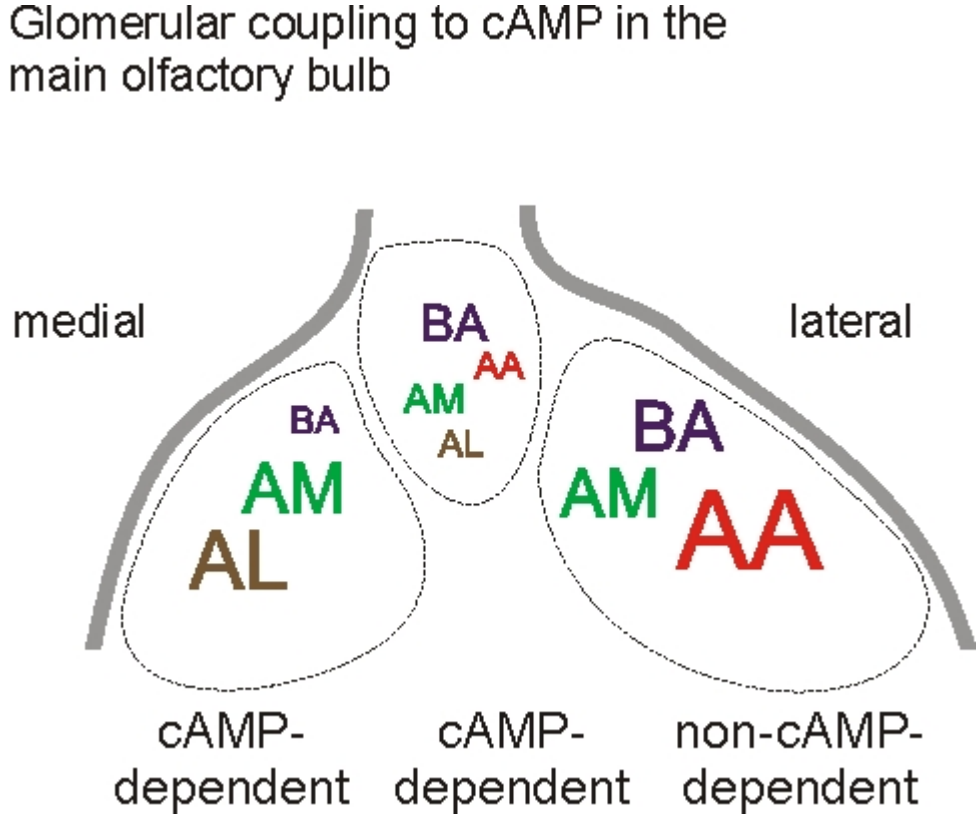
[2]Institute of Physiology, Neurophysiology and Cellular Biophysics, Humboldtallee 23, 37073 Göttingen, Germany

[3] DFG Research Center for Molecular Physiology of the Brain, Göttingen, Germany

[4] DFG Priority Program/ SPP 1392

The main olfactory system of larval *Xenopus laevis* is made up of at least two subsystems consisting of subsets of olfactory receptor neurons (ORNs) with different transduction mechanisms. One subset lacks the canonical cAMP transduction pathway and responds to amino acid odorants. This subset projects to glomeruli in the lateral olfactory bulb (OB). The second subset contains ORNs, which possess the cAMP-dependent transduction mechanism and project to glomeruli located medially in the OB.

Here we allocate different waterborne odorants to the above mentioned subsystems using functional $Ca^{2+}$ imaging on acute slices of the olfactory system. We recorded odorant-induced responses on glomerular level and on level of ORNs and discuss coupling to the cAMP-dependent transduction pathway. We found, that the great majority of odorant-sensitive ORNs responded exclusively to one odorant. Furthermore, we show axonal projections from ORNs, which were retrogradely labelled by biocytin electroporation into the lateral and medial glomerular clusters respectively.



# Mechanisms of odor-guided orientation behavior in ants

Stefanie Neupert[1], Christoph Johannes Kleineidam[1]

[1]University of Konstanz, Department of Biology, Universitätsstraße 10, 78464 Konstanz, Germany

The ants' ability to recruit nestmates along a pheromone trail to a food source is one impressive example of insect pheromone communication. The trail pheromone activates the ant to search for food and guides the ant to the food source. While the ant is orienting on the trail, it encounters changing concentrations of the pheromone on the antennae. What orientation mechanism do ants use to navigate on the trail?
We investigate the orientation mechanisms by stimulating independently both antennae of a tethered ant. The orientation behavior of the ant in this open-loop condition is measured with a 'Buchner ball'. An air stream lifts up a small Styrodur ball and the stationary ant rotates the ball, depending on the direction she wants to move. An optical sensor of a computer mouse detects the movements of the ball, which are recorded on a PC.
Stimulation of both antennae simultaneously but with different concentrations of the trail pheromone resulted in a high variance of walking direction among all ants. Analyzing the walking behavior of individual ants to a sequence of stimulation, where both antennae simultaneously received a pheromone pulse with different concentration, revealed that some ants use the concentration differences for orientation, others don't. The same is true when the sequence of stimulation was with the same concentration on both sides but time shifted-pulses were presented. Some ants use the temporal structure of successive stimulation of both antennae, others don't. We then combined time-shifted pheromone pulses and concentration differences and found that a concentration difference of 10X ($10^{-6}$ versus $10^{-7}$) resulted in an offset of the ant's heading direction towards the higher concentration. However, concurrent time-shifted pulses influenced the walking direction consistently in some ants. Our results show that i) ants can use both, intensity differences and time differences of odor stimuli for orientation, ii) individuals rely upon one or the other and adjust their walking direction accordingly, and iii) ants are able to simultaneously use both, the concentration differences and the sequence of odor reception on the antennae for odor-guided behavior.

# Mind the gap: olfactory trace conditioning in honeybees

Paul Szyszka[1], Christiane Demmler[1], Mariann Oemisch[1], Ludwig Sommer[1], Stephanie Biergans[1], Benjamin Birnbach[1], Ana F. Silbering[2], C. Giovanni Galizia[1]

[1]University of Konstanz, Department of Biology - Neurobiology, 78457 Konstanz, Germany

[2]University of Lausanne, Center for Integrative Genomics, Génopode, 1015 Lausanne, Switzerland

Trace conditioning is a form of classical conditioning, where a neutral stimulus (CS) is associated with a following appetitive or aversive stimulus (US). Unlike in standard delay conditioning, in trace conditioning there is a stimulus free gap between CS and US, and thus a post-stimulus neuronal representation of the CS (i.e. a trace) is required in order to be associated with the US. The properties of such stimulus traces are not well understood, nor are their underlying physiological mechanisms. Using a combined behavioral and physiological approach, we characterized trace conditioning of the proboscis extension reflex in honeybees with odors as CS and sucrose reward as US. In behavioral experiments we found that a single odor presentation created a stimulus trace, which contained information about the molecular odor identity. This trace conveyed odor information about stimulus onset, and was robust against interference by other odor stimuli. In trace conditioning, memory acquisition decreased with increasing CS-US gap length. Importantly, the maximum effective interval was not fixed, but could be extended by previous experience. Furthermore, trace memory acquisition improved when an additional different odor was presented within the stimulus-free gap between the CS and US. We tested whether projection neurons in the primary olfactory brain area, the antennal lobe, contain a CS trace. Based on physiological measurements we found that CS traces are not encoded in persistent projection neuron calcium or spiking activity. Our data suggest that olfactory trace conditioning is a less automatic and reflexive form of learning than classical delay conditioning, indicating that odor traces might be involved in higher-level cognitive processes as for example olfactory working memory.

# Mitochondrial role in the calcium homeostasis in mouse olfactory sensory neurons

Daniela Fluegge[1], Lisa Moeller[1], Sophie Veitinger[1], Jennifer Spehr[1], Silvia Cainarca[2], Sabrina Corazza[2], Marc Spehr[1]

[1]RWTH Aachen, Chemosensation, Worringer Weg 1, 52074 Aachen, Germany
[2]Axxam, San Raffaele Biomedical Science Park, Milan, Italy

Ionized Calcium is an important messenger in the signal transduction cascade of olfactory sensory neurons (OSNs). Upon odor stimulation, Ca2+ enters OSNs via cyclic nucleotide-gated channel opening and accumulates in the cytoplasm. In many neurons, increased intracellular Ca2+ is compartmentalized by the endoplasmic reticulum and mitochondria.
Here, we investigate mitochondrial function during OSN activity in main olfactory epithelium tissue slices. Using a combination of bioluminescence imaging and transgenic mice which stably express a Ca2+-sensitive photoprotein selectively in mitochondria, we establish a novel imaging approach to record Ca2+ signals in OSN mitochondria at high temporal resolution.
We show that mitochondria play a role in olfactory Ca2+ signaling. During odorant responses in canonical OSNs, both cytosolic and mitochondrial Ca2+ increases. In both the dendritic knobs and somata, odor-mediated cytosolic Ca2+ signals are significantly changed when mitochondrial Ca2+ uptake is inhibited. Furthermore, we show activity dependent mitochondrial redistribution to dendritic knobs. On-going
electrophysiological recordings from identified OSNs aim to reveal the functional consequences of mitochondrial perturbation on the odor-mediated electrical output signal.
Employing this novel system, we envisage obtaining significant new insights into the role of mitochondrial Ca2+ signaling in the olfactory system.

# MUPP1 - mediator of the olfactosome

Sabrina Baumgart[1], Ruth Dooley[2], Hanns Hatt[1], Eva Maria Neuhaus[3]

[1]Department of Cell Physiology, Ruhr-University Bochum, Bochum, Germany.
[2]Molecular Medicine Lab RCSI, Beaumont Hospital, Dublin, Republic of Ireland.
[3]NeuroScience Research Center, Charité, Charitéplatz 1, 10117 Berlin, Germany.

The complex olfactory signal transduction pathway enables mammals to detect and discriminate between thousands of different odorants. But how the individual components of this complex signaling cascade get into close proximity to each other is an unsolved question. We recently showed that a PDZ protein called MUPP1 (multiple PDZ domain protein 1) is an interaction partner of olfactory receptors and thereby a putative mediator of a so called olfactosome (Baumgart , FEBS journal, Dec 2009). This scaffolding protein consists of 13 single PDZ domains and is known to be a mediator of diverse GPCR based signalling networks.
We demonstrated that this scaffolding protein is highly expressed in the dendritic knobs and cilia of olfactory sensory neurons of mice. Further, we could show that different ORs are able to interact with MUPP1. In a new peptide microarray approach we investigate putative interactions of a great variety of ORs of all different known subfamilies in the mouse genome. In addition, we investigate other signaling components for their putative presence in the MUPP1 based complex by protein microarrays, as well as by direct protein-protein interaction assays.

# Neuropeptides of identified local interneurons in the antennal lobe of *Periplaneta americana*

Debora Fusca[1], Susanne Neupert[2], Joachim Schachtner[3], Reinhard Predel[2], Peter Kloppenburg[1]

[1]Biocenter Cologne, Institute for Zoology, Center for Molecular Medicine Cologne (CMMC), and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Zülpicher Str. 47b, 50674 Cologne, Germany

[2]Institute for Zoology, University of Jena, Erbertstrasse 1, 07743 Jena, Germany

[3]Animal Physiology, University of Marburg, Karl-von-Frisch Str. 8, 35043 Marburg, Germany

A multitude of potential neurotransmitters and neuromodulators, including neuropeptides, have been detected in the antennal lobe (AL), the first synaptic relay of the central olfactory pathway in the insect brain. However, the functional role of neuropeptides in this system has yet to be revealed. An important prerequisite for understanding the role of neuropeptides is to match the functionally different cell types in the antennal lobe with their peptide profiles by using electrophysiological recordings combined with immunohistochemical studies and/or single cell mass spectrometry. The olfactory system of *Periplaneta americana* is particularly well suited to accomplish this goal, because several physiologically distinct neuron types can be unequivocally identified. In the AL, we identified two main local interneuron (LN) types with distinctive physiological properties: 1) type I LNs which generated Na+ driven action potentials upon odor stimulation and exhibited GABA-like immunoreactivity (IR) and 2) type II LNs, in which odor stimulation evoked depolarizations, but no Na+ driven action potentials due to the lack of voltage dependent transient Na+ currents. Furthermore, 90 % of type II LNs did not exhibit GABA-like IR. Towards our aim to analyze the neuropeptide inventory of the *P. americana* AL, we first systematically analyzed different parts of the AL by MALDI-TOF mass spectrometry to obtain the complete set of neuropeptides present. Altogether, 50 ion signals could be assigned to known members of 8 neuropeptide families (AST-A, AST-Bs [MIP], AST-C, SIFamide, AT, FMRFamide related peptides (FaRFa; [myosuppressin [MS], short neuropeptides F [sNPF], extended FMRFamides]), crustacean cardioactive peptide [CCAP], TKRPs. Secondly, a combination of immunocytochemistry and mass spectrometric profiling of defined antennal lobe compartments was used to reveal the spatial distribution of neuropeptide containing cells.
To match the previously identified LN types with their biochemical profiles, we used whole-cell patch-clamp recordings and single cell stainings combined with immunocytochemical experiments. We used antisera against GABA and the acetylcholine synthesizing protein choline acetyltransferase (ChAT) as well as antisera against tachykinin-related peptides (TKRPs) and allatotropin (AT). ChAT IR was found in uniglomerular projection neurons (uPNs) and in a subpopulation of type II LNs. A large subpopulation of the GABA-like immunoreactive (ir) type I LNs were also AT ir, while only a subgroup of TKRP ir cell bodies showed GABA-like IR. TKRP IR could be detected in subpopulations of type I and type II LNs.

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# Neuropeptides of the Insect Mushroom Body

Marlene Binzer[1], Martin Kollmann[1], Carsten M. Heuer[1], Joachim Schachtner[1]

[1]Philipps Universität Marburg, Tierphysiologie, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany

Among the signaling molecules involved in neuronal communication, neuropeptides present the largest and most diverse group. Neuropeptides have often been considered as cotransmitters that are released in concert with a principal transmitter. To date, neuropeptides are accepted as molecules responsible for shaping the activity pattern of neuronal circuits and thus as being of major importance for the functional condition and output pattern of the central nervous system. Several studies have shown that (neuro-)peptides act as key players in the coordinated action of endocrine events and behavioral actions, e.g. during the insect molt or in the regulation of circadian control. Furthermore, neuropeptides are thought to be involved in processes related to neuronal plasticity, the substrate for learning and memory.

Mushroom bodies (MBs) are ancient brain centers that occur in taxa as distantly related as annelids (Lophotrochozoa) and arthropods (Ecdysozoa). In insects, these paired protocerebral neuropils act as higher integrative centers that are intimately connected with primary olfactory neuropils. They are primarily regarded as sites of learning and memory formation but may also serve a general function in the control of behavior. MB architecture among insects is diverse and correlates with the amount of (multimodal) sensory information that the MBs integrate and thus to the complexity of information they process. The functional components of the MBs are the parallel organized intrinsic MB neurons, the Kenyon cells. Studies in a variety of insects have demonstrated the presence of neuropeptides in either intrinsic or extrinsic MB neurons, suggesting an important functional aspect of these neuropeptides in information processing in the MB. To further understand the role of neuropeptides, we investigate neuropeptides in the MBs of the emerging model organism *Tribolium castaneum* and compare it to other insect species, including holometabolous and hemimetabolous taxa, as well as ancestral representatives. Neuropeptide distribution is examined in immunohistological preparations by confocal laser scanning microscopy. To assess the full range of the MB neuro-peptidome, we analyze isolated MB tissue by means of MALDI-TOF mass spectrometry.

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# Octopamine causes rises of cAMP in antennae of the hawkmoth *Manduca sexta* and the cockroach *Leucophaea maderae*

Thomas Schendzielorz[1], Wladimir Peters[1], Monika Stengl[1]

[1]University of Kassel, Animal Physiology, Heinrich-Plett-Str. 40, 34132 Kassel, Germany

In the hawkmoth *Manduca sexta* it is assumed that octopamine (OA) concentrations show circadian rhythms in the hemolymph. Furthermore, infusion of this biogenic amine in long-term tip recordings of trichoid sensilla caused time-dependent rises in the speed and sensitivity of bombykal detection. Because OA action was only partly mimicked by infusion of membrane-permeable cAMP-analogs, apparently more than one OA receptor is present in the hawkmoth antenna. While three different types of insect OA receptors are known, so far, only the OA a-adrenergic-like receptor was identified in *M. sexta*, which usually does not couple to adenylyl cyclase.
In this study we used RIA-assays to analyze the effect of OA on adenylyl cyclase activity in lysates of the antennae of the holometabolous hawkmoth *M. sexta* and the hemimetabolous cockroach *Leucophaea maderae* at different times of day. The OA effects on the cAMP concentration in antennal lysates of *L. maderae* were measured at ZT 5, before the beginning of the activity phase, and at ZT 10, during the main mating phase of the cockroach. Application of 50 µM OA to the antennal lysates of the cockroach increased cAMP concentrations significantly at both ZTs. Similarly, OA also increased cAMP concentrations in antennal lysates of *M. sexta*. Both, at ZT 9 during the resting phase of the moth and at ZT 22 during the activity phase 5 µM OA increased cAMP concentrations significantly. However, in the hawkmoth the OA-effect was stronger at the resting phase. Even 1 nM OA in the incubation buffer was able to increase cAMP concentrations in antennal lysates of *M. sexta*. These results indicated that OA induces cAMP dependent modulation of insect odour transduction via a ß-adrenergic-type OA receptor which still needs to be identified. [Supported by DFG STE531/20-1]

# Odor discrimination times and their dependence on odorant intensity in GluA2 knockout mice

Elena Kudryavitskaya[1], Thomas Kuner[1]

[1]Institute of Anatomy and Cell Biology, University of Heidelberg, Functional Neuroanatomy , Im Neuenheimer Feld , 69120 Heidelberg, Germany

Stimulus intensity is an important determinant for sensory perception, learning and behavior. In the olfactory system, optical in vivo imaging experiments have shown that decreased odor concentrations produce more sparse representations in the olfactory bulb (OB). Furthermore, the pattern and strength of activation seems to inversely correlate with odor discrimination time (DT). Hence, we examined the question if DT depends on odor concentration. We tested this in C57BL6 mice containing a conditional GluA2 allele (control, representing wild type) and in mice with a granule cell (GC)-specific deletion of GluA2. The latter were used, because they exhibit a gain-of-function phenotype in odor discrimination tests (increased Ca2+ influx into the GC, increased inhibition of mitral cells and faster DT of similar binary mixtures) that may be accompanied by an as yet unknown loss of function, such as an altered detection treshold and intensity-dependence of odor discrimination.

In this study, we used 16 male GluA2lox/lox mice, 8 of which were injected with AAV1/2-Cre-2A-Venus into the GCs of the OB to obtain a GC-specific deletion of GluA2 (GluA2-/-GCL). As a control, the GC layer of 8 mice was injected with AAV1/2 -Venus. All mice were trained using olfactometry in a go/no-go operant conditioning paradigm to discriminate among simple monomolecular odors of amyl acetate (AA) versus ethyl butyrate (EB) and their binary mixtures with similar ratio: 0.6% AA + 0.4% EB v/s 0.4% AA + 0.6% EB. The mice were trained to performance levels above 90% correct responses. After that we gradually decreased the odor concentration of odor pairs (mixtures of AA and EB ) from 1 % vol (in mineral oil) to 1 x 10-6 % vol. Each mouse was given a minimum of 600 trials on each odor concentration and DT was determined once stable high performace was reached.

In control mice, the DT increased from 350 Â± 6 ms at 1% vol dilution to 450 Â± 22 ms at 10-4 %vol, while the accuracy remained high. Hence, mice require more time to discriminate less intense stimuli at maximal accuracy.

In GluA2-/-GCL mice, the DT increased with decreasing odor concentrations from 327 Â± 6 ms to 430 Â± 23 ms at the 10- 4 % vol dilution, with accuracy slightly decreasing. Thus, similar to controls, mice lacking GluA2 in GCs also require more time to discriminate more diluted odors. These results imply that GluA2-/-GCL mice maintain normal odor detection tresholds, leaving the question open if the gain-of-function phenotype of these mice is accompanied by an evolutionary unfavourable loss-of-function.

When directly comparing DT between control and GluA2 -/-GCL mice, a significantly shorter DT for binary mixture discrimination was only found at 1% vol, but not at the more diluted odors, although a tendency for shorter DTs was evident. This might be related to the observation that higher concentrations activate more glomerular units in the OB. Then, increased inhibition in GluA2-/-GCL mice might exert a stronger effect than at lower concentrations, when the fewer units are activated.

In summary, we found evidence that a decrease in stimulus intensity results in longer DTs. Our observation of increased DTs at lower intensities of the stimulus are consistent with the finding that mitral cell responses change both quantitatively and qualitatively with odor concentration. Nevertheless, the neuronal mechanism underlying concentration-independent identification of odors remains unknown.

# Odor segmentation from temporally incoherent mixtures in honeybees

Jacob S. Stierle[1], Stephanie Biergans[1], C. Giovanni Galizia[1], Paul Szyszka[1]

[1]University of Konstanz, Neurobiology, 78457 Konstanz, Germany

Natural olfactory stimuli are highly discontinuous in space and time, leading to intermittent odor pulses. Most odors that emanate from a natural odor source consist of many components with a stable concentration ratio and form a perceptual entity, which does not allow to identify the single components from it (configural odor mixture perception). On the other hand, if odors from two sources intermingle, the concentration ratio of the resulting mixture is highly transient. Thus, the temporal concentration fluctuations between the components of a mixture may be used as information about whether there is a single or whether there are several odor sources. Previous studies on odor mixture processing mainly considered mixtures with stable concentration ratios. Typically, odor mixtures lead to mixture interactions, which is in line with configural odor mixture perception.

We investigated whether honeybees can use temporal differences between two components of an odor mixture to perceive it as two independent odors (elemental odor mixture perception). As stimuli we used binary odor mixtures where the components were presented either simultaneously (perfect mixture) or with different time intervals between the odor onsets (imperfect mixture). Behavioral experiments showed that an interval of only 5 ms between two components of a mixture improved the honeybees' ability to perceive them as individual odors.

We then searched for a neural correlate of this elemental mixture perception and asked whether projection neurons (PNs) in the antennal lobe are capable of resolving short time delays in the onsets of odor components. Using *in vivo* calcium imaging we found that processing of imperfect mixtures (5 to 600 ms delay between the components) involved more inhibitory interactions than the processing of perfect mixtures. Currently we are testing possible functional consequences of these inhibitory interactions. Taken together, these results suggest that honeybees may use short temporal differences in stimulus coherence for odor segmentation.

# Odorant receptor coding genes of the tobacco hornworm (*Manduca sexta*)

Ewald Grosse-Wilde[1], Christopher Koenig[1], Christian Klinner[1], Merid Negash Getahun[1], Linda S. Kuebler[1], Bill S. Hansson[1]

[1]Max-Planck-Institute for Chemical Ecology, Evolutionary Neuroethology, Hans-Knoell-Str. 8, 07745 Jena, Germany

With the goal to further our understanding of the olfactory sense in complex behaving moth, we recently analysed the antennal transcriptome of the tobacco hornworm (*Manduca sexta*). We were able to identify members of all gene families involved in the detection of odorants, including 49 genes encoding putative odorant receptors. Expression profiling and comparison with other lepidopteran species allowed us to identify odorant coding genes with either sex-specific expression patterns or unusually conserved representation in lepidopteran species. Here we present an extensive analysis of these genes.

# Odour Discrimination and Odour Generalisation in Olfactory Learning of *Drosophila melanogaster*

Jonas Barth[1], Moritz Hermann[1], Andre Fiala[1]

[1]Georg-August University Goettingen, Molecular Neurobiology of Behavior

In associative learning animals can connect sensory stimuli with a reward or a punishment. Thereby, the animal learns to predict the consequences of a specific sensory stimulus. A fruit fly, for example, can associate an odour with a punishing electric shock. However, the animal has to evaluate the sensory input and act accordingly, also if the stimulus is slightly different than the learned stimulus. Thus, the fly has to be able to react to similar odours with a learned response (generalization). However, similar stimuli might have different consequences depending on the learning procedure. Therefore, the animal has to be able to differentiate between similar stimuli, that have been learned differentially (discrimination learning).

The fruit fly *Drosophila Melanogaster* is a suitable model system to study generalisation and discrimination of sensory stimuli as the flies are capable of olfactory learning. If an odour (conditioned stimulus, CS) is presented to the flies and paired with an electric shock (unconditioned stimulus, US), they will learn to avoid this odour when tested in a T-maze situation. Usually, a subset of monomolecular odours is used for olfactory learning (e.g. Methylcyclohexanol or 3-Octanol). In a discriminative learning procedure, the fly is encountered with two different odours, one is paired with the electric shock (CS+) and the other is not (CS-). In the subsequent T-maze test, the flies can decide to approach or avoid the CS- or CS+. A generalisation effect can be observed, if flies are trained with an odour CS1 and tested with another odour CS2. If the flies generalize between the two odours, they do not show a different behaviour towards the two odours. Experiments were performed in our lab to test the generalization of two distinct but structurally similar odours. In addition, it was tested whether the odours can be distinguished by the flies if they are trained in a discriminative way (one odour is CS+, the other CS-). First results show that for similar odorants generalisation and discrimination can be achieved depending on the training protocol and test situation.

# Olfactory sensitivity: modification by physiological status in *Drosophila*

Farhan Abu[1], Markus Knaden[1], Bill S. Hansson[1]

[1]MPI for Chemical Ecology, Neuroethology, Hans-Knoll str-8, 07745 Jena, Germany

Olfactory information is essential for many fly behaviors and is processed at different levels of the nervous system. While physiological status is known to modulate the olfactory sensitivity, few behavioral studies have revealed starvation-dependent modulation of olfactory sensitivity1, 2, 3 & 4, but the mechanism and the pathway is unknown. To study starvation mediated modifications of fly olfactory sensitivity we selected the odors on the basis of their source either food or non-food related and also perceived by morphologically different sensilla types. We started with group fly behavior T-maze experiments grown on relatively odor free synthetic medium that limits odor exposure for sensory deprivation. Single-unit recordings of odor responses were collected from a defined class of ORNs (eg. ab2A which response to ethyl acetate ,EA)5. We assessed differences between the ensemble spike-response patterns to different odors under 28 Hrs starved and satiated physiological conditions. Both starved and satiated flies has different basal firing pattern of the ORNs, also, our results indicated that ORN firing patterns of starved flies show increased sensitivity to all odors (Ethyl acetate, 2,3 Butanedione) with a different temporal response dynamics than the satiated. Starvation causes an increase in both odor sensitivity and acuity. These results demonstrate quantitatively, starvation-dependent functional modification at the level of behavior as well as at the level of spike generation and patterning in ORNs.

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# Olfactory sensory neurons expressing the OR37 subfamily: connectivity to higher brain centres

Andrea Bader[1], Heinz Breer[1], Jörg Strotmann[1]

[1]University of Hohenheim, Institute of Physiology, Garbenstraße 30, 70599 Stuttgart, Germany

Olfactory sensory neurons (OSNs) which express a particular odorant receptor (OR) gene are usually widely dispersed throughout the olfactory epithelium (OE) of mammals. In contrast, neurons expressing a member of the OR37 subfamily are concentrated in a small patch located in the centre of the OE. Each OSN population expressing an OR37 subtype sends its axons to only one glomerulus in the olfactory bulb (OB), whereas OSNs expressing other OR types typically project to two glomeruli, one located in the medial and one in the lateral aspect of each bulb. Thus also on the level of the OB the cells expressing OR37 receptors exhibit a quite unusual feature. A detailed molecular characterization of the OR37 receptors revealed that they are not only expressed in cells with a unique topographic organization, but display several other special features, as well. Only this group of ORs is characterized by an insertion of six amino acids in the third extracellular loop. Comparative analyses have shown that orthologous receptors exist in diverse mammalian species. Surprisingly the coding sequence of the OR37 genes displays a high degree of conservation and bioinformatic data revealed that they apparently are under a negative selective pressure, a feature that is quite unusual for OR genes. Due to these unusual properties it seems conceivable that the OR37 receptors are tuned to special ligands and therefore have an exceptional function in monitoring the chemical environment of the animal. Towards an understanding of their functional role, information regarding the higher brain centres to which the OR37 odor information is conveyed, could be extremely valuable. Toward this goal a transgenic approach was employed, in which OSNs expressing mOR37C coexpress wheat germ agglutinin (WGA). WGA is specifically transported via synapses and therefore can be used as a transsynaptic marker; thus by visualizing the WGA protein, neurons connected to the OR37C subpopulation can be identified. In the OB, one glomerulus in the ventral domain was strongly immunoreactive for WGA. By crossing WGA transgenic animals to a mouseline in which cells express mOR37C together with tauGFP, it could be shown that WGA positive axons specifically converge in this particular glomerulus. Close to it, several glomeruli with weaker WGA immunoreactivity were visible. It is known that glomeruli formed by OSNs expressing the different members of the mOR37 subfamily are located in immediate vicinity; nevertheless each glomerulus receives input with very high precision from only one, receptor-subtype specific population. Based on this fact it seemed to be conceivable that those glomeruli with weaker WGA immunoreactivity represent other OR37 subtypes. In fact, one of these glomeruli was shown to be mOR37A indicating that the transsynaptic marker has been transferred to glomeruli formed by other OR37 subpopulations. Odor information from the olfactory bulb is conveyed to various higher brain centres by mitral/ tufted cells, the principle projection neurons. These cells do transmit olfactory information to different brain areas known as the olfactory cortex. Interestingly, WGA immunoreactivity could not be detected in these parts of the brain. It was however visible in the medial amygdala and the hypothalamus, brain areas known to be involved in mediating social communication and behaviour.

# Optogenetic generation of spatio-temporal activity patterns in the mouse olfactory bulb.

Alexander Lehmann[1], Anna D'Errico[1], Martin Vogel[1], Hartwig Spors[1]

[1]Max-Planck-Institut für Biophysik, Molecular Neurogenetics, Max-von-Laue-Straße 3, 60438 Frankfurt am Main, Germany

Odor stimuli elicit spatio-temporal patterns of neuronal activity in the olfactory bulb (OB). It remains open if spatial or temporal components of mitral cell (MC) activity or both are used by downstream cortical circuits to identify odorants. Artificial generation of activity patterns across the OB will allow tests to assess if cortical neurons and finally animal behavior are tuned to specific features of these spatio -temporal patterns. Pattern generation on different levels of neuronal processing in the OB, i.e. the input level (sensory neuron axon terminals) or the output level (MCs) will furthermore allow testing hypotheses of neuronal computation in the OB. Initially we address the questions: Can we drive MCs belonging to individual glomeruli with sufficient temporal precision and specificity? How do MCs interact that receive input from different glomeruli? Can we find activation patterns driving individual neurons with limited a priori knowledge of potential optimal stimuli?
We started using optical stimulation in a transgenic mouse line that expresses Channelrhodopsin-2 (ChR2) in MCs (Arenkiel et al, Neuron 2007). Patterns of blue light (465 nm, 1.2-6 $mW/mm^2$) were projected onto the OB surface using a digital mirror based projection system (1024 x 786 pixels, 8 kHz temporal resolution) while recording from individual MCs in loose patch configuration in urethane anesthetized Thy1-ChR2 mice (line 18). In this setting stimulation efficiency depends on the respiratory phase. Illumination of discrete areas of the size of individual glomeruli (50-100 µm) was sufficient to drive MC activity with up to 90% efficacy and latencies ranging from 10 to 15 ms. Interaction between glomerular units as measured by significant changes in evoked firing rate was rare. Increasing the number of stimulated glomeruli increased the LFP power in the gamma band and increased precision of the individual APs. This effect could easily be evoked by stimulation of distant glomeruli. Thus while inhibitory interactions appear to be sparse between MCs belonging to different glomeruli there is still a significant effect of stimulation of additional glomeruli on the spiking activity of an individual MC. In summary experiments demonstrate that optical stimulation of individual functional modules in the olfactory bulb is feasible and will allow to provide well controlled artificial inputs to test hypotheses of olfactory coding and processing.

# Organization of deutocerebral neuropils in representatives of the Chilopoda (Myriapoda)

Andy Sombke[1], Bill S. Hansson[2], Steffen Harzsch[1]

[1]Ernst Moritz Arndt Universität Greifswald, Zoologisches Institut und Museum, Cytologie und Evolutionsbiologie, Johann Sebastian Bach Strasse 11/12, 17487 Greifswald, Germany

[2]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Hans Knöll Strasse 8, 07745 Jena, Germany

Considering that the olfactory sense of myriapods has evolved independently from that in hexapods and crustaceans, the question arises if and how myriapods have solved the tasks of odor detection and odor information processing in air. Behavioral experiments indicate that chilopods are able to perceive airborne stimuli, both from live prey and prey extracts. Comparative studies between these taxa with terrestrial life style provide a powerful means to understand how the arthropod nervous system evolved in response to new environmental and ecological challenges. In general, the architecture of the myriapod central nervous system is insufficiently understood. In a set of experiments with representatives from all five chilopod groups, we analyzed the organization of deutocerebral neuropils with serial semi-thin sectioning and whole-mount preparations combined with 3D reconstructions, antennal backfilling with neuronal tracers, and immunofluorescence combined with confocal laser-scanning microscopy. The primary chemo- and mechanosensory centers are well developed in these organisms. Although, the shape and composition differs when compared to those of hexapods and malacostracan crustaceans, the presence of distinct neuropils for chemo- and mechanosensory qualities in all three mandibulate groups could indicate a common architectural principle within the Mandibulata.

# Organization of the antennal lobe in desert ants of the genus *Cataglyphis*

Sara Mae Stieb[1], Christina Kelber[1], Rüdiger Wehner[2], Wolfgang Rössler[1]

[1]University of Würzburg, Behavioral Physiology and Sociobiology, Am Hubland, 97074, Würzburg, Germany

[2]Brain Research Institute, University of Zürich, Switzerland

Foraging desert ants of the genus *Cataglyphis* possess remarkable navigational capabilities. They forage solitary and use predominantly visual cues to precisely return back to their often inconspicuous nest entrances after foraging over distances of up to several hundred meters (Wehner 2003). Although all *Cataglyphis* species lack a trail pheromone or recruiting behavior, behavioral studies on *Cataglyphis fortis* could show that olfactory cues are used during foraging to pinpoint food sources (Wolf et al. 2000) and the nest entrance (Steck et al. 2009). Furthermore, foragers can learn olfactory landmarks in the vicinity of the nest for homing (Steck et al. 2010). Our study focused on the neuro-anatomical organization of the first olfactory neuropil – the antennal lobe (AL) – to find out about potential olfactory adaptations within different *Cataglyphis* species.

By using 3D-reconstructions (with AMIRA) of the ALs, we compared the number, size and spatial arrangement of glomeruli – the functional units of the AL – among *C. fortis*, *C. albicans*, *C. bicolor*, *C. rubra*, and *C. noda*. Compared to more olfactory guided ants like leaf-cutting ants (Kelber et al. 2009; Kuebler et al. 2010) or carpenter ants (Zube and Rössler 2008; Nishikawa et al. 2008), all tested *Cataglyphis* species show a reduced number of glomeruli. For a more close comparison, we reconstructed glomeruli in the ALs of two *Formica* species belonging to the same subfamily (Formicinae). In the two largely olfactory guided *Formica* species we found a higher number of glomeruli compared to the conditions in the highly visual desert ants. These results indicate that interspecific variations in the number of glomeruli may reflect the importance of olfactory-guided behavior.

Within the genus *Cataglyphis*, the ALs of *C. fortis* exhibit two special features. First, they possess with around 198 the lowest number of glomeruli among all tested *Cataglyphis* species, and second, a conspicuously enlarged glomerulus is found next to the antennal nerve entrance. At this point we can only speculate about the possible function of this glomerulus. *C. fortis* inhabits microhabitats that are avoided by all other *Cataglyphis* species, namely salty areas or even salt-pans. Living under such extremely harsh conditions might result in highly adapted foragers not only in their visual navigation features, but also in their olfactory system.

Due to the exceptional position of *C. fortis*, we analyzed their AL structure in more detail. Backfill stainings revealed that the AL of *C. fortis* workers is innervated by four distinct sensory tracts formed by axons of incoming olfactory receptor neurons that divide the AL into four glomerular clusters. In addition, we compared the AL structure between all castes – workers, queens and males – of *C. fortis*. Males possess with 150 a significantly smaller number of glomeruli compared to females. Additionally they have a macroglomerulus at a position different from the enlarged glomerulus in females that is presumably involved in sex-pheromone communication.

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# Pheromone responses in antennal trichoid sensilla of the hawkmoth *Manduca sexta* and their modulation by cAMP and DAG.

Andreas Nolte[1], Christian Flecke[2], Monika Stengl[3]

[1]Universität Kassel, Abteilung Tierphysiologie, Heinrich-Plett-Straße 44, 34132 Kassel, Deutschland

The males of the hawkmoth *Manduca sexta* are able to detect sex pheromones with specific pheromone sensitive trichoid sensilla on their antennae. These sensilla are innervated by two olfactory receptor neurons (ORNs). One of the ORNs responds to the main pheromone component bombykal (BAL) of the pheromone blend, released by the females. The involved signal transduction cascades in insect olfaction are still not completely understood. But there is evidence for the involvement of both cAMP and DAG in the pheromone-dependent signal transduction cascade in a time dependent manner. In extracellular tip recordings of pheromone-sensitive trichoid sensilla of the hawkmoth antenna we investigated BAL responses with a non-adapting stimulation protocol (dosage 10 or 1 µg BAL, duration 50 ms, interstimulus interval 5 min) for 3 hours at Zeitgebertimes (ZT) 1-4 (beginning of day = end of activity phase) and 8-11 (rest phase). The passive perfusion of 100 µM of the membrane-permeable cAMP analog 8bcAMP into the sensillar lymph of the trichoid sensilla at ZT 1-4 had an increasing effect on the sensillar potential (SP) amplitude, but there was no significant change in the action potential (AP) frequency of the first 5 interspike intervals at the same time. The perfusion of 50 µM forskolin, an adenylyl cyclase activator, also leads to an increased SP amplitude at ZT 1-4. In contrast to 8bcAMP there was an increased AP frequency at the beginning of forskolin recordings. Perfusion of 100 µM of the membrane permeable DAG analog 1,2-Dioctanoyl-sn-glycerol inhibited both the BAL-dependent action potential response as well as the SP amplitude at ZT 8-11. These results support the hypothesis of BAL-dependent activation of a phospholipase C (PLC). PLC increases the $IP_3$-concentration and leads to an activation of $IP_3$-dependent $Ca^{2+}$-channels. The increase of intracellular $Ca^{2+}$ and DAG is suggested to decrease the sensitivity of the signal transduction cascade via activation of protein kinase C. Octopamine-dependent increase of cAMP may affect the sensitization of the pheromone transduction in a time-dependent manner.

# Pheromone-plant odour interactions and mating effects in the antennal lobe of Agrotis ipsilon males

Nina Deisig[1], Sylvia Anton[1], Christophe Gadenne[1]

1)INRA Versailles, UMR 1272 Physiologie de l'Insecte: Signalisation et Communication, Route de St Cyr , 78026 Versailles cedex, France

Most animals, including Lepidoptera, use odours in different behavioural contexts, e.g., to find a mating partner, food sources, a habitat, or oviposition sites. Moths exhibit a pronounced sexual dimorphism, where males detect and process sensory information from plant odours and sexual pheromones by means of two relatively distinct olfactory pathways. Female moths emit a blend of sexual pheromone when situated on plants (host or non-host) and males need to extract this pertinent information from the plant-dominated olfactory environment. Plant odours can either facilitate the localization of females (synergy) or mask the female pheromone (suppression). In addition, behavioural responses to olfactory stimuli depend on the physiological state and it was recently shown that mating modifies the pheromone -guided behaviour in male A. ipsilon. Behavioural responses of males to the female pheromone are strongly reduced after mating, and at the same time, central neurons within the antennal lobe strongly decrease their sensitivity in mated males.
Using in vivo calcium imaging with a bath applied dye, we here compared antennal lobe glomeruli activity induced by plant odour, pheromone and their mixture in virgin and mated 5-day old males. As in other moth species, plant odours activate ordinary glomeruli, while the pheromone blend strongly activates the macroglomerular complex (MGC), situated at the entrance of the antennal lobe. Addition of plant odour to the pheromone blend strongly reduces (suppresses) pheromone-evoked activity in the MGC, whereas plant odour-evoked activity in ordinary glomeruli was not modified when the pheromone blend was added, independently of the mating state. Mating did not affect pheromone -evoked MGC activity. However, mating led to stronger plant odour-evoked responses in ordinary glomeruli when high doses were employed. In conclusion, within the male-specific part of the antennal lobe (MGC), strong pheromone/plant-odour interactions exist, which are independent of the males' mating status. Furthermore, we found an influence of mating on plant- odour responses within the sexually isomorphic glomeruli of the antennal lobe.

# Plant odour-pheromone interactions in the olfactory pathway of moths

Sylvia ANTON[1], Jan Kropf[1], Simon Vitecek[1], Philippe Lucas[1], Christophe Gadenne[1], Romina B. Barrozo[1]

[1]INRA, UMR 1272 PISC, Route de St Cyr, 78000 Versailles, France

Most animals rely on olfaction to find e.g. their sexual partner, food or a habitat. The olfactory system meets the challenge of extracting meaningful information from a noisy odorous environment. In moths, females emit a sexual pheromone in an environment with abundant plant volatiles. Plant odours could thus either facilitate the localization of females or mask the female pheromone. We studied how mixtures of a behaviourally-attractive plant odour, heptanal, and the sex pheromone are encoded at different levels of the olfactory pathway in virgin males of the noctuid moth *Agrotis ipsilon*. We used recordings from individual sensilla to study responses of olfactory receptor neurons and intracellular recordings of antennal lobe output neurons when stimulating with heptanal, the sex pheromone and their mixture.

Our findings indicate different origins of mixture interaction in a multi-level approach: plant odours inhibit pheromone reception at the level of antennal receptor neurons and this effect persists without evident further integration throughout neural elements transmitting information to the protocerebrum. Plant odour processing, on the other hand, is modulated by pheromones primarily at the central level. Whereas interactions happen at the reception level in the case of pheromone sensilla, information on sex pheromone and plant odours arrives in parallel through different input channels and interactions between the two stimuli within

# Post Metamorphic Plasticity of Numbers of Peptidergic Neurons in the Antennal Lobe of *Tribolium castaneum* (Coleoptera)

Peter Christ[1], Martin Kollmann[1], Silke Redelfs[1], Joachim Schachtner[1]

[1]Philipps Universität Marburg, Tierphysiologie, Karl-von-Frisch-Str. 8, 35043 Marburg, Germany

The major pest of stored grain, the red flour beetle *Tribolium castaneum* is emerging as a further standard insect model organism beside *Drosophila*. With the recently fully sequenced genome, its susceptibility for transgenetic approaches such as directed gene expression and powerful reverse genetics based on systemic RNAinterference, (RNAi), and its longevity, *T. castaneum* offers an excellent system to study development and plasticity of the olfactory system. Recent studies revealed a massive increase of brain size incl. antennal lobes (AL) and mushroom bodies (MB) within the first days of adult life, indicating a pronounced sensitive phase. To further reveal mechanisms involved in the post metamorphic phase, we examined whether there are changes in the number of peptidergic AL cells. We compared the numbers of tachykinin-immunoreactive (TK-ir) AL neurons of females and males directly after adult eclosion (A0) and seven days after eclosion (A7). We found (1) a sexual dimorphism at A0 concerning numbers of TK-ir cells, with females having more TK-ir AL neurons than males. (2) In both sexes, the numbers of AL TK-ir cells increased from A0 to A7. Similar to the situation in A0 animals, A7 females showed more AL TK-ir cells than males. (3) Females isolated shortly before A0 showed no increase in the number of TK-ir cells. This finding suggests that the increase of TK-ir cells between A0 and A7 in females may depend on the perception of the pheromone signal and thus favors an activity dependent mechanism for the maturation of a peptidergic system in the AL of the red flour beetle.

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# Processing of Complex Host Blends in the Manduca Antennal Lobe

Linda S Kuebler[1], Marco Schubert[1], Silke Sachse[1], Shannon B Olsson[1], Bill S. Hansson[1]

[1]Max-Planck-Institut for Chemical Ecology, Evolutionary Neuroethology, Hans Knöll Straße 8, 07745 Jena, Germany

Natural odor cues are often multi component blends and animals typically perceive their olfactory environment as a complex mixture. In insects, the initial representation of odors occurs in the first olfactory center of the brain, the antennal lobe (AL). Afferent input determined by the response profile of olfactory sensory neurons (OSNs) is modified via interneuronal connections (LNs) in the assembly code and carried by projection neurons (PNs) to higher order brain centers. The resultant neural representation of an odor mixture may either retain the mere sum of single-odor information of blend components, or reveal non-linear interactions different from the sum due to processing in the AL network.

Our goal is to reveal mechanisms of host odor information processing at different levels in the AL of the hawk moth, *Manduca sexta*. Electrophysiological studies revealed that 82% of the individual in vivo recorded projection neurons (PNs) and local interneurons (LNs) showed non-linear spike frequencies in response to the blend versus its individual compounds. To assess the network as a whole, optophysiological studies in the AL were used to assess calcium activity to combinations of up to seven host volatiles. We simultaneously measured projection neuron output in concert with the compound signal dominated by sensory neuron input across the glomerular array. By comparing patterns and intensity of activation between the two processing levels we can determine the relative interactions between input and output blend information within a single animal. Our combined physiological approach results in a highly combinatorial, non-linear process for coding complex host blends in *Manduca sexta*.

# Projection patterns of sensilla basiconica and differences in the dual olfactory pathway of honeybee workers and drones

Jan Kropf[1], Kathrin Bieringer[1], Christina Kelber[1], Wolfgang Rössler[1]

[1]University of Wuerzburg, Biozentrum Zoology II, Am Hubland, 97074 Wuerzburg, Germany

Insect antennae are covered with different types of sensilla housing olfactory receptor neurons (ORNs) which transmit olfactory input to the antennal lobe (AL). The AL represents the first central relay station for processing of olfactory information in the insect brain. As a general rule, ORNs expressing the same olfactory receptor project to the same olfactory glomerulus in primary olfactory centers, and odors are encoded in a spatial activation map of glomeruli. In the honeybee (Apis mellifera), ORNs project via four different sensory tracts to four corresponding glomeruli clusters (T1-T4), where they synapse on local interneurons and projection (output) neurons (PNs). PNs convey the olfactory information to the mushroom bodies (MBs), higher sensory association centers and sites associated with learning and memory. In Hymenoptera, PNs project to the MBs via two parallel tracts forming a dual olfactory pathway, the medial and the lateral antennal lobe - protocerebral tract (m- and l-APT). The role of parallel olfactory pathways in odor coding is unclear. We tested different hypotheses for the function of a dual pathway to the MBs: 1. the two pathways may carry information about different subsets or classes of odors. 2. The two pathways may transmit information about different coding aspects of similar odors. The social lifestyle shared by social Hymenoptera involves many pheromonal and colony-specific odors that play an important role in the regulation of social organization. Honeybee drones almost exclusively serve reproductive tasks and are not involved in social tasks. Drone ALs were shown to contain less glomeruli compared to the female castes (Sandoz 2006, J Exp Biol). Besides, drone antennae lack sensilla basiconica (SB) and the reduction of AL glomeruli mostly affects the T3 cluster (Nishino et al. 2009 J Comp Neurol). Interestingly, the T3 cluster was shown to be mainly innervated by PNs of the m-APT in honeybee workers (Kirschner et al. 2006, J Comp Neurol). We tested the hypothesis that SB preferentially project to the T3 cluster and their absence in drones explains the reduction in the number of T3 glomeruli. We labeled the axonal projections of ORNs in small numbers of SB as well as in individual SB on the worker antenna. Furthermore, we labeled the APTs in drones to test whether only the m-APT proportion of glomeruli is reduced as it was previously shown in ants (Zube and Rössler 2008, ASD). Parts of the T3 cluster are lacking in honeybee drones (Nishino et al. 2009, J Comp Neurol), thus we expected less PNs in the mAPT of drones compared to workers. Brains were analyzed as whole mounts under the confocal microscope. Image stacks of sensillum stainings were further processed using the VOI method to obtain a map of the AL with the most frequently innervated glomeruli (Kelber et al. 2006, J Comp Neurol). The results from sensilla stainings indicate that SB associated ORNs mainly project into the T3 cluster of AL glomeruli. APT labelings in drones indicate that the m-APT innervates less glomeruli compared to the m-APT in workers. The two results combined suggest that in contrast to the broad projections of sensilla placodea (Kelber et al. 2006, J Comp Neurol), information transmitted by SB associated ORNs is preferentially processed via the m-APT. The absence of SB and T3 glomeruli in drones may indicate that the m-APT may carry specific information about colony odors in addition to other odors.

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# Random inhibition separates odor representations in a model of the *Drosophila* antennal lobe

Henning Proske[1], Marco Wittmann[1], C. Giovanni Galizia[1]

[1]University of Konstanz, Biology, Universitätsstr. 10, 78464 Konstanz, Germany

In the insect antennal lobe odors are represented as spatiotemporal patterns of neuronal activity. These patterns are influenced by a dense network of lateral excitatory and inhibitory connections. While in *Drosophila melanogaster* most neurons project in a global fashion anatomically, the question is open as to what connectivity pattern among glomeruli would yield a functionally most efficient network. For example, glomeruli responding to similar odors might be de-correlated at the output level by reciprocal inhibitory connections. Here we seek to answer the question if there are coding advantages with lateral interactions that are based on stimulus features as compared to a completely random connectivity.

We used both an algebraic as well as a numeric approach to characterize the relationship between connection architectures and the performance of the resulting networks to separate the representation of odor stimuli. Our numerical models utilize linear algebraic matrix multiplications to efficiently and exhaustively explore the parameter space of the tested networks. The DoOR database of olfactory receptor neuron responses (http://neuro.uni-konstanz.de/DoOR) provided input activity patterns to the network that captured the statistical properties of real-world odorant stimuli.

Our results suggest that networks based on input correlations and spatial distance between glomeruli do not yield advantages in their ability to increase discriminability between odors when compared to randomly constructed inhibitory networks. We found that with increasing total inhibition glomerular activation patterns became sparser allowing for a more reliable identification of odors. However, the effect came at the price of a reduced number of odors that could be distinguished upstream of the network. This effect could be modulated dynamically by changing the efficacy or number of inhibitory connections. Such a mechanism might be useful for insects to quickly adapt to different odorant environments and to modulate input statistics during plastic changes in the insect brain.

# Ratio Coding and Dynamic Range in the Pheromone System of the Moth

Thomas Nowotny[1], Christopher L. Buckley[1], Andrei Zavada[1]

[1]University of Sussex, Informatics, Falmer, BN1 9QJ Brighton, United Kingdom

Information coding in the olfactory system of insects has a long tradition in a handful of main animal models: Locust, honeybee, Drosophila, moths, and cockroach. In the last 3 years our group has concentrated on the specific problem of coding in the pheromone sub-system, in particular, the macro-glomerular complex (MGC) of moths. Contrary to earlier reports in locust [1], honeybee [2] and Drosophila [3], we were unable to observe oscillations in the local field potential in the MGC. We take this as an indication that the coding strategy in the MGC of moths may differ from the popular account of coding of odour information in locust [4, 5]. We have identified two important aspects of information coding in the MGC which I will discuss in this presentation:

1. On the level of individual glomeruli in the MGC, the extreme sensitivity of the moth to conspecific pheromone blends implies a wide dynamical range for the recognition of the concentration of the chemical components. This wide dynamical range is implied by behaviour but has also been directly observed experimentally on the level of projection neurons in the MGC [6]. The common view that the dynamic range arises in the chemical transduction pathways within the ORNs is not enough as the dynamic range appears to be dependent on neuromodulation in the MGC [6].

2. On the level of the whole MGC network, the fact that sexual pheromones of moths are blends of two to three separate chemicals at fixed concentration ratios, implies that male moths need to recognise pheromone blend component ratios, in particular when sympatric, related species use pheromone blends that only differ in their component ratios [7]. This ratio recognition ability has to be concentration independent.

On the single glomerulus level we propose a model of nearly-critical rate dynamics. In a multi-scale model encompassing conductance based formulations, rate reductions and further mean field approximations we can consistently demonstrate that

1. the observed rate patterns in LN and PN responses can be explained by a generic network of inhibitory LNs

2. the LN network can form an implicit disinhibition pathway that could explain the late excitatory response of the PNs that is to a degree inconsistent with dominantly direct ORN input to PNs.

3. the dynamic range of PN responses becomes maximised in this model when we approach the critical point in the model dynamics where the baseline state becomes unstable.

On the network level we have formulated a model of neuronal competition for pheromone blend ratio recognition. The prototype model demonstrates component ratio recognition for two pheromone components in a 1:1 ratio. The core finding in this part of the work were that

1. in a minimal model of three individual LNs which receive inputs from either of the re-ceptor populations, or both, and compete through lateral inhibition (figure 1) the coding inadvertently reverts to a latency-to-first spike code.

2. the latency code is not beneficial for ratio recognition across a wide range of concentrations.

3. populations of neurons allow a more gradual competition (rate based coding) which improves ratio recognition, in particular its stability across concentrations.

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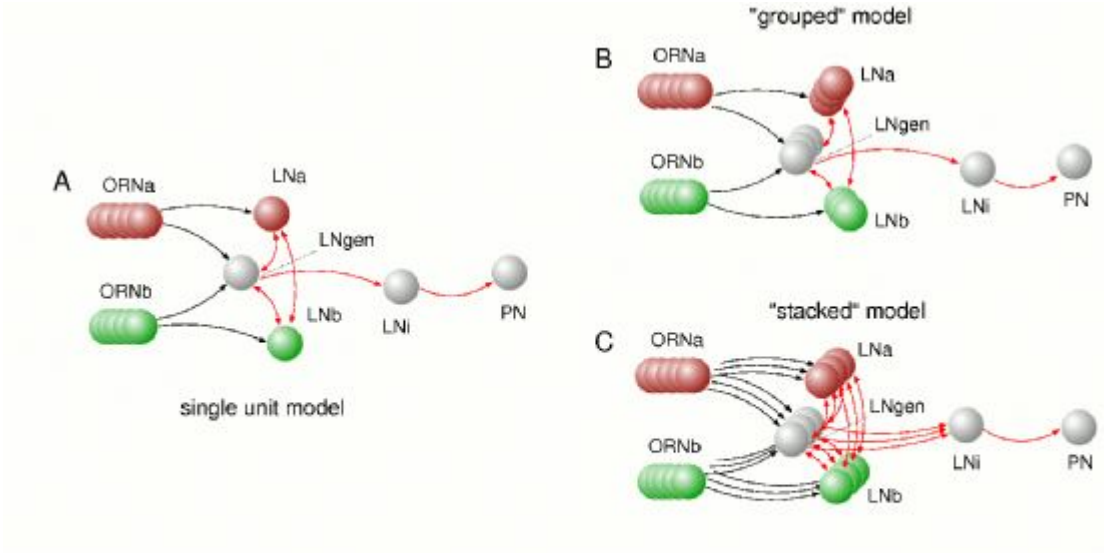


Figure 1: Minimal single LN model (A) and two more realistic (and more successful) populations models (B and C) for pheromone blend component concentration ratio recognition.

# Relevance of olfactory cues during group recruitment in the desert ant *Ocymyrmex robustior*.

Nadine Wenzler[1], Sara Mae Stieb[1], Wolfgang Rössler[1], Rüdiger Wehner[2]

[1]University of Würzburg, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany

[2]Brain Research Institute, University of Zürich, Switzerland

The Namib Desert provides a harsh and nutrient-poor habitat for the myrmicinae ant, *Ocymyrmex robustior*. Starting from their underground sandy nests situated in sandflats, these solitarily foraging ants search for dead insects available in this hot environment (B. Bolton, A.C. Marsh 1989). In contrast to the ecologically equivalent Saharan ants of the genus *Cataglyphis*, these ants perform a special form of group recruitment. When an *O. robustior* worker detects a rewarding food source, it runs back to the nest to recruit other workers inside the nest. A group of up to 20 workers pours out of the nest entrance, moves tightly around the recruiting ant, and then follows the latter from the nest in direction of the food source, which can be located up to fifty meters away. In our analyses we examined the olfactory recruitment behavior of *O. robustior* and implied histological data about anatomical differences of the antennal lobe, the olfactory organization center. The current account represents the first demonstration that also highly thermophylic desert ants can use chemical cues for recruiting nestmates to occasionally occurring patches of food items.

Using slow motion video analysis, we show that only the leading ant runs in a straight line towards the food source. Due to the extremely high surface temperatures of up to 70 C°, the ants reach forward speeds of up to 0.4 m per second. The leading ant intermittently touches the ground with the tip of its gaster, most probably depositing a pheromone. The recruited ants follow the leading ant with high speed, and occasionally even pass the recruiter. In the latter case they stop and get back behind the leading ant. First results demonstrate that the recruited ants do not follow the recruiter in a strait (trail-like) way, but zig-zag, with high speed, around the putative trail. This behavior resembles much more the zig-zag flight behavior of male moths towards a sex-pheromone source than the usual trail-following behavior of, e.g., harvester ants, and, therefore, may indicate that the ants try to stay in contact with a probably volatile pheromone plume produced by the leading ant.

Using immunolabeling and 3D-reconstructions, we quantified and compared the antennal lobe structure, in particular the number, size and spatial arrangement of olfactory glomeruli of three diurnal desert ant species - *Cataglyphis fortis*, *Ocymymrex robustior* and *Camponotus detritus*. Only the first of these three species represents a strictly solitary forager relying predominantly on visual cues for long-distance navigation, but all share analog habitats in the Namibian and Saharan Desert and therefore represent an interesting model for potential adaptations in the olfactory system.

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# Response profiles of the Drosophila olfactory receptors Or42b and Or69a - knocking on the DoOR.

Jennifer S Ignatious Raja[1]

University of Konstanz, Neurobiology, D-78467 Konstanz, Germany

*Drosophila* is able to detect and discriminate a wide range of odor molecules in the environment by the olfactory receptors (ORs) expressed in the olfactory receptor neurons. The perception of odor molecules is essential for *Drosophila* to find food sources and suitable sites for egg laying and for mating. Drosophila is an excellent model animal to study various aspects of olfaction. For these studies the response profiles of the ORs to a wide range of odor molecules should be known. Several labs have measured the response profiles of many ORs by using various methods (e.g. calcium imaging, electrophysiology). Our group recently developed a database called DoOR (Database-of-Odor-Responses; see http://neuro.uni -konstanz.de/DoOR) by integrating all data sets (single sensillum recordings and *in vivo* calcium imaging) published until 2010. DoOR provides information about many *Drosophila* OR response profiles. However some ORs have not yet been characterized or have only been tested with a limited number of odor stimuli. Among these, no response profile is available for dOR69a, and only few odor-responses have been published for dOr42b.We measured the response profile (~100 odorants) of these two *Drosophila melanogaster* olfactory receptors – Or69a and Or42b by *in vivo* calcium imaging. The calcium sensor GCamP was specifically expressed in ORNs carrying Or69a or Or42b respectively by using the UAS/Gal4 system. We recorded odor-evoked calcium increases in the dendrites and somata through the intact cuticle of the antennae (see also Galizia et al, 2010, Chem. Senses 35: 551–563, 2010). Among the best ligands for Or69a we found 4-Methylphenol, a-Terpineole, ß-Citronellol and Ethyl-3-hydroxyhexanoate. The response profile of Or42b consisted of both excitatory and inhibitory responses with 3- Octanol, Linalool and 2- Heptanol being among the inhibitory stimuli and 3-Hexanone, ethyl-3-Hydroxybutanoate and 3-Pentene-2-one belonging to the excitatory ligands. These two data sets will be included in DoOR to fill gaps in the database, ultimately striving towards the complete olfactome for *Drosophila*.

# Sensory Innervation of the Antennal Lobe in Leaf-Cutting Ant Workers (*Atta vollenweideri*)

Christina Kelber[1], Wolfgang Rössler[1], Christoph Johannes Kleineidam[2]

[1]University of Würzburg, Biozentrum, Zoologie II, Am Hubland, D-97074 Würzburg, Germany
[2]University of Konstanz, Department of Biology, Universitätsstraße 10, D-78464 Konstanz, Germany

In ants, the sense of smell plays an important role in intraspecific communication, nestmate recognition and foraging. Therefore, the olfactory system in ants is highly developed, and the first olfactory neuropile, the antennal lobe, shows a complex architecture with a large number of functional units (glomeruli). In leaf-cutting ants (*Atta vollenweideri*), 390-450 glomeruli can be found. Two different antennal lobe phenotypes have been described: small workers show the low-number phenotype (LN-phenotype) with about 390 glomeruli and large workers show the high-number phenotype (HN-phenotype), with about 450 glomeruli (Kelber et al., Developmental Neurobiology 2010). Based on their sensory innervation (sensory tracts T1-T6), glomeruli can be grouped into different clusters. In LN-phenotype workers, we found a reduced number of glomeruli in the T4-cluster, located at the dorsal region of the antennal lobe. We then addressed the question from which olfactory sensilla and receptor neurons (ORNs), respectively, the glomeruli of the different clusters are innervated. On *A. vollenweideri* antennae, two sensillar types are abundant: the thick, stump -like sensilla basiconica and the long, curved sensilla trichodea curvata. We selectively stained the ORNs of single sensilla of both types and reconstructed all glomeruli that showed an innervation. Subsequently, we used the sensory tracts to assign the glomeruli to one of the six clusters. We further analyzed whether each ORN innervates only a single glomerulus.

For single sensilla trichodea curvata (n=6), we found between four and 48 ORNs terminating in the antennal lobe and innervating glomeruli of all six clusters. ORNs from each single sensillum innervated at least three of the six different clusters. The innervation patterns indicate that the glomeruli of the clusters T1-T4 are innervated more often than the clusters T5 and T6. About five percent of the arborizations were found in the T6-cluster, although it contains about 30 percent of all glomeruli. For the sensilla basiconica (n=5), we found between 11 and 53 innervated glomeruli in the antennal lobe. All glomeruli with arborizations of ORNs were located exclusively in the dorsal T6-cluster. Thus, the T6-glomeruli are innervated from ORNs of both, the sensilla basiconica and the sensilla trichodea curvata. Whether the same type of ORN can be associated with both types of sensilla, and innervating the same glomeruli in the T6-cluster is not known.
We conclude that both types of olfactory sensilla house multiple ORNs. Our finding is consistent with the finding of multiple ORNs in the honeybee and other ant species supporting the idea that this is a common character in Hymenoptera. For over 100 ORNs of both sensillar types, the axons could be traced from the arborizations in the glomeruli retrogradely to the sensory tract and sometimes even back to the antennal nerve. In all these cases, the axons of the ORNs terminated only in a single glomerulus.

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# Sensory reception and transmission of the primer pheromone ethyloleate in the honeybee

Thomas S. Muenz[1], Alban Maisonasse[2], Erika Plettner[3], Yves LeConte[2], Wolfgang Rössler[1]

[1]University of Würzburg, Biozentrum, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany
[2]INRA UMR 406, Abeilles et Environment, Laboratoire Biologie et Protection de l'Abeille, Site Agroparc, 84914 Avignon, France
[3]Simon Fraser University, Department of Chemistry, Burnaby BC, Canada V5A 1S6

In honeybee colonies the female worker caste expresses a pronounced polyethism. Summer bees perform a rich behavioural repertoire ranging from various indoor duties (e.g. feeding larvae or building combs) to foraging nectar, pollen and water outside the hive. Switching between these behaviours depends on age and goes along with plastic changes in the synaptic architecture as well as the volume of the mushroom-body calyces.
However, division of labour in a honeybee colony is not purely age-dependant. In fact, the transition from nurse bees to foragers may be modulated to enable the colony to respond in a flexible manner to environmental changes by shifting work force between indoor and outdoor duties. Worker-worker interactions have long been suspected to play a major role in modulating the work-force distribution. These interactions have been narrowed down to most likely short range or contact chemical cues from older worker bees engaged in foraging activities.
One of these chemical cues is ethyloleate (EO) which has been described to delay the onset age of foraging (Leoncini et al., PNAS, 2004). EO is found at high concentrations only with foraging bees, thus, making it a colony wide accessible signal for work force distribution. Therefore, the concentration of EO can be regarded as a primer pheromone that accelerates or delays the onset of foraging in a concentration dependant manner.
It was suggested that EO may be transmitted via trophallaxis. However, chemical analyses demonstrate that EO is at unexpected high concentrations on the cuticle, and absent in the regurgitate. Here we investigated whether EO is received as an olfactory cue. A first indication for this hypothesis was delivered by electroantennography (EAG) recordings of whole antennae using EO as a stimulus. Furthermore, $Ca^{2+}$-imaging at the level of the antennal lobe (AL) using selective loading of projection (output) neurons with calcium sensitive dyes was used to analyse the neuronal representation and processing of EO in AL glomeruli. Preliminary results indicate that EO is processed in glomeruli of the T1 cluster and potentially the T3 cluster of glomeruli. Furthermore, we applied EO as an olfactory stimulus in a classical conditioning paradigm, conditioning of the proboscis extension response, to test whether EO is internally represented at the level of perception. Taken together, the results provide evidence that EO is at least partly received, processed and perceived as an olfactory cue suggesting that it may mediate plastic changes in brain and behaviour during the transition of nurse bees to foragers via the olfactory pathway.

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# Serotonin and FMRFamide in regulation of development of the bay mussel *Mytilus trossilus* in various salinity conditions.

Elena E. Voronezhskaya[1], Vyacheslav A. Dyachuk[2], Olga V. Yurchenko[2], Evgeniya E. Vekhova[2], Marina Yu. Khabarova[1], Elizaveta G. Fofanova[1], Evgeny G. Ivashkin[1]

[1]Institute of Developmental Biology RAS, Vavilov str, 26, 119334 Moscow, Russia
[2]Institute of Marine Biology FEB RAS, Palchevsky str, 17, 690041 Vladivostok, Russia

Earlier, we have demonstrated that serotonin (5-HT) is produced by apical neurons of freshwater gastropod larvae and regulates developmental tempo; whereas FMRFamide (FMRFa) is involved in osmoregulation and its expression can be modulated by neomycin (NM). In the presented study we investigated how changes in salinity, addition of neomycin and ampicillin affected larval growth and expression of FMRFa and 5-HT within the larval nervous system of the marine mussel *Mytilus trossilus*. Larvae were cultivated in normal (33‰), low (8‰) and high (40‰) salinity from 40 to 120 hours after fertilization. At 120 hours, they were fixed for immunostaining. Immunolabeling in standard conditions and laser scanning microscopy were used to measure the levels of expression of FMRFa and 5-HT.

The lowest FMRFa level was observed in 8‰ while no difference was found between 33‰ and 40‰. Incubation in NM increased the level of FMRFa while ampicillin did not affect it. To the contrary, the level of 5-HT expression was the lowest in 40‰. The rate of survival was minimal in 33‰ and maximal in 8‰ with NM. Independent of addition of NM and ampicillin, the average veliger size was the biggest in the highest salinity. However, in 8‰ with NM, the shells of 5% of animals were two times bigger then in average, and their shape resembled that of pediveligers.

We suggest that FMRFa is involved in osmoregulation in marine bivalve larvae and NM modulates the level of FMRFa expression, whereas 5- HT regulates larval growth. Low salinity in combination with NM results in significant increase of survival rate.

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# Sexual dimorphism in the olfactory system of the solitary bee, *Eucera berlandi* (Hymenoptera: Apidae)

Martin Streinzer[1], Christina Kelber[2], Sarah Pfabigan[1], Christoph J. Kleineidam[2,3], Johannes Spaethe[1,2]

[1]Department of Evolutionary Biology, Faculty of Life Sciences, University of Vienna, Vienna, Austria

[2]Department of Behavioral Physiology and Sociobiology, Biozentrum, University of Würzburg, Würzburg, Germany

[3]Department of Biology, University of Konstanz, Konstanz, Germany

In the eusocial honeybee, *Apis mellifera*, males exhibit an extremely specialized olfactory system, which is used for mate detection. They possess enlarged antennae equipped with a 7-fold number of poreplate sensilla (PPs) compared to the worker antenna. In the antennal lobe (AL), 4 macro-glomeruli are present, which are assumed to process the sex pheromone compounds. The total number of glomeruli is reduced in males by a third, which presumably results in a reduced complexity of their olfactory perception.
Unlike most other bee species, which have a solitary lifestyle, the honeybee colony provides males with a social support system. Males are unable to feed from flowers. Instead, they are fed by workers in the hive where they also find rest and shelter. However, whether this social backup facilitated the evolution of the highly specialized male olfactory system is still under debate.
Here we present comparative data from the solitary bee, *Eucera berlandi* (Hymenoptera, Apidae). In this species, the sexual dimorphism of the antennae is more pronounced than in honeybees. We found striking differences between males and females, both in the in the periphery of the olfactory system as well as in the brain. A nearly 10-fold increase of PPs and a 3-fold increase in the number of olfactory receptor neurons (ORNs) suggest that the male antenna has a highly increased sensitivity. Further, 4 macro -glomeruli are present in the male AL, which account for more than one third of the total AL volume. The volume of these glomeruli are about 10 to 30 times greater than the median glomerulus volume and suggests that the olfactory system has an increased sensitivity for particular substances, probably sex pheromone compounds. However, in females no enlarged glomeruli were present. We found ca. 100 glomeruli in males and about 130 in females. The sex-specific differences indicate that there is a reduced complexity in the male olfactory system.
In contrast to honeybees, males of *E. berlandi* feed themselves, rest on flowers during the night and have a completely independent lifestyle from the females.
Based on our data, we conclude that the male olfactory system of *E. berlandi* and *A. mellifera* show similar adaptations for female detection. Therefore, the evolution of highly sensitive antennae, macroglomerular complexes and a reduced glomerular number are not restricted to the eusocial bees, but are also present in solitary bees.

# Small size, huge amazing complexity: the antennal lobe of *Camponotus* ants

Fernando J. Guerrieri[1], Juergen Rybak[1], Maik Althans[1], Marcus Stensmyr[1], Bill S. Hansson[1]

[1]Department of Evolutionary Neuroethology, Max Planck Institute for Chemical Ecology, Hans-Knoell-Strasse 8, 07745 Jena, Germany

Anatomical, digital atlases of insect antennal lobes have served as a tool for studies on morphology and physiology of the olfactory neural pathway (e.g. Honey bee, Galizia et al. 1999; Heliothis virescens moth, Loefaldli et al. 2010). Camponotus ants have very complex antennal lobes (about 400 glomeruli vs. 40 in Drosophila, 60 in moth species or 160 in the honey bee). Recognising the anatomical structure is the first step to unraveling the adaptive meaning of such complexity. We reconstructed the antennal lobe anatomy of the ant Camponotus aethiops mid-size workers. Ant brains were dissected and fixed in paraformaldehyde/glutaraldehyde, which enhanced the background contrast among glomeruli, neuronal tracts. Afterwards, image stacks of the antennal lobes were obtained by confocal microscopy. We observed that the structure was similar among individuals, presenting 392 glomeruli in 4 clusters. This allowed the identification of a group of 4 relatively large glomeruli placed directly by the entrance of the AL i.e. the antennal nerve root thus serve as landmark glomeruli. These glomeruli could be precisely identified because of their similar size and relative position relative to adjacent glomeruli and depth inside the antennal lobe. Ongoing work will allow identifying the glomerular clusters corresponding to sensory tracts connecting the antennal nerve with the antennal lobe, in order to achieve a complete anatomical atlas with regard to sensory projection represented herein.
Such an atlas will serve as a database and will be used be used to map functional features obtained by optical imaging, electrophysiology onto the antomical framework e..g. Namiki & Kanzaki 2008)

Galizia et al. (1999) Cell Tiss. 295: 383-394

Loefaldli et al. (2010) Front. Syst. Neursci. 4: 5

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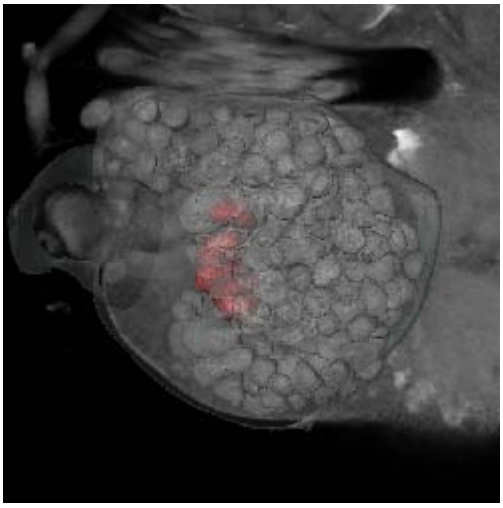

# Temperature dependent representation of a low volatile recruitment signal in the antennal lobe of *Apis mellifera*

Mirjam Rittmeyer[1], Andreas S. Brandstaetter[2,3], Christoph J. Kleineidam[1]

[1]University of Konstanz, Department of Biology, Universitätsstr. 10, D-78464 Konstanz, Germany
[2]University of Würzburg, Biozentrum, Zoologie II, Am Hubland, D-97074 Würzburg, Germany
[3]CNRS (UPR 9034), Evolution, Genome and Speciation, Avenue de la Terrasse, F-91198 Gif-sur-Yvette, France

Honeybees use multi-modal signals (vibration, tactile and olfactory signals) during the waggle dance to recruit other bees. The dancing bee releases several long-chain hydrocarbons (alkanes and alkenes) that have low volatility. She might increase the release of these compounds by increasing her body temperature during the waggle dance. This may allow followers to detect these hydrocarbons despite of their low volatility as air-borne odors, thus facilitating their recruitment in the dance. We tested this hypothesis by measuring the neuronal representations of three components and their mixtures at various temperatures. Information about odor stimuli is processed in the antennal lobes (AL), the primary olfactory centers of the bee brain, where different odors are represented as odor-specific patterns of glomerular activity. Using calcium-imaging, an in vivo optical recording technique, we found overlapping but still distinct activity patterns in the AL in response to the three long-chain hydrocarbons and their mixture when they were presented using a contact-less dummy heated to 35°C. Thus, all three components are odors that can be perceived by bees. For two components, an increase in temperature during stimulation resulted in a second activation of glomeruli after the initial response. Our results support the idea that dancing bees may communicate a recruitment odor during the dance by heating up their body temperature.

# Terrestrial adaptations of olfactory systems – A comparative neuroanatomical study of terrestrial and marine members of the Meiura

Jakob Krieger[1], Florian Seefluth[1], Renate E. Sandeman[2], David C. Sandeman[3], Bill S. Hansson[4], Steffen Harzsch[1]

[1]Ernst Moritz Arndt Universität Greifswald, Cytologie und Evolutionsbiologie, Johann Sebastian Bach-Straße 11/12, 17487 Greifswald, Germany

[2]Justus-Liebig-Universität Gießen, Fachbereich 06 Psychologie und Sportwissenschaft, Abteilung für Entwicklungspsychologie, Otto-Behaghel-Strasse 10F, D-35394 Giessen, Germany

[3]Wellesley College, 106 Central Street, Wellesley College, Department of Biological Sciences, Wellesley, MA 02481-8203, USA

[4]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Beutenberg Campus, Hans-Knöll-Str. 8, D-07745 Jena, Germany

The conquest of land probably occurred convergently at least five times within malacostracan Crustacea. In this context, we are interested in terrestrial adaptations of the sensory organs and the nervous system of different crustacean taxa in comparison to their nearest marine relatives. Our aim is to analyse the central olfactory pathway of crustaceans in order to gain a better understanding of the different adaptation strategies for conquering land within the Meiura. To that end, we examined histological sections of the brains of the marine *Carcinus maenas* (Brachyura), *Pagurus bernhardus* (Anomura) and the terrestrial *Gecarcoidea natalis* (Brachyura) and *Birgus latro* (Anomura) with immunohistochemical agents raised against synaptic proteins and allatostatin. In addition, we constructed a three dimensional model of the brain and selected brain structures of *B. latro*. Our results provide evidence for an extensive elaboration of the first antennae (antennules) and olfactory lobes in terrestrial anomurans in comparison to their marine relatives. In contrast, in terrestrial Brachyura the first antennae and olfactory lobes are highly reduced in size as compared to marine brachyurans.

In conclusion, our data suggest that terrestrial anomurans evolved an elaborated sense of aerial olfaction whereas in terrestrial brachyurans the deutocerebral olfactory pathway has been eroded away.

# The ants' ability to sense current temperature

Manuel Nagel[1], Christoph J. Kleineidam[1]

[1]University of Konstanz, Department of Biology, Universitätsstraße 10, 78464 Konstanz, Germany

How good are insects in measuring temperature? As insects are ectothermal animals with a small body mass, their body temperature is close to environmental conditions. Social insects protect themselves from a harsh environment by building elaborated nests with microclimatic control. Within the nest, they care for their virtually immobile brood by translocating it. The extremely fine-tuned brood-tending behavior of ants illustrates how social insects can cope with the challenge to provide suited conditions for the brood. In behavioral tests, temperature differences of only 0.2°C can be discriminated by ants (1). The thermal preference of brood-tending ants depends on the time of the day, in order to match rather the expected than the current temperature condition for the brood. In addition, the thermal preference of a brood-tending ant is influenced by its own thermal experience during pupal development (2).
It is unknown, how thermal-guided behaviors are controlled, and how the sensory system receives thermal information that allows such fine-tuned behavior.
Recently, we described in detail the sensilla coeloconica, which are capable of detecting transient temperatures (3,4). In this study, we describe the sensitivity of a receptor neuron associated with the sensilla coelocapitula that is able to measure the current temperature.
Our results, based on extracellular recordings of the sensory neuron, show that an increase of only 0.16°C causes a decrease in neuronal activity of 10%. These neurons are comparable to a thermometer because they do not adapt to the current temperature. Traditionally, neurons with such a response characteristic are termed cold-neurons. We currently investigate whether the dose-response curves are shifted during the course of the day (circadian modulation). Our hypothesis is that the before mentioned behaviors are reflected in changes in sensitivity of the receptor neurons.

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# The evolution of olfaction in hermit crabs

Katrin C. Groh[1], Marcus C. Stensmyr[1], Ewald Große-Wilde[1], Bill S. Hansson[1]

[1]Max Planck Institute of Chemical Ecology, Evolutionary Neuroethology, Hans-Knöll-Straße 8, 07745 Jena, Germany

Chemodetection is ancient and present from the oldest organisms like bacteria and fungi up to mammals. Among this, olfactory and gustatory senses are central for the detection of nutrients, conspecifics and dangers, and their characteristics depend on the stimuli patterns of the inhabited environment. Arthropods and especially insects show specific adaptations due to their dependence on perceiving chemical cues. This adaptability can be retraced to the early stages of arthropod evolution. Based on fossil records it can be assumed that the hexapods split from an arthropod progenitor and left the marine habitat in the very late Silurian 416 million years ago. Independently from the insects, at least five crustacean lineages succeeded in the transition from water to land. This change leads to an adaptation shifting the range of chemical stimuli solved in water to air-borne substances. Despite the long time of independent development, insects and crustaceans show a parallel evolution, sharing a connatural organization of olfactory organs and brain architecture. Considering a common aquatic ancestor and the morphological similarities it is likely to assume a similar but independently developed way to detect air-borne volatiles. To gain insight into the evolution of the olfactory sense we investigated the antennal transcriptomes of two hermit crabs, the land -living Coenobita clypeatus and the marine Pagurus bernhardus by paying special attention on candidates of chemosensory genes.

# The function of MsexOR-2 in pheromone transduction of the hawkmoth *Manduca sexta*

Nico W. Funk[1], Ewald Große-Wilde[2], Bill S. Hansson[2], Dieter Wicher[2], Monika Stengl[1]

[1]University of Kassel, FB 10 Mathematics and Natural Sciences, Animal Physiology, Heinrich-Plett-Str. 40, 34132 Kassel, Germany
[2]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Hans-Knöll-Str. 8, 07745 Jena, Germany

*Manduca sexta* male moths detect minute traces of female sex pheromone blend filaments with remarkable sensitivity more than one km downwind of the calling female. On the males antennae long sensilla trichodea are innervated each by one pair of olfactory receptor neurons (ORNs) which express pheromone receptors in the outer dendritic membrane. One ORN of each pair responds to the main component of the pheromone blend E10, Z12 hexadecadienal (bombykal). Weak pheromone stimuli activated a phospholipase Cß dependent signal transduction cascade leading to transient $Ca^{2+}$-influx. This rapid $Ca^{2+}$-influx triggered a cascade of $Ca^{2+}$-dependent ion channels which resulted in de- or hyperpolarizing receptor potentials. Strong, minute-long pheromone stimuli elevated intracellular cGMP levels which decreased the sensitivity and speed of pheromone pulse resolution. In contrast, octopamine-dependent cAMP concentration rises increased the amplitude of the sensillar potential as well as the spontaneous activity of the ORNs and appeared to be involved in disadaptation or sensitization of the pheromone transduction cascade.
Recently an olfactory co-receptor was found, which is highly conserved among different insect species. This co-receptor was termed OR83b in the vinegar fly *Drosophila melanogaster* and MsexOR-2 in *M. sexta*. For *M. sexta* this co-receptor was shown to co-localize with pheromone receptors in sensilla trichodea on the male antenna. In a heterologous expression system OR83b acted as an ion channel. Odorant application to the co-expressed OR and OR83b resulted in a faster, shorter ionotropic current and a slower, prolonged current which appeared to be gated metabotropically via activation of an adenylyl cyclase.
In this study we analyzed the function of MsexOR-2 in a heterologous expression system. HEK293 cells co-expressing MsexOR-2 and the putative bombykal receptor MsexOR-1 were used for patch-clamp and calcium imaging experiments. Stimulation of co-transfected cells with $10^{-12}$ – $10^{-6}$ M bombykal resulted in a non-specific cation conductance in approximately 10 % and calcium concentration rises in approximately 50 % of the experiments. Delays as well as duration of the observed current increases were in a range of several minutes, suggesting metabotropically activated currents. Calcium concentration rises had different kinetics in different cells. We observed slow, continuous rises in the intracellular calcium concentration over the course of several minutes in some cells and rapid, high peaks with durations of several seconds in others. In some cases the peaks were superimposed on the slower rises within the same cells. The delays of the calcium concentration rises were in a range of seconds to minutes. Furthermore, we observed similar increases in calcium concentrations after stimulation with membrane permeable cyclic nucleotide analogs in about 50 % of the experiments. Currently we are testing whether MsexOR-1 alone or MsexOR-2 alone promotes odor- or cAMP/cGMP-dependent responses.
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# The Grueneberg ganglion – a dual sensory organ?

Katharina Mamasuew[1], Nina Hofmann[1], Heinz Breer[1], Joerg Fleischer[1]

University of Hohenheim, Institute of Physiology, August-von-Hartmannstrasse 3, 70599 Stuttgart, Germany[1]

The Grueneberg ganglion (GG) is a cluster of neuronal cells in the anterior nasal region which project axonal processes to the olfactory bulb and express the olfactory marker protein (OMP). Moreover, GG neurons are endowed with distinct olfactory receptors, indicating that the GG is not a homogenous population of neurons and that it may operate as a chemosensory subsystem. In search of stimuli activating the GG, we have found that a large subset of murine GG neurons is activated by cool ambient temperatures. These coolness-induced responses were particularly intense in neonates and restricted to a subset of GG neurons.
Since a line of evidences suggests that the GG serves a chemosensory function, it was assessed for responsiveness to an array of odorants. These approaches revealed that GG neurons were activated by a limited number of chemically related odorous substances, most notably dimethylpyrazine. Responses induced by these odorants were dose-dependent and confined to subsets of GG neurons. The odor-evoked responses were detectable in early postnatal stages but not in the GG of juvenile or adult mice. However, responsiveness to GG-activating odorous substances was not restricted to the GG but also occurred in the main olfactory epithelium.
The observation that GG neurons are activated by cool temperatures and given odorants indicates that these cells might function as dual sensors. In fact, cool temperatures were found to enhance odor-evoked responses of GG neurons. These findings support the concept that the GG of neonatal mice operates as a dual sensory organ which is activated by both odorous compounds and cool ambient temperatures.
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# The molecular and phenotypic characterization of the *TßH* gene in *Drosophila melanogaster*

Manuela Ruppert[1], Henrike Scholz[1]

[1]Institute of Animal Physiology, AG Scholz, Zülpicher Straße 47b, 50674 Köln, Germany

Octopamine is a widely used neurotransmitter, neuromodulator and neurohormone in invertebrates. The Tyramine-ß-hydroxylase (TßH) enzyme is the key limiting factor in the synthesis of Octopamine. In *Drosophila melanogaster* the *TßH* gene was initially characterized and $TßH^{M18}$ mutants lacking measurable amounts of Octopamine are female sterile (Monastirioti *et al.,* 1996). Previously we have shown that mutants have a reduced ethanol tolerance and initial olfactory startle response (Scholz *et al.,* 2000; Scholz, 2005) suggesting that octopaminergic neurotransmitter system is involved in the regulation of ethanol tolerance and olfactory startle response. We molecularly characterized the *TßH* gene and figured out that the gene encodes at least two transcripts. In addition we analyzed the nature of the TßH mutation and found that a coding exon is deleted. In $TßH^{M18}$ mutants the transcription level is reduced as analyzed by quantitative Real-Time PCR and also the protein levels as analyzed by western blot analysis. However transcript and protein are still detectable. The transcript levels can be induced by heat shock and this heat shock expression might rescue ethanol tolerance defect observed in adult $TßH^{M18}$ mutant flies. Using different GAL4 driver lines we could show that neurons mediating ethanol tolerance and preference can be separated suggesting that ethanol preference and tolerance are not genetically linked.

# The molecular basis of sex pheromone detection in *Heliothis virescens*

Pablo Pregitzer[1], Danuta Schymura[1], Heinz Breer[1], Jürgen Krieger[1]

[1]University of Hohenheim, Institute of Physiology, Garbenstrasse 30, 70599 Stuttgart, Germany

Many insects use complex sex pheromone blends for mate attraction. The remarkable ability of male moths to accurately detect lowest concentrations of the female-released pheromone components is based on the cellular and molecular equipment of specifically tuned olfactory "hairs" on the antennae. These sensilla trichodea house the dendrites of olfactory receptor neurons (ORNs) each endowed with distinct pheromone receptors in their membranes. The dendrites are bathed in aqueous sensillum lymph containing high concentrations of specific pheromone binding proteins (PBPs), supposed to capture the hydrophobic pheromones from the air and to mediate the transfer towards the pheromone receptors. We have identified a group of relatively conserved pheromone receptors of *Heliothis virescens*, which are expressed in ORNs of pheromone-responsive sensilla. In functional calcium imaging studies with receptor expressing cell lines it was found that the receptor type HR13 mediates a response to the major component of the female sex-pheromone, Z11-hexadecenal. Experiments employing PBPs indicated that HR13 interplays with PBP2, but not with PBP1, in the detection of Z11-hexadecenal and that PBP2 may contribute to the sensitivity of the pheromone detection system. In addition to pheromone receptors and PBPs, a possible role of "sensory neuron membrane proteins" (SNMPs) and the ubiquitous olfactory co-receptor type OR83b in pheromone detection has been suggested. Two-colour fluorescence *in situ* hybridization experiments with specific probes on antennal sections revealed that SNMP1 is co-expressed with HR13 in one of the generally two ORNs of a single sensillum. The HR13- and SNMP1-expressing ORNs were found to also express the receptor type HR2 (=OR83b) and were surrounded by support cells expressing PBP2. Together our results suggest that PBP2 and HR13, as well as HR2 and SNMP1 are involved in the detection of the major component of the female sex-pheromone by the *H. virescens* males. In ongoing work attempts are made to reconstitute the identified elements in heterologous expression systems and to analyze their specific functions in the pheromone detection process.

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# The OR37 subfamily: establishment of the clustered expression pattern

Jörg Strotmann[1], Andrea Bader[1], Verena Bautze[1], Desiree Haid[1], Heinz Breer[1]

[1]University of Hohenheim, Physiology, Garbenstrasse 30, 70593 Stuttgart, Germany

Odorant receptors (ORs) are encoded by the largest gene family in vertebrate genomes, comprising of more than 1000 members. Each olfactory sensory neuron (OSN) in the olfactory epithelium (OE) selectively expresses one particular receptor gene from this large repertoire; moreover, only one of the two alleles is chosen per cell. OSNs which express a distinct OR gene are usually broadly dispersed throughout the OE; in contrast, cells expressing a member of the OR37 subfamily show a unique pattern: they are located exclusively in a small central patch of the OE. The regulatory mechanisms which govern OR gene choice of individual OSNs in a topographically restricted manner are still largely elusive. To obtain more insight into the underlying principles we have employed a transgenic approach in mice that allowed to permanently label all cells that selected a defined OR gene for expression. For this purpose a knock-in mouse line was generated in which expression of one member of the OR37 subfamily (OR37C) leads to the co-expression of Cre-recombinase (OR37C-IRES-Cre). By crossing this line to Cre-reporter mouse lines, cells could be visualized that transcribed OR37C at any time during development. As expected, labelled OSNs were found in the central patch which is typical for OR37 expression. Surprisingly, however, numerous additional OSNs were found which were broadly dispersed throughout the OE. Using in situ hybridization, mRNA for OR37C could only be detected in those cells located in the typical 'OR37 patch', suggesting that all ectopic OSNs had ceased OR37C expression. The question whether these cells may undergo premature apoptosis was addressed by immunohistochemical analysis for active caspase-3; none of them, however, expressed this pro-apoptotic marker. A close examination of the ectopically positioned OSNs revealed that they all extended an axon towards the olfactory bulb (OB), and indeed many glomeruli could be detected which contained a few labelled fibers. The location of these glomeruli in the medial and lateral domains of the bulb indicated that these represented glomeruli that receive input from OSN populations expressing other ORs than OR37C. Altogether, these data indicate that OSNs which initially express OR37C outside the typical patch do not continue, but switch to the expression of a different OR gene, suggesting the involvement of a feedback mechanism downstream of gene choice that restricts OR37C expression to the central patch.

# The role of NKCC1 in chloride homeostasis in trigeminal sensory neurons of mice

Debbie Radtke[1], Nicole Schöbel[1], Jennifer Spehr[2], Hanns Hatt[1]

[1]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstr. 150, 44780 Bochum, Germany [2]RWTH-Aachen, Institut für Biologie II, Aachen, Germany

The trigeminal system is known to play an important role in chemo- and thermosensation as well as the perception of pain. Recent studies have shown a connection between intracellular chloride accumulation and pain perception by neurons of the dorsal root ganglia (DRG), but little is known about the chloride homeostasis in neurons of the trigeminal ganglia (TG).

The intracellular chloride concentration is mainly controlled by cation-coupled chloride cotransporters. The $Na^+K^+2Cl^-$ cotransporter NKCC1 accumulates $Cl^-$ intracellularly and is highly expressed in embryonic neurons of the central nervous system. During late embryonic to early postnatal development NKCC1 is downregulated accompanied by an upregulation of $Cl^-$-extruding cotransporters. Due to this "chloride switch" opening of $Cl^-$ conductances leads to hyperpolarization of adult central neurons. However, some peripheral neurons like olfactory sensory neurons and neurons of the DRG maintain high intracellular $Cl^-$ levels even in adulthood resulting in cellular depolarization after opening of $Cl^-$ conductances.

Here, we show that isolated neurons of wild type (WT) mice display robust $Ca^{2+}$ transients upon GABA stimulation in $Ca^{2+}$-imaging experiments. In neurons of NKCC1$^{-/-}$ mice, however, these responses are dramatically diminished with respect to the number of responsive cells and signal amplitude.

Furthermore, we used the chloride imaging technique to investigate changes of intracellular $Cl^-$ levels upon GABA stimulation of TG neurons of WT and NKCC1$^{-/-}$ mice. In WT mice, 82% of the neurons showed a chloride efflux upon GABA stimulation, whereas in neurons of NKCC1$^{-/-}$ mice this number is reduced to 33%. Only 4% of the WT neurons displayed an inlux of chloride compared to 28% of the neurons of NKCC1$^{-/-}$ mice.

Additionally, we determined the intracellular $Cl^-$ concentration of neurons of WT and NKCC1$^{-/-}$ mice using the double-ionophor technique. NKCC1$^{-/-}$ mice neurons showed a significantly lower $[Cl^-]_i$ than neurons of WT mice.

We conclude that NKCC1 is the main $Cl^-$ accumulating transporter in TG neurons. Further investigations aim at clarifying the role of NKCC1 in trigeminal pain perception and inflammatory processes.

# The voltage-gated sodium channel Nav1.7 is essential for odour perception in mice

Jan Weiss[1], Martina Pyrski[1], Eric Jacobi[1], Bernd Bufe[1], Trese Leinders-Zufall[1], John N. Wood[2], Frank Zufall[1]

[1]Universität des Saarlandes, Physiologisches Institut, D-66424 Homburg, Germany

[2]Molecular Nociception Group, Department of Biology, University College London, London WC1E 6BT, UK

Loss of function of the the gene *SCN9A*, encoding the a-subunit of the voltage-gated sodium channel Nav1.7, causes congenital inability to experience pain in humans (1). Several lines of evidence point to the possibility that Nav1.7 also plays a critical role for odour perception. To investigate this question, we generated mice with a tissue-specific deletion of Nav1.7 in all olfactory sensory neurons (OSNs) and combined gene expression studies with cellular electrophysiological and behavioural analyses. We found that Nav1.7 occupies a critical location in the olfactory pathway, namely in the olfactory nerves and glomeruli, presynaptically to the first synapse in the olfactory pathway. To examine whether presynaptic activity of Nav1.7 underlies transmitter release in the olfactory glomerulus, we prepared acute olfactory bulb tissue slices and combined focal electric stimulation of the olfactory nerve layer with whole-cell patch-clamp recording and intracellular staining of visually identified mitral/tufted (M/T) cells. In the absence of Nav1.7, postsynaptic responses in M/T cells and juxtaglomerular interneurons could not be elicited. Consistent with these findings, several behavioural tests showed that tissue specific, Nav1.7-deficient mice are anosmic. Thus, the presence of Nav1.7 in OSN axons is an essential and non-redundant requirement for information transfer from OSN terminals to neurons in the olfactory bulb. Our work generates new insight into normal and pathologic function of the olfactory system. The Nav1.7 sodium channel constitutes the first genetic link between pain sensation and odour perception.

(1) Cox et al., An *SCN9A* channelopathy causes congenital inability to experience pain. Nature 444, 894-898 (2006).

# Time-dependent differences in the pheromone transduction of the hawkmoth *Manduca sexta*

Christian Flecke[1], Andreas Nolte[1], Petra Gawalek[1], Monika Stengl[1]

[1]Universität Kassel, FB 10, Biologie, Tierphysiologie, Heinrich-Plett-Strasse 40, 34132 Kassel, Germany

The male responsiveness to pheromone expresses circadian rhythms in many moth species. Evidence is increasing that rhythms in pheromone sensitivity are not only present in higher order neuropils like the antennal lobe but exist also at the level of peripheral olfactory receptor neurons (ORNs) (Saifullah and Page 2009, J Biol Rhyhtms 24:144-52). Furthermore, previous studies suggest that the stress hormone octopamine (OA) plays a major role in the regulation of rhythms in pheromone responsiveness and also in the sensitivity of ORNs. To search for time-dependent differences in pheromone sensitivity extracellular recordings from single pheromone-sensitive trichoid sensilla (tip recordings) of nocturnal male *Manduca sexta* were performed during three Zeitgebertimes (ZTs, ZT 22-1, 1-4 and 8-11, ZT 0 = lights on; ZT 17 = lights off). Using a non-adapting stimulation protocol the sensilla were stimulated (duration 50 ms) with 1 or 10 µg of bombykal (BAL) every 5 min for 180 min of the recording duration. To test time-dependent effects of biogenic amines OA (1 mmol $l^{-1}$) was applied by perfusion of the sensillar lymph. In addition, to elucidate circadian rhythms in pheromone sensitivity long-term tip recordings with a minimum duration of 24 hours were performed under normal light-dark cycle (L:D 17:7) conditions and were started during the end of the photophase (ZT 13-14). The sensilla were stimulated with 1 µg BAL with an inter-stimulus interval of 20 minutes. The responses to BAL stimulation and the spontaneous action potential (AP) activity of the ORNs were analyzed. In the comparison of the three different ZTs it is apparent that during the scotophase at ZT 22-1 the responses are much stronger than at ZT 8-11. The ORNs adapt with beginning of the photophase on the level of the sensillar potential amplitude and in the AP response. Furthermore, the mean spontaneous AP frequency declines with beginning of the photophase caused by decreases in the number of bursts and in the number of single APs. In contrast, preliminary results indicate that in the 24-hour recordings which started during the photophase no circadian rhythms in the strength of BAL responses are present. These results are in accordance with a study in *Drosophila melanogaster*, where circadian rhythms were only found in the amplitude of spontaneous APs but not in responses to odor stimulation (Krishnan et al. 2008, Curr Biol 18:803-07). However, for *M. sexta* and *Trichoplusi ni* circadian rhythms in the concentration of OA in the hemolymph and brain were found, which correlated with rhythms in pheromone responsiveness (Lehman 1990 Abstr Soc Neurosci 16:1334; Linn Jr et al. 1996, J Insect Physiol 42:881-91). Furthermore, the time-dependent adaptations in ORNs were antagonized by the application of OA during the photophase and it was shown that OA injected into the hemolymph sensitizes ORNs of moths (Pophof 2000, J Comp Physiol A 186:307-13). The immobilization of the moth during the recordings could have prevented locomotion-dependent increases in the OA hemolymph concentration with beginning of the scotophase and due to this an OA-dependent increase in the pheromone sensitivity. We hypothesize that time-dependent changes in the OA hemolymph concentration are necessary for the expression of circadian rhythms in pheromone sensitivity. Current experiments in which long-term recordings begin at the middle of the scotophase test this hypothesis. [Supported by DFG grant STE531/13-1,2]

# Towards a physico-chemical description of vertebrate olfactory receptive space

Jan Soelter[1], Jan Schumacher[2], Hartwig Spors[2], Michael Schmuker[1,3]

[1]Freie Universität Berlin , Neuroinformatics & Theoretical Neuroscience, Königin-Luise-Str. 1-3 , 14195 Berlin, Germany
[2] Department of Molecular Neurogenetics, Max Planck Institute of Biophysics, Frankfurt, Germany
[3] Bernstein Center for Computational Neuroscience Berlin, Berlin,Germany

The olfactory system extracts information from chemical cues in our environment. In contrast to other sensory systems, the receptive space of the olfactory system lacks a parameterized description of stimuli. It is also unclear whether the target of olfactory receptor neurons in mammals, the olfactory bulb, displays a chemotopic organization, such that chemical properties are mapped to certain areas of the olfactory bulb.

In a first approach to address these questions we characterize the physico-chemical properties of effective stimuli to specific GFP -labeled glomeruli in the mouse. Due to the genetic label, we can identify the glomeruli across animals, and we measure their response to odor stimulation using optical signal imaging. We perform virtual and biological screening in an iterative fashion in order to fully characterize the physico-chemical "receptive field" of the glomerulus under investigation.

In biological screening, we employ robot-assisted, computer-controlled odor delivery to obtain a large number of odor response measurements. In virtual screening, we obtain an activation model using pattern recognition algorithms, which allows us to predict the glomerular response to untested odorants based on their physico-chemical properties. We test these predictions in the next iteration of biological screening. We continue this iterative screening scheme until our model shows sufficient prediction accuracy. After two iterations, our predictive models start to converge towards a set of chemical properties that is suited to predict glomerular responses.

We are currently extending our approach to predict the physico -chemical receptive fields of non-labeled glomeruli.

# Transduction components in Grueneberg ganglion neurons

Joerg Fleischer[1], Katharina Mamasuew[1], Nina Hofmann[1], Verena Kretschmann[1], Ruey-Bing Yang[2], Martin Biel[3], Heinz Breer[1]

[1]Institute of Physiology, University of Hohenheim, Stuttgart, Germany
[2]Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
[3]Department Pharmazie, Zentrum für Pharmaforschung, Ludwig-Maximilians-Universität, Munich, Germany

The detection of odors and pheromones in mammals is mediated by chemosensory neurons of the main olfactory epithelium (MOE) and the vomeronasal organ (VNO) which generally express the olfactory marker protein (OMP). We have found that OMP is also expressed in cells of the so-called Grueneberg ganglion (GG), a cluster of neuronal cells in the vestibule of the anterior nasal cavity; this finding suggests an olfactory function of the GG. Chemosensory responsiveness of nasal neurons is based on the expression of olfactory signaling elements including odorant and vomeronasal receptors. To scrutinize the concept that GG neurons may serve a chemosensory function, they were subjected to molecular phenotyping. These approaches revealed that a distinct vomeronasal receptor type designated as V2r83 was expressed in the majority of murine GG neurons. V2r83-negative GG neurons were observed to express members of the trace amine-associated receptor (TAAR) family of olfactory receptors.
V2r83-positive but not TAAR-expressing GG neurons were found to be activated by cool ambient temperatures. Attempts to unravel the signaling mechanisms underlying coolness-induced GG responses revealed that the coolness-sensitive TRP ion channel TRPM8 was not expressed in the GG. Instead, coolness-responding GG neurons were endowed with elements of a cGMP-mediated cascade, including the transmembrane guanylyl cyclase subtype GC-G, the phosphodiesterase PDE2A and the cyclic nucleotide-gated ion channel CNGA3. Experiments with CNGA3-deficient mice demonstrated that this ion channel is required for coolness-evoked responses in the GG, indicating that a cGMP pathway is indeed involved in responsiveness to cool ambient temperatures.
Expression of chemosensory transduction elements such as olfactory receptors suggests that the GG, in addition to coolness, might also be activated by odorous cues. In fact, we have recently identified a limited set of defined odorants which induce responses in GG neurons. Responsiveness to these odorous substances was confined to the GC-G-/CNGA3-expressing subpopulation of GG neurons. Subsequent experiments showed that GC-G and CNGA3 are important for odor-induced signaling in these cells.
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# Transient potassium currents in identified olfactory interneurons of the cockroach antennal lobe

Sabine Schleicher[1], Cathleen Rotte[1], Lars Paeger[1], Peter Kloppenburg[1]

[1]Biocenter Cologne, Institute for Zoology, Center for Molecular Medicine Cologne (CMMC), and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, Zülpicher Strasse 47b, 50674 Cologne, Germany

Local interneurons (LNs) mediate complex inhibitory and excitatory interactions between the glomerular pathways, ultimately restructuring the olfactory information in the antennal lobe and shaping the tuning profile of projection neurons. In *Periplaneta americana*, we found three types of LNs with fundamentally different intrinsic firing properties, implying that these neurons serve distinct functions in the olfactory system. Type I LNs fire $Na^+$-driven action potentials in response to odour stimulation. Type II LNs lack voltage dependent transient $Na^+$ currents and accordingly could not trigger synaptic release by action potentials. By their morphology and physiological properties, we further differentiated two subtypes of non-spiking LNs, namely LN IIa and LN IIb. Considering that the electrophysiological properties are determined by cell type specific ion channel composition, our long term goal is to elucidate the distinct sets of ionic currents of each cell type. Recent studies revealed that in the non-spiking LN II, consistent with graded transmitter release, the voltage dependence for activation of $I_{Ca}$ was shifted to more hyperpolarized membrane potentials. Here we have started to characterize fast inactivating voltage gated potassium currents ($I_A$). The voltage dependence for half maximal activation and inactivation, and the inactivation kinetics of $I_A$ during a voltage step differed significantly between the distinct cell types. Whereas the $I_A$ in LN type I and IIa was fast inactivating, it displayed very slow inactivation kinetics in LN IIb. Despite obvious differences in the waveform, amplitude and voltage dependence, $I_A$ showed similar concentration-response relations in all cell types. With respect to the strong differences in the biophysical properties of $I_A$ in the LN subtypes, which are probably due to the endowment of the cells with different channel types, we aim to pharmacologically dissect $I_A$, ideally to reveal the molecular identity of the underlying channel.

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# TRANSITION FROM MARINE TO TERRESTRIAL ECOLOGIES: CHANGES IN OLFACTORY AND TRITOCEREBRAL NEUROPILS IN LAND-LIVING ISOPODS

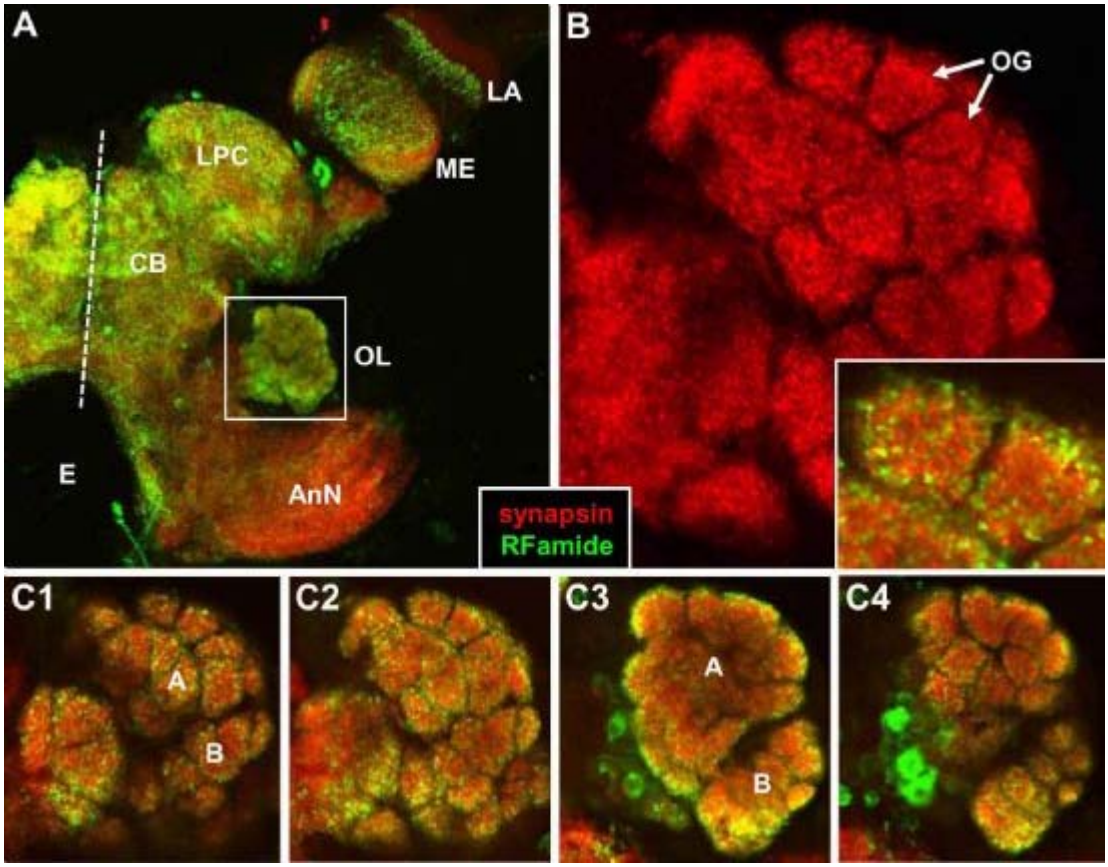
Steffen Harzsch[1], Verena Rieger[1], Jakob Krieger[1], Nicholas Strausfeld[2], Bill S. Hansson[3]

[1]University of Greifswald, Zoological Institute and Museum, Cytology and Evolutionary Biology, Soldmannstrasse 23, 17498 Greifswald, Germany

[2]Department of Neuroscience and Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA

[3]Max Planck Institute for Chemical Ecology, Department of Evolutionary Neuroethology, Beutenberg Campus, Hans-Knöll-Str. 8, D-07745 Jena, Germany

In addition to the ancestors of insects, representatives of five lineages of crustaceans have colonized land. Whereas insects have evolved sensilla that are specialized to allow the detection of airborne odors and have evolved olfactory sensory neurons that recognize specific airborne ligands, there is so far little evidence for convergent evolution in terrestrial crustaceans. Studies of terrestrial anomurans ("hermit crabs") show that they possess olfactory sensilla, the aesthetascs, that differ little from those of their marine relatives. Here we enquire whether terrestrial Isopoda have evolved a solution to the problem of detecting far-field airborne chemicals. The figure shows the olfactory lobes of the marine isopod crustacean Idotea baltica visualized by immunolocalization of synaptic proteins (confocal laser-scan microscopy) and FMRFamide-related neuropeptides in brain whole-mounts. Despite the presence of a well developed central olfactory pathway in marine isopods, we show that conquest of land of Isopoda has been accompanied by a radical diminution of their first antennae and a concomitant loss of their deutocerebral olfactory lobes and olfactory computational networks. In terrestrial isopods, but not their marine cousins, tritocerebral neuropils serving the second antenna have evolved radical modifications. These include a complete loss of the stomatopod/malacostracan pattern of somatotopic representation, the evolution in some species of amorphous lobes and in others lobes equipped with microglomeruli, and yet in others the evolution of partitioned neuropils that suggest modality -specific segregation of second antenna inputs. Evidence suggests that Isopoda have evolved, and are in the process of evolving, several novel solutions to chemical perception on land and in air. Acknowledgements: This study was funded by the Max Planck Society and by the grant I/84 176 from the Volkswagenstiftung.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# Tyramine ß-Hydroxylase is required for ethanol preference in *Drosophila melanogaster*

Andrea Schneider[1], Marvin Vollbach[1], Henrike Scholz[1]

[1]Institute of Animal Physiology , AG Scholz, Zülpicher Str. 47b, 50674 Köln, Germany

Preference guides an animal to a food source or mating partner. Ethanol preference attracts adult flies of *Drosophila melanogaster* to move towards ethanol-containing food sources, e.g. fermenting fruits. We are interested in identifying the neuronal basis and mechanisms underlying ethanol preference of *Drosophila melanogaster*. Adult flies show dose dependent preference to ethanol containing food sources. Flies are attracted to low ethanol concentrations present in nature, however high concentrations are aversive (Ogueta *et al*., 2010). By using different olfactory mutants we can show that ethanol preference is caused by an olfactory stimulus. Previously in collaboration with the Heisenberg lab we have found that the octopaminergic neurotransmitter system is important for positive associative olfactory learning (Schwaerzel *et al*., 2003). Here, we show that the octopaminergic neurotransmitter system plays an important role in odor-evoked ethanol preference. The *Tyramine-ß-hydroxylase (TßH)* mutant $TßH^{nM18}$ lacking the neurotransmitter Octopamine fails to develop ethanol preference. The loss of preference can be restored by expressing *TßH* in a subset of around 20 TßH positive neurons. Consistent with the role of these neurons in preference neuronal silencing in the adult fly brain completely abolishes ethanol preference. Furthermore the activation of these neurons can induce preference. In summary, we show that TßH is necessary and sufficient to mediate ethanol preference in adult flies and that ethanol preference bases on neuronal adaptation of modulatory neurons.

# Variation in the human olfactory subgenome and its implications for olfactory perception

Jonas Kuklan[1], Caroline Flegel[1], Kaveh Baghaei[1], Ivonne Wallrabenstein[1], Martin Grobosch[1], Marilen Demond[1], Günter Gisselmann[1], Hanns Hatt[1]

[1]Ruhr-Universität Bochum, Lehrstuhl für Zellphysiologie, Universitätsstr. 150, Gebäude ND4/133, 44780 Bochum, Germany

The olfactory receptors (ORs) are a family of G-Protein coupled receptors that provide the molecular basis for the detection of volatile odorant molecules by the central nervous system. In humans, the OR gene family comprises about 400 functional genes and about 600 non-functional pseudogenes. Furthermore, the OR gene family shows a high degree of genetic variability between individuals. Common single nucleotide polymorphisms (SNPs) and copy number variants (CNVs) lead to specific patterns of functional and non-functional OR genes in each individual subject, resulting in "different noses for different people" (Menashe et al., Nat Genet, 2003).
At the same time, it has long been known that people differ in their ability to perceive certain odorants. In the most striking cases, some people completely lack the ability to detect certain odorants, although their sense of smell functions normally in general. This phenomenon is known as specific anosmia and in some cases has been shown to have a genetic basis.
We used a range of methods from molecular biology to psychophysical studies to investigate the effects of genetic variance in the human olfactory subgenome on the physiological function of the olfactory system. We obtained genomic DNA samples from subjects with a specific anosmia. We then used massively parallel sequencing, DNA microarray and real time quantitative PCR techniques to identify genetic polymorphisms in the olfactory subgenome that show an association to these specific anosmias. Experiments were performed on pools of DNA samples and we employed a variety of bioinformatic tools to analyze the resulting complex datasets. With this combination of methods from molecular biology and bioinformatics, we tested the hypothesis that specific anosmias are correlated to the occurrence of genetic polymorphisms in the olfactory subgenome.
In a second set of experiments, we sought to characterize the effects of SNPs inside the coding regions of OR genes on the functionality of that OR protein in vitro. We established optimized protocols for the heterologous expression of ORs in HEK293 cells and employed calcium imaging methods to assess the ORs' functionality. With this approach, we found evidence that naturally occurring SNPs can reduce the ligand affinity of the affected OR protein. We were also able to identify putative ligands for a number of especially interesting ORs, those coded for by segregating pseudogenes (SPGs). SPGs are functional genes in most people, but SNPs or frameshift mutations disrupt their coding regions and render them dysfunctional in parts of the population. Accordingly, SPGs are thought to be prime candidates for genes which underlie the occurrence of specific anosmias.
In addition to our investigations of the ORs, we looked at a second family of G-Protein coupled receptors expressed in the olfactory epithelium, the trace-amine associated receptors (TAARs). We characterized the ligand-binding qualities of these receptors by expressing them heterologously in Xenopus laevis oocytes. We focused our investigations on the response of TAARs to volatile amines for which specific anosmias are known and found that these amines can activate TAARs, suggesting that TAARs may play a role in specific anosmias for these compounds.

# Visualization of taste receptor-expressing cells in the central nervous system

Anja Voigt[1,2], Ellen Schöley-Pohl[1], Sandra Hübner[1], Jonas Töle[1], Ulrich Boehm[2], and Wolfgang Meyerhof[1]

[1]Department of Molecular Genetics, German Institute of Human Nutrition Potsdam-Rehbrücke, Germany

[2]Institute for Neural Signal Transduction, Center for Molecular Neurobiology Hamburg, Germany

Taste buds are specialized morphological structures in the oral cavity that detect chemical signals and transmit this information to the brain. Within taste buds, distinct subpopulations of cells that express different types of taste receptors mediate detection of the five taste modalities sweet, bitter, umami, sour, and salty. The expression, distribution and function of taste receptors in gustatory tissues have been extensively studied. However, taste receptors are also expressed in non-gustatory tissues including the gut, testis, pancreas, thymus, nasal respiratory epithelium, and airways. Recently, taste receptor mRNA has also been detected in the brain. However, due to low expression levels of taste receptor genes in nervous tissue their detection remains challenging. Furthermore, the individual taste receptor expressing cells in the brain have neither been identified nor characterized.
To investigate taste receptor expression in the central nervous system with increased sensitivity we first generated gene-targeted mice with modified taste receptor loci. These animals express Cre recombinase in the bitter taste receptor *Tas2r131* locus or in the locus for the specific Tas1r1 subunit of the umami taste receptor. To visualize Tas2r131 and Tas1r1 expressing cells, we bred Tas2r131$^{Cre}$-mice and Tas1r1$^{Cre}$-mice with Rosa26-reporter mice expressing a fluorescent protein after Cre-mediated activation. Fluorescent cells were easily identified in brain sections of offspring animals with the activated reporter. Littermates with inactive reporter did not display fluorescent cells. Interestingly, Tas2r131 and Tas1r1 expressing cells were found in brain regions well known to contain neurons responding to gustatory stimulation, such as the nucleus of the solitary tract and the parabrachial nucleus. Furthermore, fluorescent cells were also detected in various other brain areas including the reticular formation, hypothalamus, hippocampus, periaquaeductal gray, colliculus, basal ganglia, and cerebral cortex. In the hypothalamus, fluorescent cells were detected in the dorsomedial and ventromedial hypothalamic nucleus, as well as in the lateral hypothalamic area, i.e., regions crucially involved in feeding behavior and energy homeostasis. These findings suggest a role for taste receptors in regulating energy balance.

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# Prolonged odor information in the antennal lobe of *Drosophila melanogaster*

Alja Lüdke[1], C. Giovanni Galizia[1], Paul Szyszka[1]

[1]University of Konstanz, Department of Biology - Neurobiology, 78457 Konstanz, Germany

Animals can react to sensory information either instantaneously or with a certain delay after the end of a stimulus, as in trace conditioning or tasks that require working memory. Thus, sensory systems must keep a neural representation of a stimulus after its termination (i.e. a stimulus trace). Using *in vivo* calcium imaging, we investigated whether and how olfactory information is kept in the first olfactory brain area, the antennal lobe, of *Drosophila*. We measured spatio-temporal patterns of odor responses and post-odor activities in olfactory glomeruli, which are the functional units of the antennal lobe. Using the GAL4/UAS system, with OR83b and GH146 as driver lines, we selectively measured receptor neurons and projection neurons, respectively. Odors evoked stimulus specific response patterns of activated and inhibited glomeruli, which corresponded to the previously described combinatorial response patterns (see http://neuro.uni-konstanz.de/DoOR). After odor offset the odor response patterns turned into prolonged patterns of activated and inhibited glomeruli. These post-odor activity patterns were different to the initial odor response pattern, though still being odor specific. Variation of the stimulus length had only minor effects on the post-odor activity patterns. Taken together, these results show that there is a physiological odor trace in the antennal lobe of *Drosophila*. Whether this physiological trace is the substrate of the behavioral trace remains to be determined.

# Purinergic modulation of network activity in the olfactory bulb

Daniela Hirnet[1], Christian Lohr[1]

[1]Universität Hamburg, Abteilung für Tierphysiologie, Martin-Luther-King Platz 3, 20146 Hamburg, Germany

ATP and its metabolites, in particular adenosine, are ubiquitous co-transmitters and neuromodulators, participating in synaptic transmission as well as in neuron-glia interactions. The purinergic signalling system is unique with regard to its complexity and specificity, which is achieved by tissue specific combinations of transmitters - ATP, ADP and adenosine - with a variety of receptor subtypes, second messenger systems, transporters and enzymes. Members of the purinergic signalling machinery are highly expressed in the olfactory bulb of rodents, suggesting purinergic modulation of the olfactory information processing. Recently, we found functional evidence for purinergic cell-cell communication in the olfactory bulb: In addition to the principal neurotransmitter, glutamate, the olfactory receptor neurons (ORN) release ATP, which elicits calcium signalling in astrocytes in the glomerular layer. In addition, ATP is degraded to adenosine that stimulates glial A2A receptors. In the present study, we focus on the effect of adenosine on the neuronal network activity in the olfactory bulb. We monitored the activity of olfactory bulb neurons by recording postsynaptic whole-cell currents of mitral cells, the output neurons of the olfactory bulb. Bath application of adenosine reversibly reduced the frequency of spontaneous synaptic inputs in mitral cells. DPCPX, a specific antagonist of the A1 receptor subtype, blocked the effect of adenosine. We used paired-pulse stimulation of receptor axons and imaging of vesicle fusion in OMP-synapto-pHluorin mice to test the effect of adenosine on synapses between receptor axons and mitral cells. Adenosine neither changed the amplitude of the EPSC response nor the paired-pulse ratio as compared to control stimulations. Similarly, vesicle fusion in presynaptic structures of ORNs did not change in the presence of adenosine. After pharmacological isolation of glutamatergic inputs by gabazine, adenosine failed to decrease the frequency of spontaneous (glutamatergic) inputs in mitral cells. In contrast, adenosine still exerts its depressing effect on the spontaneous GABAergic synaptic inputs in mitral cells after blocking the glutamatergic transmission by D-APV and NBQX. This indicates that in the olfactory bulb purinergic modulation of olfactory information processing is mediated by adenosine aiming at GABAergic interneurons via A1 receptors.

# Poster Topic

## T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

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T20-2C Sensory basis of wind orientation in desert ants
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*Bruno Pradier, Ildikò Racz, Monika Jeub, Astrid Markert, Daniela Mauer, Valérie Gailus-Durner, Helmut Fuchs, Martin Hrabé de Angelis, Danny Huylebroeck, Heinz Beck, Andreas Zimmer*

T20-6C THE ROLE OF CB2 RECEPTORS IN INFLAMMATORY PAIN

*Ildikó Rácz, Astrid Markert, Jürg Gertsch, Andreas Zimmer*

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*Marcus Grohmann, Tanja Nußbaum, Ralf Hausmann, HaiHong Wang, Ronald Naumann, Heike Franke, Günther Schmalzing*

T20-8C Trigeminal sensory interneurone responses to skin stimuli and their inhibitory modulation in hatchling tadpoles of *Xenopus laevis*
*Edgar Buhl, Stephen R. Soffe, Alan Roberts*

T20-9C Variability in the encoding of low- and high-frequency whisker vibrations in the barrel cortex of the awake rat.
*Sina Sieler, Maik C. Stüttgen, Cornelius Schwarz, Andreas K. Engel, Christiane Vahle-Hinz*

# A novel kind of sensilla described from ground-dwelling stick insects

Florian Walker[1], Oliver Mai[2], Andreas Stumpner[2], Ralf Heinrich[2], Sven Bradler[2]

[1]Computational Neuroscience, Department Biology II, Ludwig-Maximilians-Universität München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

[2]Abt. Neurobiologie, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Georg-August-Universität Göttingen, Berliner Str. 28, 37073 Göttingen, Germany

We report a novel kind of sensilla found in ground-dwelling stick insects (insect order Phasmatodea). The sensilla are located on the venter of the prothorax, where they are restricted to certain prominent areas and occur in high density. Each sensillum is longitudinally furrowed with an apical mushroom-like enlargement. The central projections innervate the ventral association centre (VAC) of the prothoracic ganglion in a particular pattern. The exact function of the "mushroom-sensilla" is yet unknown, but neuroanatomical and first electrophysiological studies indicate mechanoreception. The most parsimonious evolutionary scenario favours multiple convergent origins of these specialized sensilla resp. sensory areas. On the other hand, their consistent specific morphology and localization strongly suggests homology of the sensilla among stick insects and a single origin. Under the assumption of homologous "mushroom -sensilla", several hypotheses have to be considered, e.g. multiple reductions, alternatively few reacquisitions in subordinate lineages after ancestral loss must have taken place. The latter hypothesis implies that "mushroom-sensilla" re-evolved at least three times in ground-dwelling phasmatodeans of the Caribbean (Cladomorphinae, Pseudophasmatinae) and the Orientalic region (Heteropteryginae). The latter view is in accordance with the well supported finding that ground -dwelling stick insects evolved repeatedly from tree-dwellers in the isolation of islands (Buckley et al. ProcB 2009).

# A novel splice variant of NaV1.8 voltage-gated sodium channel from human dorsal root ganglion neurons leading to skipping of exon 11

Jana Schirmeyer[1], Enrico Leipold[1], Stefan H. Heinemann[1], Christian Mawrin[2], Matthias Platzer[3], Karol Szafranski[3]

[1]Friedrich Schiller University Jena, Center for Molecular Biomedicine, Department of Biophysics
[2]University Magdeburg, Department of Neuropathology
[3]Fritz Lipmann Institute Jena, Leibniz Institute for Age Research

NaV1.8 voltage-gated sodium channels are expressed in afferent neurons of dorsal root ganglia (DRG) where they are involved in the perception of inflammatory and chronic pain. Various cellular mechanisms, e.g. auxiliary subunits and posttranslational modifications, as well as mRNA processing, allow for specific functional regulation of these channels. We isolated mRNA from human, rat, and mouse DRG tissue samples and screened them for alternatively spliced isoforms of SCN10A, the gene encoding NaV1.8, using 454 sequencing technology.

We identified a novel splice variant of human SCN10A, which results from skipping of exon 11. The protein encoded by the new splice variant will lack 98 amino acid residues (from serine 488 to serine 585) in the cytoplasmic loop between domains I and II. This region is highly involved in channel regulation as it contains various putative phosphorylation sites. The abundance of the splice event was independently analyzed by capillary electrophoresis of fluorescence-labeled RT-PCR products. The relative amount of NaV1.8_Δe11 compared to NaV1.8 wild-type mRNA in human adult DRGs was 5% in three individual samples. The splice event could be detected in neither rat nor mouse DRG tissue, and thus is not conserved among rodents and human.

We constructed hNaV1.8_Δe11, the gene product of the short splice variant, using a PCR-based strategy. To examine the influence of the splice event on channel function we expressed both isoforms of hNaV1.8 in neuroblastoma cells (Neuro-2A) and compared their functions using the whole-cell configuration of the patch-clamp method. All experiments were performed in the presence of 100 nM extracellular tetrodotoxin (TTX) to suppress TTX-sensitive currents endogenous to Neuro-2A cells. Mutant and wild-type channels reached similar current densities at test pulses to 0 mV (62.5 ± 15.6 pA/pF, n = 13 for hNaV1.8_Δe11 and 62.9 ± 13.1 pA/pF, n = 15 for hNaV1.8 wild type), indicating that the splice event has no effect on the production of functional channel proteins in this expression system. Voltage dependence and kinetics of channel opening and inactivation was assayed by tailored pulse protocols, however, in none of the measured parameters the alternatively spliced isoform deviated from the control channel (n = 13 for hNaV1.8_Δe11, n = 15 for hNaV1.8 wild type, t-test: P-values > 0.25).

As reported in Zhang et al. (J Cell Sci 121 (19): 3243. (2008)), exon 11 contains an ER retention signal involved in the regulation of the cell surface expression of NaV1.8 channels. We therefore compared the expression of hNaV1.8_Δe11 and wild type in HEK293 cells because hNaV1.8 channels are hardly expressing in these cells. In three of 23 cells transfected with hNaV1.8_Δe11 we detected an average maximum current density of 21.3 ± 6.4 pA/pF at pulses to 0 mV; for hNaV1.8 wild type we only found specific $Na^+$ current in one cell with 23 pA/pF out of 24 cells tested.

The splice event leading to NaV1.8_Δe11 channels thus does not appear to have an obvious impact on channel function under the given experimental conditions. Different approaches may help to identify effects on targeting and clustering of NaV1.8 channels or on its modulation by phosphorylation, processes that are possibly influenced by skipping of exon 11.

# Behavioural modifications in ephrinA5 knockout mice

Julia Landmann[1], Daniel Pensold[1], Melanie Wüstenhagen[1], Jürgen Bolz[1]

Friedrich-Schiller-Universität Jena, Institut für Allgemeine Zoologie und Tierphysiologie, Erbertstrasse 1, 07743 Jena, Germany

Recent studies showed that the Eph/ephrin family of guidance molecules is involved in the development of the somatosensory system, regulating the formation of thalamocortical connections and the construction of layer-specific cortical circuits. The thalamocortical projections in somatosensory and cingulate cortical areas were reported to be miswired in ephrinA5 deficient mice. Furthermore, structural alterations of spiny stellate cells in layer 4 of the somatosensory cortex, caused by the reduced thalamic input, were observed in the knockout animals. To test a potential behavioural consequence of the miswiring in ephrinA5 deficient mice, we performed a set of different behavioural experiments. First, we conducted an approach to test for somatosensory accomplishments, which indeed resulted in poorer performances of the ephrinA5 knockout mice in comparison to wild type mice. Moreover, this effect could be mimicked with control animals induced by bilateral whisker deprivation, suggesting that defects in the somatosensory system are responsible for the observed phenotype of ephrinA5 deficient mice. We also tested for motor achievement and anxiety applying the open field test, to verify whether alterations in the limbic or motor system provoke the somatosensory defects observed in the mutant mice. However we could not observe any motor defects of the ephrinA5 knockout mice. Our findings suggest that ephrinA5-deficiency leads to vibrissae-related tactile insufficiencies and anxiety -like behaviour, which are based on modest cortical somatosensory and/or limbic changes rather than motor deficits.

# Central projections of antennal hair fields and descending interneurons in stick insect brain and suboesophageal ganglion

Jens Goldammer[1], Volker Dürr[2]

[1] Animal Physiology, Biocenter Cologne, Zülpicher Str. 47b, 50674 Cologne, Germany[2]Department of Biological Cybernetics, University of Bielefeld, Universitätsstr. 25, 33615 Bielefeld, Germany

The stick insect Carausius morosus continuously moves its antennae during locomotion to explore the environment for obstacles within the action range of the front legs. Each antenna is movable by means of two single-axis hinge joints, the proximal head-scape- and distal scape-pedicel joint. Close to the distal margin of these joints, seven hair fields (HF) are arranged with a variable number of sensilla. Ablation experiments of HFs revealed various changes in the antennal working range, indicating their function as joint angle sensors. Furthermore, descending brain interneurons (DINs) transmit short-latency information from antennal mechanoreceptors to motor centers of the thoracic ganglia.

So far, neuroanatomical studies on antennal HF projections within the brain and suboesophageal ganglion (SOG) are sparse (see Adv. Insect Physiol. 32:49-205). Here, we examine the central projection pattern of all antennal HFs with respect to differences in arborisation pattern, terminal neuropil region and vicinity to DINs. Through double-labeling experiments of HFs with, e.g. antennal flagellar afferents, other pedicellar mechanosensory sensilla or antennal motor nerves, we obtained more insights into the organization of the antennal mechanosensory motor center (AMMC): for instance, all afferents of the seven HFs have very similar and strongly overlapping terminal arborisations. Whole-mount preparations showed beyond extensive ramifications within the dorsal lobe (DL) of the deutocerebrum with collaterals descending to the SOG where they terminate in a postero-ventro-medial region.

For stick insects it is largely unknown how many DINs are present within the brain and SOG and how many DINs project to the ventral nerve cord. To reveal the number and distribution of DINs we performed backfills of neck connectives and of connectives between pro- and mesothoracic ganglia. Stainings of neck connectives showed at least 190 pairs of brain DINs, whereas backfills of connectives between pro- and mesothoracic ganglia displayed a reduced amount of at least 78 labeled pairs of cell bodies. Additionally, double-labeling of DINs and HF afferents showed that DIN dendrites arborise in close vicinity of HF central projections and arborisation volumes of afferents and DINs strongly overlap. This supports the idea that short- latency information transfer from HFs to DINs involves only few synaptic relay connections within the deutocerebrum.

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# Ciliated sensory organs in Chaetognaths

Verena Rieger[1], Yvan Perez[2], Carsten HG Müller[1], Steffen Harzsch[1]

[1]Ernst-Moritz-Arndt Universität Greifswald, Zoologisches Institut und Museum, Cytologie und Evolutionsbiologie, Johann Sebastian Bach Str. 11/12, 17497 Greifswald, Germany

[2]Université de Provence, Institut Méditerranéen d'Ecologie et de Paléoécologie UMR CNRS 6116 3 Pl Victor Hugo Case 36, 13338 Marseille cedex 3, France

Chaetognaths are very abundant marine predators. They can be found in varying depths and habitats and feed mainly on zooplankton. Several sensory organs are used for orientation and prey detection. In addition to a pair of eyes that enable phototaxis and the retrocerebral organ, the function of which is yet unclear, Chaetognaths possess two major ciliated organ systems. These ciliated organs, the ciliary fence receptors and the corona ciliata, are the focus of our study. We examined mainly two species, the epibenthic *Spadella cephaloptera* and the pelagic *Sagitta setosa*. Whole mounts were processed with immunhistofluorescence methods to detect tubulin and cell nuclei and explore details of the structure and innervation of the organs in question. The ciliary fence receptors are known to have a mechanosensory function, detecting vibrations to coordinate hunting or escape movements [1]. They are relatively small but distributed in large numbers along the entire body. A fence receptor consists of a row of tubulin-containing cilia that originate from a dense cluster of epidermal cells. Their axons project in bundels towards the ventral nerve centre. The corona ciliata is a single organ, consisting of a loop of short ciliae. The function of this organ could not yet be elucidated and was the subject of a variety of speculations [1]. As with the fence receptors the corona is formed by a large number of densely packed cells. The corona ciliata, like the eyes, is connected to the posterior domain of the chaetognath brain. However, unlike other sensory systems, some of the cells in the corona are constantly replaced which is characteristic for chemosensory organs in a variety of other species and might be a hint as to its function. The comparison between the two species shows a similarity in fine structure but, especially for the corona ciliata, a wide variety of form. Shape, number and distribution of the studied organs are species specific [2] and they can be easily highlighted by our techniques. This makes them a useful tool for taxonomic studies. Our study was supported by the German Research Society, SPP "Metazoan Deep Phylogeny" (Grant HA 2540/7-1/2).

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# Cues of vibrotactile signals used for discrimination in the rat vibrissal system

Christian Waiblinger[1], Cornelius Schwarz[1,2]

[1]Systems Neurophysiology Group, Werner Reichardt Centre for Integrative Neuroscience, University of Tübingen, Germany, [2] Department for Cognitive Neurology, Hertie Institute for Clinical Brain Research, University of Tübingen, Germany

The rat's vibrissal system is an important model system to study sensory processing. Here we asked the question how vibrotactile whisker signals reach perception. We make use of a head-fixed behaving rat preparation that allows us to measure discriminability of precisely applied pulsatile whisker deflections. Pulsatile stimuli are characterized by physical properties like kinematic events (e.g. high velocity events), frequency (spectral composition) and intensity (e.g. mean absolute velocity). In a previous study we showed that discrimination using intensity-matched stimuli breaks down in the frequency range between 60 and 90 Hz (Gerdjikov et al., 2010). These earlier results were acquired using a go/no go psychophysical paradigm with presentation of one single stimulus per trial that required a memory component for discrimination. In addition, before confronted with the intensity-matched stimuli, the animals were trained first on stimuli that differed in intensity, raising the possibility that the failure to discriminate depended on the training history of the animals. In the present study we address these issues. First, using a novel yes/no psychophysical task and intensity-matched stimuli to train the animals, we readily observed the inability of the rats to discriminate stimuli within the range of 60- 90 Hz. Second, using a detection-of-change task which requires only a minimum of memory contribution because one pulsatile stimulus at 90 Hz is presented as a background stimulation and then switches seamlessly to the test stimulus (30-90 Hz), we were able to show that discrimination in this range is readily possible. Taken together, these results point to the possibility that comparison of sensory evidence against memory is done using intensity cues. Pure sensory comparisons, on the other hand, may have access to a richer set of stimulus characteristics like spectral cues and/or kinematic events.

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# Dissecting transducer adaptation in a Drosophila mechanosenory cell

Georg Raiser[1]

[1]JFB-Institute for Zoology and Anthropology, University of Göttingen, Cellular Neurobiology, Herrman-Rein Str.3, 37073 Göttingen, Germany

Despite many years of research, the molecular structure of mechanosensitive transducer machineries remains unknown. The macrochaete bristles of Drosophila melanogaster offer an elegant way to access a mechanosensitive system with little invasiveness at the level of a single cell.
The mechanotransduction of these cells is shown to be dependent on the product of the nompC gene, a putative mechanotranduction channel: While a null mutation in the gene causes a complete loss of the receptor potential, a point mutation in the predicted channel pore shows altered adaptation kinetics. By recording these mechanically evoked currents, we now identified novel mutations that affect adaptation The respective functional defects are quantified and the mutations are linked to identified genes.

# Encoding of tactile stimuli in sensory neurons of the medicinal leech *Hirudo medicinalis*

Friederice Pirschel[1], Jutta Kretzberg[1]

[1]Carl-von-Ossietzky Universitaet Oldenburg, Computational Neuroscience, Carl-von-Ossietzky Strasse 9-11, 26111 Oldenburg, Germany

How is it possible to encode several stimulus properties simultaneously with only a small number of sensory neurons?
The CNS of the leech Hirudo medicinalis is one of the smallest neuronal networks, with only three types of mechanosensory neurons located in each ganglion, and only four respectively six individual neurons of each type. In this study we analyze the responses of two of these cell types, P cells ("pressure") and T cells ("touch"), to pressure stimuli applied to the skin of the leech.

In response to a tactile stimulus of the skin, the leech bends away from the mechanical pressure. This behavior, called "local bend response" [Baca et al., 2005], is a good example for the tight connection between sensory input and behavioral motor output in the leech's CNS. It results from the contraction of the muscles on the side touched and the elongation of the muscles on the other side [Kristan et al., 2005]. The sensory neurons which are mainly responsible for this reaction are the P cells ("pressure"). The role of a second type of sensory neurons, the T cells ("touch"), is still unresolved [Lewis and Kristan, 1998]. Based on responses of only two P cells the leech can discriminate locations of tactile stimuli which are only 9° of the body circumference apart [Thomson and Kristan, 2006].

In our experiments, we recorded from P and T cells intracellulary while stimulating the skin mechanically. We applied tactile stimuli with varying properties such as the intensity, the duration and the location. The neuronal responses were analyzed in respect to the four features response latency, spike count, mean of interspike intervals and 1st interspike interval. Based on these response features, we performed stimulus reconstruction based on the maximum likelihood method.

We found that in comparision to the P cells, the T cells respond with shorter latencies. The interspike interval patterns of T cells and P cells differ considerably.
For P cell activity, we show that the intensity of the tactile stimulus influences all four response features. With rising intensity the spike count increases, whereas the latency, the average interspike interval and the 1st interspike interval decrease. The stimulus location influences all the features depending on the location relative to the receptive field center of the cell. Longer stimulus duration leads to a greater spike count, while the other response properties are uneffected.
Both for stimulus intensity and location the 1st interspike interval leads on average to the best stimulus reconstruction performance, followed by the latency and the spike count. Whereas stimulus intensity and duration can be reconstructed well based on single cell responses, at least two cells with overlapping receptive fields are needed to precisely encode stimulus location [see also Thomson and Kristan, 2006].

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# MECHANOSENSITIVITY IN THE ENTERIC NERVOUS SYSTEM

Gemma Mazzuoli[1], Michael Schemann[1]

1 Technische Universitaet Muenchen, Human Biology, Liesel-Beckmann Strasse, 85354 freising, Germany

The enteric nervous system (ENS) regulates reflex pathways that control gut motility and mucosal transport function independently from the central nervous system. This intriguing capability is among others due to coding of mechanical stimuli by enteric neurons. Mechanotransduction mechanism remains vastly unknown despite the evidence that ENS neurons appear to respond to sustained distension. Up to now, the most common theory postulate mechanosensitivity as a property of specialized intrinsic primary afferent neurons (IPANs). This concept has been challenged from studies revealing mechanosensitivity in a different class of neurons and outlining a possible multifunctional role of neurons in the ENS. Our research is aimed to further investigate the existence and the properties of enteric neurons responding to mechanical stimuli that mimic contractile activity rather than using sustained stretch. Using fast Neuroimaging technique based on a voltage sensitive dye we identified mechanosensitive enteric neurons which fired action potentials in response to ganglion deformation. Experiments were performed in guinea pig and mouse fresh preparation from myenteric plexus ileum, in organotypic culture of ileum segments with DiI retrograde tracing and in primary culture of enteric neurons. Different techniques were applied to deform neurons: von Frey hairs or pressure pulses of small volumes of Krebs solution. Results revealed that in both species around 25% of all neurons responded. The discharge pattern suggests that mechanosensitive neurons behave like rapidly adapting mechanosensors that respond to dynamic changes. We therefore referred to these neurons as Rapidly Adapting Mechanosensitive Enteric Neurons (RAMEN). Deformation evoked spike discharge is not changed by synaptic blockade with omega-conotoxin or low Ca++/high Mg++, defunctionalisation of extrinsic afferents with capsaicin or muscle paralysis with nifedipine, suggesting direct activation of RAMEN. Mechanosensitivity was observed in 31% and 47% of retrogradely traced interneurons and motor neurons, respectively. RAMEN belong to a new population of mechanosensitive neurons which differ for neurochemical code and electrophysiology from IPANs. This work provides strong evidences that mechanosensitivity is a feature of different classes of enteric neurons supporting the concept of multifunctional mechanosensitive neurons which may fulfill sensory, integrative and motor functions. Finding similar results in guinea pigs and mice drove us to the conclusion that this concept could be transferred across species. Future goals are oriented to understand if we can translate this concept to the human gut as well as to deeply investigate RAMEN mechanotransduction modality.

# A large-scale behavioral screening for neurons responsible for electric shock and sugar response in *Drosophila*

Vladimiros Thoma[1], Christine Damrau[1], Hiromu Tanimoto[1]

[1]Max Planck Institite of Neurobiology, Laboratory of Behavioral Genetics, Am Klopferspitz 18, D-82152 Martinsried, Germany

The ability to sense a wide range of stimuli is of vital importance for all animals. Sensory input from the environment is processed by higher-order neurons and can eventually lead to appropriate behavioral responses through motor neurons. *Drosophila* is an increasingly popular model organism for the analysis of such neuronal circuits thanks to its genetic toolbox and the anatomical simplicity of its nervous system. In this study, we have conditionally blocked different groups of cells using more than 1000 GAL4 drivers and screened them for various defects in sugar and electric shock responses. Our screening assays allow both high throughputs and the evaluation of a wide range of accompanying behaviors. By combining our results with anatomical data of GAL4 expressing cells, we have narrowed down our search to approximately 100 lines for sugar responses and 100 lines for electric shock responses. We here discuss about candidate neurons that are required for these appetitive and aversive stimulus responses.

# Information transmission is limited by entropy in spider mechanoreceptors

Keram Pfeiffer[1], Päivi H. Torkkeli[1], Andrew S. French[1]

[1]Dalhousie University, Deptartment of Physiology and Biophysics, 5850 College St, B3H1X5 Halifax, Canada

Most neurons propagate information in the form of encoded in action potentials. While the binary nature of action potentials allows for robust information transmission over large distances, it also creates a cost, because the durations and refractory periods of action potentials set an upper limit to the entropy rate; i.e. the amount of information that can be encoded per unit time. Another factor that can limit transmission through an information channel, such as a neuron, is the inherent noise of the system. In this study we asked whether noise or entropy limits information transmission during sensory transduction and encoding in mechanosensory neurons of the lyriform organ VS-3 of the spider *Cupiennius salei*.

Information transmission through a neuron can be assessed by measuring information capacity or entropy. While the former can be estimated from the signal-to-noise ratio and measures the maximum information rate that could theoretically be transmitted, the latter measures the total information that is present in a signal from any source.

Receptor current, receptor potential and action potentials in VS-3 neurons were recorded using voltage clamp and current clamp recordings during repeated stimulation with identical sequences of random mechanical stimuli. Information capacity was estimated from the signal-to-noise ratio at each stage by averaging responses to repeated identical stimulation sequences, and between stages from the coherence function.

To make equivalent comparisons of entropy values at all stages of transduction and transformation we developed a new entropy measure that is suitable for analog signals as well as digitally filtered action potentials, by implementation of a context-free data compression algorithm. All signals were normalized and digitized at 10 bit (1024 levels) resolution. Each of the 1024 levels representing the digitized signal was treated as an independent symbol in a discrete time series. Data compression was performed by repeatedly replacing pairs of symbols that occurred with the greatest frequency by additional symbols, until no further compression was achieved. The compression entropy, $E_c$, was then obtained from:

$$E_c = N \log_2 M$$

where N = number of symbols in the compressed message, M = number of different symbols in the message.

We found that entropy, rather than noise, limited information transmission at each stage. While little information was lost during the transduction from mechanical stimulus to receptor current and then to receptor potential, the action potential signal contained only about 10% of the original information.

Our data show that the entropy that can be encoded into a neural signal limited information transmission by this sensory system, and that the overall information transmission limit was imposed by the parameters of the action potential encoding, rather than inherent noise.

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# Information-theoretic analysis of whisker-responsive trigeminal ganglion neurons to white noise stimulation

Andre Maia Chagas[1,3,4], Biswa Sengupta[2,3], Maik Stuettingen[3,5], Cornelius Schwarz[3,4]

[1]Graduate School of Cellular and Molecular Neuroscience, Eberhard Karls University Tübingen, Germany
[2]Neural Circuit Design Group, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, UK
[3] Dept. Cognitive Neurology, Hertie-Institute for Clinical Brain Research, Eberhard Karls University Tübingen, Germany
[4]Systems Neurophysiology, Centre for Integrative Neurosciences, Eberhard Karls University Tübingen, Germany
[5]Dept. Biopsychology, Ruhr University Bochum, Germany.

Rodents acquire tactile information using whiskers, whereby tactile perception is constrained by the information transmitted through (at least) two classes of primary trigeminal ganglion afferents that innervate the whisker follicles. We employed extracellular single unit recordings from the trigeminal ganglion of anesthetized animals in response to band-limited white noise stimulation of the corresponding whisker (low cut-off frequency 100 Hz). Recorded spike trains were analyzed using standard information theoretic measures yielding the total information carried under no assumptions of what and how information is represented by the afferents (direct method), as well as the channel capacity (upper bound), and stimulus reconstruction methods (lower bound). Preliminary results obtained from n=15 trigeminal ganglion cells show that primary afferents respond to whisker deflections in a highly reliable manner and that they are able to convey information about the stimuli at high transmission rates (~200 bits/sec). Comparison of the channel capacity and reconstruction quality confirms that the two classes of primary afferents reliably encode whisker frequencies related to textures between 50-100 Hz. Information coding was fairly linear in this frequency range. In contrast, frequencies typically related to whisker movement (5-10 Hz) were found to be less well encoded and rely more on non-linear encoding.

# Intermittent Theta-Burst Stimulation Applied by TMS Weakens Inhibitory Sensory Activity in Rat Barrel Cortex

Andreas Thimm[1], Klaus Funke[1]

[1]Ruhr-University Bochum, Neurophysiology, Universitaetsstrasse 150, 44780 Bochum, Germany

Our previous studies already demonstrated that repetitive transcranial magnetic stimulation (rTMS) of the rat cortex modifies the expression of distinct proteins related to the activity of inhibitory interneurons [1,2]. Especially the intermittent theta-burst protocol (iTBS) was found to not only weaken the expression of the gamma-aminobutyric acid (GABA) synthesising enzyme glutamic acid decarboxylase GAD67, but also that of the calcium-binding protein parvalbumin (PV) which is expressed in fast-spiking interneurons. The expression of calbindin and calretinin - calcium-binding proteins primarily found in other classes of interneurons - was either less or not affected, respectively. These findings indicate that rTMS may be able to modulate the activity of distinct types of interneurons depending on the stimulation protocol applied.
With the assumption that iTBS primarily weakens the activity of the PV-expressing, fast-spiking interneurons, we now tested how iTBS modifies the intensity of distinct forms of sensory evoked inhibitory interactions in the rat barrel cortex. We established input-output relationships on the basis of stimulus strength (speed of whisker deflection) and response amplitude to estimate changes in tonic inhibition possibly affecting response threshold and gain [3]. We further tested the strength and time course of recurrent inhibition by applying stimuli at different intervals (10, 20, 100 ms) to the same whisker. Moreover, lateral inhibition was tested by stimulating the dominant and one adjacent whisker at different intervals (0, 20, 100 ms). Piezoelectric actuators (Physik Instrumente, Karlsruhe, Germany) were used to achieve ramp-like deflections of single whiskers. Simultaneous recordings of three single units at the representation of the whisker D2 or D3 were performed in the anesthetized rat (urethane, 1,5 g/kg) using a bundle of three tungsten electrodes (FHC, 1 MOhm). After obtaining baseline values of neuronal responses, rats received five blocks of iTBS (10 bursts of 3 pulses/60ms [50 Hz] at 200 ms intervals [5 Hz] repeated 20 times every 10 seconds [600 stimuli]) at intervals of about 20 minutes (3000 stimuli in total). Recordings of neuronal activity were performed between iTBS blocks and up to four hours after termination of iTBS.
Compared to baseline level, iTBS increased mean sensory responsiveness to single stimuli by an almost constant amount (offset) without changing the gain, indicating a reduction in tonic inhibition [3]. Suppression of sensory responses was most effective when preceded by stimulation of the same (dominant) or a neighbouring whisker 20 ms earlier. This inhibition was significantly reduced following iTBS. Simultaneous stimulation of the two adjacent whisker resulted in response enhancement while the 100 ms interval had little effect at all. These response types were little affected by iTBS. Our results indicate that iTBS induced a fast reduction in cortical inhibition of the tonic and recurrent type which is most likely mediated by the perisomatic action of the PV-type interneurons.
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# Network analysis of the pain system in transgenic mice by fMRI and graph theory

Andreas Hess[1], Silke Kreitz[1], Cornelia Heindl-Erdmann[1], Roland Axmann[2], Josef Penninger[3], Georg Schett[2], Brune Kay[1]

[1]Institute for Pharmacology, Pharmacological Imaging and Image Analysis, Fahrstrasse 17, 91054 Erlangen, Germany
[2]Internal Medicine 3, Rheumatology and Immunology, Friedrich-Alexander-University of Erlangen-Nuremberg, Erlangen, Germany
[3]Institute of Molecular Biology of the Austrian Academy of Sciences, Vienna, Austria

This functional magnetic imaging study investigated the functional implications of genetic modification and pharmacological intervention on cerebral processing of heat-induced nociception in mice. Resting state and stimulus evoked BOLD signals are evaulated. Comparing dynorphin-overexpressing dream-/- mice, with strongly reduced activity in the pain system, with wild-type mice, smaller activated cortical and limbic brain structure sizes could be observed. Moreover, significantly reduced blood oxygenation level-dependent signal amplitudes were found in pain-related brain structures: sensory input, thalamic regions, sensory cortex, limbic system, basal ganglia, hypothalamus and periaqueductal grey. Administration of the specific κ-opioid-receptor antagonist nor-binaltorphimine (BNI) to dream-/- mice reversed this reduction to wild-type level in the same brain structures. Interestingly, under BNI treatment the relative short period of pain exposure during the fMRI experiment lead to faciliation of the somatosensory pain network in the transgenic mice rendering their network properties to the wild-type connectivity pattern.

# Nitric oxide in the antennal mechanosensory neuropil of the cricket brain

Nicole Naumann[1], Klaus Schildberger[1], Gay Holstein[2], Paul Anthony Stevenson[1]

[1]University of Leipzig, Institute for Biology, Talstr. 33, 04103 Leipzig, Germany
[2] Department of Neurology, Mount Sinai School of Medicine, New York, New York 10029

The antennal mechanosensory neuropile (vfa - ventral flagella afferents) in the cricket brain is the first station for processing tactile antennal information gathered during locomotion, courtship and aggressive behaviors. As nitric oxide (NO) is implicated in modulating sensory processing, we sought to reveal NO release site in the vfa using NADPH-histochemistry, and antibodies against nitric oxide synthase (NOS) and citrulline, the byproduct of NO synthesis. All methods labelled essentially the same profiles in the vfa, whereby citrulline-immunocytochemistry produced the most vivid label. The most prominent structure is a ring-like neuropil derived from antennal afferent fibres. Though diffuse in appearance when viewed by standard confocal microscopy, the higher resolution of STED- (Stimulated Emission Depletion) confocal microscopy revealed the ring to comprise a meshwork of citrulline-like immunoreactive granules (100 -200 nm) encompassing non- labelled profiles, which we presume to be synaptic varicosities. Citrulline immunocytochemistry also labelled a local brain interneurone with prolific branches in vfa, but none of the descending brain interneurones that project in vfa. Potential targets of NO were revealed using a cGMP-antibody, with which we identified 3-4 local interneurones that ramify the ring-neuropil in vfa. We propose that NO released from the terminals of antennal afferents and the local brain interneurone modulates primary synaptic transmission in the vfa.

# Photodynamic targeting of mitochondria in cultured sensory neurons reveals ROS-induced neuronal signaling

Ben Novak[1], Nicole Schoebel[2,3], Roxane Schulten[1], Saskia Kortmann[1], Hanns Hatt[2,3], Hermann Luebbert[1,3]

[1]Ruhr-University , Department of Animal Physiology, Universitätsstraße 150, 44780 Bochum, Germany
[2]Ruhr-University , Department of Cell Physiology, Universitätsstraße 150, 44780 Bochum, Germany
[3]DFG Graduiertenkolleg 736, Development and Plasticity of the Nervous System

Reactive oxygen species (ROS) form as a cellular byproduct of oxidative metabolism and have to be controlled carefully in order to guarantee cellular survival. Many diseases, especially of the nervous system, are driven by excess ROS formation or a cellular inability of proper ROS regulation. Mitochondrial degeneration is often the immediate cause of such dispositions. As mitochondria have been described as important regulators of sensory neuron excitability and signaling, dysfunction of these organelles will result in sensory malfunctions and pain.
Although various test systems have been established in order to simulate oxidative stress in cell cultures, in many of them ROS production is an effect secondary to respiratory chain uncoupling. Inspired by the photodynamic treatment of cancer, where a mitochondrial localized photosensitive ROS-donor molecule is employed to destroy mitochondria in tumor cells, we have exploited the properties of 5- aminolevulinic acid (ALA) to induce photosensitizer (Protoporphyrin IX; PpIX) formation in cultured dorsal root ganglion neurons. Illumination at an adequate wavelength will activate the PpIX and release singlet oxygen, a potent radical precursor. We can thereby manipulate mitochondria by inducing different levels of ROS - dependent on the loading conditions - and trigger various subsequent cellular reactions without interfering with the respiratory chain in the first instance. Here we describe a down-stream mitochondrial impairment by studying mitochondrial membrane potential and measuring ATP levels and mitochondrial oxidoreductase function. Dependent on the 5-ALA dose applied, illumination of the neuronal cultures will result in modest, transient stress at low doses, apoptosis at medium doses and necrosis at maximum stress levels. The versatility of the experimental approach allows studying the different signaling pathways in response to ROS increase.
In cancer treatment the illumination that causes oxidative stress is frequently associated with pain perception. Therefore, we studied DRG neuron activation in response to photodynamic treatment using calcium imaging. We observed an immediate activation of neurons, indicated by a cytosolic $Ca^{2+}$ rise that was dependent on extracellular $Ca^{2+}$ and voltage gated channels. These observations may lead to a better understanding of the role of ROS in PNS disorders, neuropathic pain and the painful side-effects of skin PDT.

# Pronociceptive Effects of Prostacyclin ($PGI_2$) in spinal nociceptive Processing

Claus-Dieter Schuh[1], Christian Brenneis[1], Bona Linke[1], Klaus Scholich[1], Gerd Geisslinger[1]

[1]Klinikum der Goethe Universität,Pharmazentrum Frankfurt/ZAFES Institut für klinische Pharmakologie, Theodor Stern Kai 7, 60590 Frankfurt, Germany

Lipid Mediators formed from arachidonic acid by cyclooxygenases (COX) play important roles in inflammatory processes and nociceptive transmission. The COX enzyme converts arachidonic acid to the instable intermediate prostaglandin $H_2$, which can be metabolized by inducible microsomal prostaglandin $E_2$ synthase 1 (mPGES1) or prostacyclin synthase (PGIS) to $PGE_2$ or $PGI_2$ respectively. Inflammatory processes e.g. in the mouse hind paw induce COX2 expression in the spinal cord and thereby increase the level of prostaglandins.
We aimed to investigate the role of prostacyclin ($PGI_2$) and its receptor (IP) in spinal nociceptive transmission. Liquid chromatography coupled mass spectrometry (LC-MS/MS) revealed that $PGI_2$ level significantly increased in spinal tissue one hour after zymosan injection in one hind paw. We next showed by fluorescence resonance energy transfer (FRET)-based cAMP imaging, that the IP agonist cicaprost can induce cAMP synthesis in a subset of embryonic spinal cells. The same cells were shown to be NMDA -responsive, suggesting the presents of functional Gs coupled IP-receptor in spinal neurons. Importantly, cicaprost treatment of embryonic spinal cord cultures increased PKA-dependent phosphorylation of the NMDA receptor subunit 1 (NR1) at Serin 897, which is known to sensitize NMDA stimulation. Finally, incubation of spinal cord slices of adult mice with cicaprost resulted in increased glutamate-release indicating an increased activity of excitatory neurons.
In summary, we showed that peripheral inflammation increases spinal $PGI_2$ levels and activation of spinal IP-receptors increases PKA-dependent phosphorylation of NMDA receptors and potentiate excitatory activation.

# Representation of thermal information in the antennal lobe of leaf-cutting ants

Markus Ruchty[1], Fritjof Helmchen[1], Rüdiger Wehner[1], Christoph Johannes Kleineidam[2,3]

[1]Department of Neurophysiology, Brain Research Institute, University of Zürich, Winterthurerstrasse 190, 8057 Zürich, Switzerland

[2]Department of Behavioral Physiology and Sociobiology, Biocentre, University of Würzburg

[3]Department of Biology,University of Konstanz

Insects are equipped with various types of antennal sensilla, which house thermosensitive neurons adapted to receive different parameters of the thermal environment for a variety of temperature-guided behaviors. In the leaf-cutting ant *Atta vollenweideri*, the physiology and the morphology of the thermosensitive sensillum coeloconicum (Sc) has been thoroughly investigated. However, the central projections of its receptor neurons are unknown. Here we selectively stained the three neurons found in single Sc and tracked their axons into the brain of *Atta vollenweideri* workers. Each of the three axons terminates in a single glomerulus of the antennal lobe (Sc-glomeruli). Two of the innervated glomeruli are adjacent to each other and are located laterally, while the third one is clearly separated and located medially in the antennal lobe. Using two-photon $Ca^{2+}$ imaging of antennal lobe projection neurons, we studied where in the antennal lobe thermal information is represented. In the 11 investigated antennal lobes, we found up to 10 different glomeruli in a single specimen responding to temperature stimulation. Both, warm- and cold-sensitive glomeruli could be identified. The thermosensitive glomeruli were mainly located in the medial part of the antennal lobe. Based on the general representation of thermal information in the antennal lobe and functional data on the Sc-glomeruli we conclude that temperature stimuli received by Sc are processed in the medial of the three target glomeruli. The present study reveals an important role of the antennal lobe in temperature processing and links a specific thermosensitive neuron to its central target glomerulus.

# Response properties of neurons in the somatosensory cortical areas of the Etruscan shrew

Claudia Roth-Alpermann[1], Michael Brecht[1]

[1]Humboldt University Berlin, Bernstein Center for Computational Neuroscience, Philippstr. 13, Haus 6, 10115 Berlin, Germany

The Etruscan shrew *Suncus etruscus* is the smallest terrestrian mammal. Etruscan shrews are excellent hunters that recognise the shape of their prey with amazing speed and accuracy. Behavioural experiments indicate that they have a Gestalt-like representation of objects based on whisker mediated tactile cues (Anjum et al., 2006).

Cortical sensory areas have been delineated using multi-unit electrophysiological mapping of sensory responses (Roth-Alpermann et al., 2010). Large parts of Etruscan shrew cortex (i.e. 60% of the total neocortical surface) responded to sensory stimuli. We identified a small visual and a small auditory area. The majority of recording sites responded to tactile stimuli and more than half of these sites responded to macrovibrissae stimulation. These findings demonstrate a remarkable degree of tactile specialization in the Etruscan shrew cortex. In comparison to other mammals studied so far, it is clear that the Etruscan shrew is one of the most extreme tactile specialists studied to date.

We identified a topographically organized primary somatosensory cortex S1 and a secondary somatosensory cortex S2. Lateral from these areas we found cortical somatosensory regions with very large receptive fields and sometimes polysensory responses. We suppose that these regions might be higher-order processing areas.

We now seek to characterize the response properties of neurons in different somatosensory cortical regions by presenting various stimuli to the whiskers. Furthermore, we like to inquire whether neurons respond in a shape-specific manner by presenting different objects to the whiskers. Preliminary evidence suggests that neurons in different somatosensory regions have different stimulus preferences, but further experiments are necessary to corroborate these observations.

# Mechanosensitivity in isolated enteric neuronal networks

Eva Kugler[1], Gemma Mazzuoli[2], Michael Schemann[3]

[321]TU München, department of human biology, Liesel-Beckmann-Straße 4, 85350 Freising, Germany

The enteric nervous system controls vital gut functions independently from the central nervous system. Enteric neurons are situated inside the gut wall and therefore subjected to a wide variety of mechanical stimuli. Myenteric neurons have been described to have mechanosensitive properties. Our group recently demonstrated the existence of multifunctional Rapidly Adapting Myenteric Neurons (RAMEN). These neurons are able to transduce mechanical stimuli into electrical signals. The activated pathways and the role of different mechanical modalities are still unknown.

We aimed to characterize mechanotransduction and stimulus modalities of mechanosensitive enteric neurons. The studies were performed in primary cultures of myenteric neurons in order to avoid interference from other cells. Enteric neurons were stained with the voltage sensitive dye Di-8-ANEPPS and then probed with normal (compression and stretch) or shear stress. Compression was achieved by probing with a small diameter carbon fiber as von Frey hair. Stretch was induced by indentation of an elastic surface on which neurons were grown. Shear stress (range of 0.2- 1.6 Pa) was generated by exposing neurons grown in a channel to different flow rates. The evoked action potentials were detected with an ultra fast Neuro-Imaging technique.

Enteric neurons fired action potentials in response to the different mechanical stimuli: compression evoked a response in 39.0 % of the enteric neurons with a mean frequency of 3.7 Hz and a mean duration of 799 ms. No difference was detected in the response when the compression was evoked on the soma or on the neuronal processes, suggesting that the mechanical stimulus was encoded by both neuritis and cell bodies. Stretch induced a response in 46.4 % of the neurons that fired action potentials with a mean frequency of 2.4 Hz and a mean duration of 373 ms. Compression as well as stretch induced an immediate spike discharge: the first action potential could be recorded 1 ms after the onset of the stimulus. Shear stress evoked a different type of response in a rather small subset of neurons (9.9 %). The neurons fired with a much smaller frequency (1.3 Hz) and the spike discharge was slowly adapting. We established a novel method to study mechanosensitivity in isolated enteric neuronal networks. Our results suggest for the first time that enteric neurons are able to respond to different types of deformation, with shear stress evoking slowly adapting responses while compression and stretch induced an immediate transient response.

# Screening for local anesthetics which induce TRPA1-mediated entry of QX-314 into cells and the consequences for a sensory selective nerve blockade

Christian Brenneis[1], Michelino Puopolo[2], Marco Sisignano[3], David Segal[1], Gerd Geisslinger[3], Bruce Bean[2], Clifford Woolf[1]

[1]Children's Hospital Boston, Harvard Medical School, F.M. Kirby Neurobiology Center, 300 Longwood Ave, 12260 Boston, USA

[2]Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115, USA

[3]pharmazentrum frankfurt/ZAFES, Institut für Klinische Pharmakologie, Klinikum der Goethe-Universität Frankfurt, Theodor-Stern-Kai 7, 60590 Frankfurt/Main, Germany

Perineural delivery of capsaicin together with the charged sodium channels blocker QX-314 produces nociceptive selective blockade by introducing QX-314 through the TRPV1 channel pore into nociceptors. Here, we screened caine-type sodium channel blockers for their ability to activate TRP-channels in sensory neurons. We further showed how caines can promote QX-314-uptake through TRPA1 channels and characterized the modality of anesthesia induced by this. In a calcium imaging screen with primary rat dorsal root ganglion neurons, we identified in a group of 14 caine-type anesthetics bupivacaine as the most potent inducer of a ruthenium red sensitive calcium flux. Patch clamp recordings in heterologous expression system revealed that bupivacaine induces strong TRPA1- but not TRPV1-mediated inward currents. In conditions where QX-314 represents the only extracellular cation, QX-314 could carry inward currents through TRPA1 when activated by bupivacaine. Additionally, we showed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of cell lysates, that intracellular QX-314 concentrations markedly increased when TRPA1-channels are expressed and activated by bupivacaine. Finally, sciatic injections of bupivacaine together with QX-314 produced a sensory selective block. Anesthesia to heat and especially mechanical stimulation lasted for more than 12h while motor function recovered after 6h. In contrast, QX-314 alone, bupivacaine alone or a combination of QX-314 together with procaine (non TRP-activator) did not show any differences in the blockade of sensory and motor functions in the same behavioral tests. The experiements illustrates that caine-type drugs which activate TRPA1 can efficiently introduce small cationic drugs into cells. We further identified a combination of bupivacaine and QX-314 as an ideal strategy to achieve long lasting and sensory selective nerve blockade with clinical potential.

# Sensory basis of wind orientation in desert ants

Alex Scheller[1], Harald Wolf[1], Matthias Wittlinger[1]

[1]University of Ulm, Institute of Neurobiology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

Desert ants, *Cataglyphis fortis*, live in the featureless salt pans of North Africa. They use path integration as their primary means of navigation, based on a stride integration odometer and a skylight compass. In addition, wind direction may serve as an orientation cue in many situations. If the skylight compass is not available, e.g. under completely overcast skies, wind direction is used as a compass instead (Wehner & Duelli 1971, Experientia 27, 1364–1366). And wind- borne odours are regularly used by the desert ants to localise food sources (Wolf & Wehner 2005, J Exp Biol 208, 4223-4230). In particular, inconspicuous but plentiful, and thus reliably visited, food sources are not approached directly. The ants rather steer downwind of the feeder where they reliably encounter the odour plume emanating from the food. By following the plume the ants safely reach the source.

The homing paths of successful foragers, by contrast, appeared to be guided by odour cues only to a minor extent, if at all, in our present experiments (below). Ants commonly approached the nest directly, even if no obvious landmarks were available for orientation, although approaches into the downwind side of the nest were occasionally observed. It remains to be examined whether or not these direct approaches are guided by familiar, small and inconspicuous surface structures on the desert floor (Seidl &Wehner 2006, J Exp Biol 209, 3336–3344). Direct return trajectories may be elicited by strong homing motivation in animals that have collected a food item. Ants returning without food, e.g. due to the experimental disturbances, exhibited more devious homebound paths that sometimes included search loops.

The primary goal of our present study was examination of the sensory basis of wind perception with behavioural experiments, namely, the possible role of the different antennal joints in wind orientation (e.g. Linsenmair 1973, Fortschr Zool. 21, 59–79). The ants' foraging visits to a well-known food source and their return journey were recorded, with and without the potentially relevant wind receptors incapacitated. We immobilised the joints of the ant antenna with cyanoacrylate glue, investigating the three joints between head capsule, scape, pedicel, and flagellum separately and in combination.

Animals that had the two joints between head, scape and pedicel immobilised never left the nest again for foraging. Immobilisation of these antennal joints thus appears to incapacitate the ants in ways beyond interference with wind orientation, e.g. regarding social interaction in the nest.

Any other immobilisations of antennal joints resulted in more devious approaches to the feeder. In particular the odour-guided final approach from the downwind area to the food source was tortuous and winding rather than well-oriented. This is illustrated in the figure below by superposition of three sample trajectories. The foraging trip of an intact ant (solid grey line) illustrates the initial approach into the area downwind of the food, and the odour-guided final approach against the wind. Immobilising the joints between head capsule & scape (dotted line) or scape & pedicel (not shown), or the two joints between scape, pedicel and flagellum (dashed line) all compromised the accuracy of the feeder approach. In summary, our data suggest that normal antennal movements and thus all antennal segments contribute to wind orientation.

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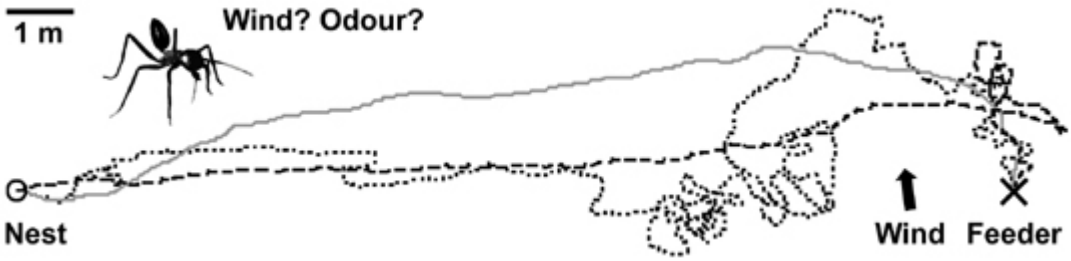
1 m
Wind? Odour?
Nest
Wind
Feeder

# Signal transmission in the rat's barrel cortex is modulated by ongoing cortical dynamics under anesthesia.

Christiane Vahle-Hinz[1], Andreas K. Engel[1]

[1]Dept. of Neurophysiology and Pathophysiology, University Medical Center Hamburg-Eppendorf, 20246 Hamburg, Germany

The encoding properties of cortical neurons are modulated by the physiological state of the cortical network, which in turn is greatly altered during general anesthesia albeit in an unknown manner. Different anesthetics may exert differential effects and may elicit fluctuations in the pattern of EEG activity, such as the well-known "ketamine-bursts". We utilized these anesthetic-induced EEG fluctuations to analyze the effects of changes in cortical dynamics on whisker-evoked responses in the barrel cortex.

Extracellular single- and multi-unit activities and local field potentials (LFPs) as well as the electrocorticogram (ECoG) were recorded in ketamine- or isoflurane-anesthetized rats. Vibratory stimuli were applied to single whiskers at frequencies of 20-700 Hz for 1 s. The frequency components of the LFP and spike response activities were analyzed from time-frequency plots obtained by applying fast Fourier transform (FFT) to the average of 50 consecutive responses, resulting in a measure of the stimulus-locked (evoked) activity. Non-locked (induced) oscillatory activity was revealed by applying FFT analysis to single trial responses before averaging. In addition, prestimulus epochs as well as the concurrent epochs of the ECoG were analyzed.

Whisker vibration elicited phase-locked LFP and spike activities continuously over the 1-s stimulation period for all frequencies tested. These sustained responses occurred, however, only during ECoG states which were characterized by decreased power in low frequency components (delta band) and additional activity in medium- (theta band) as well as high-frequency components (beta and gamma bands). An ECoG state characterized by high power in low frequency components (<4 Hz), in contrast, was reflected in low frequency LFP oscillations and ongoing spike activity at variable rates that was unrelated to whisker vibration. A burst-suppression ECoG pattern with short burst intervals was reflected in a similar pattern of LFP activity, which at stimulus onset changed for about 500 ms to high amplitude low frequency oscillations (about 15 Hz) followed by faster low amplitude activity related to stimulus frequency. The corresponding spike activity followed this pattern or was reduced to a mere on-response. When the ECoG burst intervals increased, LFP and spike activities were restricted to burst periods.

The results show that concomitant changes in ECoG, LFP and spike activities may occur periodically within minutes and arise with experimentally increased anesthetic depth. Encoding of sustained stimuli in the barrel cortex under anesthesia requires stable population activity involving frequency components in the theta, beta and gamma bands. Our results clearly demonstrate the malleability of sensory responses by ongoing cortical dynamics.

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# Single-neuron stimulation in barrel somatosensory cortex: assessing the sensory effects of action potential number and frequency

Guy Doron[1], Michael Brecht[1]

[1]Humboldt University Berlin, Bernstein Center for Computational Neuroscience, Berlin, Germany

We recently showed that individual neurons in the rat barrel somatosensory cortex can have an impact on behavioral responses in a detection task (Houweling and Brecht, 2008). In the previous study our strategy was to evoke as many action potentials (APs) as possible in a single neuron in a 200 ms period. Here we extend these findings by asking how the sensory effect of single-cell stimulation depends on AP number and AP frequency. In preparatory experiments in anesthetized rats we first established that the juxtacellular nanostimulation technique can be used to parametrically control AP frequency and AP number (Houweling et al., 2010). Specifically, we report that AP number in barrel cortex neurons varies linearly both with stimulus intensity and stimulus duration. This allows us to generate a fixed number of APs at varying frequencies, or a variable number of APs at one single frequency. Here we apply a parametric nanostimulation approach to cortical neurons of awake behaving head-immobilized rats. As before, animals are first trained to report (via tongue licks) trains of microstimulation pulses applied to the barrel cortex. Detection thresholds for microstimulation decrease over a period of a few days to currents of 2- 5 µA. Once animals reach asymptotic performance, microstimulation trials are randomly interleaved with trials in which we evoke trains of APs in single cortical neurons at different durations and current intensities. Specifically we examine the effects of AP number using current durations of 100, 200 and 400 ms, and we examine the effects of AP frequency by generating a fixed number of APs using different current intensities. Currently we are assessing the sensory effects of AP number and AP frequency in our detection task. The preliminary data suggest that single-cell stimulation effects vary with the frequency and number of evoked APs.

# Smad interacting protein-1 (Zfhx1b) affects peripheral sensitisation in acute and inflammatory pain

Bruno Pradier[1], Ildikò Racz[1,3], Monika Jeub[2], Astrid Markert[1], Daniela Mauer[1], Valérie Gailus-Durner[3], Helmut Fuchs[3], Martin Hrabé de Angelis[3], Danny Huylebroeck[4], Heinz Beck[2], Andreas Zimmer[1,3]

[1]Institute of Molecular Psychiatry, University of Bonn, Germany

[2]Laboratory for experimental epileptology, University of Bonn, Germany

[3]German Mouse Clinic, Helmholtz-Zentrum, Munich, Germany

[4]Laboratory of Molecular Biology (Celgen), Department of Molecular and Developmental Genetics, Flanders Institute of Biotechnology, Leuven, Belgium

Smad-interacting protein-1 (Sip1, Zfhx1b) is a transcription factor that plays an important role in neuronal development and is involved in the aetiology of the Mowat-Wilson Syndrome. A corresponding mouse model carrying a heterozygous Zfhx1b deletion was comprehensively analyzed in the German Mouse Clinic. The most prominent phenotype was a reduced pain sensitivity.

In this study we investigated the Zfhx1b heterozygous animals in models of acute and chronic pain. To examine the nociceptive transmission of primary sensory dorsal root ganglia (DRG) neurons we determined the neuronal activation upon painful stimulation in the spinal dorsal horn. Next, we characterised the neuronal cell population in DRGs to study the involvement of the Zfhx1b mutation in peripheral nociception.

The present data show that Zfhx1b is involved in the development of primary sensory DRG neurons, especially of C- and Ad fibres. These alterations contribute to a hypoalgesic phenotype in heterozygous Zfhx1b mice. Further, the transcription factor modulates peripheral sensitisation of thermal and chemical nociceptors under acute and formalin-induced pain conditions.

# THE ROLE OF CB2 RECEPTORS IN INFLAMMATORY PAIN

Ildikó Rácz[1], Astrid Markert[1], Jürg Gertsch[2], Andreas Zimmer[1]

[1] Institute of Molecular Psychiatry, University of Bonn, 53125 Bonn, Germany
[2] Institute of Biochemistry and Molecular Medicine, University of Bern, CH-3012 Bern, Switzerland

Infection, fracture, arthritis and autoimmune diseases elicit inflammatory pain. Inflammation can lead to a sensitization of peripheral nociceptors or can sensitize the spinal neurons causing hypersensitivity and spontaneous pain. Recently it was shown that $CB_2$ receptor agonists have analgesic effect in a tissue injury model of persistent pain. In this study we investigated the role of $CB_2$ receptors in the development of inflammatory pain. For this we examined the pain reaction of $CB_2$ receptor knockout and wild type animals in the formalin test. Furthermore, we studied the analgesic effect of a natural $CB_2$ agonist, E -ß-caryophyllene ((E) -BCP), in formalin-induced inflammatory pain. (E)-BCP is a newly identified $CB_2$ receptor agonist that is found in the essential oils of different spices and food plants. JWH -133, a highly selective $CB_2$ receptor agonist, was used as reference compound. Wild type and $CB_2$ knockout animals showed the same pain reactions in the early and late phase of the formalin test. Both strains presented a thermal hyperalgesia one hour after formalin treatment. Orally administered (E)-BCP caused a significant analgesic effect in the late phase of the formalin test in wild type animals, but had no effect in mice lacking CB2 receptors. In comparison to JWH-133, (E)-BCP was more effective in this inflammatory pain model.
Our results suggest that $CB_2$ receptors do not play a direct role in the development of formalin-induced inflammatory pain. However, $CB_2$ receptor agonists, (E) -BCP and JWH -133, are potential analgesic substances for the treatment of inflammatory pain.

# Transgenic mice expressing affinity-tagged fluorescent P2X2 receptors

Marcus Grohmann[1], Tanja Nußbaum[2], Ralf Hausmann[2], HaiHong Wang[3], Ronald Naumann[4], Heike Franke[1], Günther Schmalzing[2]

[1]Rudolf Boehm Institute of Pharmacology and Toxicology, University of Leipzig, Härtelstraße 16-18, 04107 Leipzig, Germany; [2]Molecular Pharmacology, RWTH Aachen University, Wendlingweg 2, 52074 Aachen, Germany; [3]Department of Physiology Medical School, Tongji University, Shanghai, China; [4]Transgenic Core Facility, MPI of Molecular Cell Biology and Genetics, Dresden, Germany.

Increased understanding of the neurobiology of pain gives the chance to translate these mechanistic insights into better diagnosis and treatment. Therefore knowledge of the precise tissue distribution of specific receptors is crucial to understand pain processing. The involvement of purinergic receptors in nociception has attracted prominent attention. Extensive evidence supports particular an important role for P2X2 receptors in sensory neurotransmission.

To facilitate the morphological and functional identification of neurons carrying P2X2 receptors, we have generated a transgenic mouse strain, expressing a red fluorescent P2X2 receptor subunit as fusion protein including two affinity tags under the control of its own promoter. This mouse model was generated by recombination-mediated genetic engineering using the suitability of bacterial artificial chromosomes (BACs), an efficient method to construct vectors for subsequent manipulation of the mouse genome.

The expressed fluorescence will allow for a comprehensive microscopic mapping of the distribution of P2X2 receptors in vivo and guide the identification of neurons for functional analysis. The tandem affinity tags can be exploited to isolate these receptors from native tissues for biochemical analysis and co-purification of receptor-interacting proteins for mass spectrometric identification.

In summary, this transgenic mouse model should provide a rich resource to enable new insights into P2 receptor localization and function in developmental, physiological and pathophysiological processes.

# Trigeminal sensory interneurone responses to skin stimuli and their inhibitory modulation in hatchling tadpoles of *Xenopus laevis*

Edgar Buhl[1], Stephen R. Soffe[1], Alan Roberts[1]

[1]University of Bristol, School of Biological Sciences, Woodland Road, BS8 1UG Bristol, United Kingdom

In most vertebrates the exact neuronal pathways controlling the initiation and termination of locomotion after sensory stimulation remain unclear. Hatchling tadpoles start swimming some 20-30 ms after their head skin is touched. Broader pressure to the head skin will stop ongoing swimming. These stimuli excite trigeminal sensory neurones projecting into the hindbrain. As in other vertebrates, the trigeminal touch and pressure afferents are presumed to activate trigeminal sensory pathway interneurones. Lesion studies suggest that these distribute information to both sides of the body (Boothby and Roberts, 1992) but the anatomy and response properties of these sensory pathways have not been defined. We have investigated sensory pathway neurones to reveal how trigeminal sensory input can activate reticulospinal neurones. Evidence in the tadpole has shown that some reticulospinal neurones generate the swimming rhythm and drive spinal neurone firing during swimming (Soffe et al., 2009) while others turn off swimming by releasing GABA (Perrins et al., 2002).

We recorded the responses of rostral hindbrain neurones in the 2nd to 4th rhombomeres to 0.1-0.5 ms electrical stimulation of head skin on left and right sides. We used whole-cell patch recordings under visual control in intact immobilised tadpoles while recording from ventral roots to monitor motor responses. By filling the cells with neurobiotin, we could define their morphology after the experiment. This study focused on neurones excited at short latencies (<5 ms) following head skin stimulation which are compatible with direct synaptic contact from trigeminal afferents. Nearly all these neurones fired repetitively to depolarising current injection.

EPSPs evoked in first order sensory interneurones were large (>10mV), increased with ipsilateral stimulus intensity and usually evoked firing. Neurones could be separated into 2 groups. Most responded with a single spike to a stimulus and they received early IPSPs (at 10-20 ms) which stopped further firing. Other neurones fired multiple spikes until their firing was stopped by strong IPSPs after 30-50 ms. Some neurones also responded to contralateral head skin stimulation. Neurones of both types were inhibited during fictive swimming activity which was often evoked by head stimulation. Bilateral activation of reticulospinal neurones is possible as some of the recorded neurones had an ipsilateral descending axon whereas others crossed to the other side to ascend and descend.

Our study begins to define the response properties of trigeminal sensory pathway interneurones and shows that their firing to sensory stimulation is controlled by inhibition. This inhibition can occur at short latency indicating that some primary sensory trigeminal interneurones are inhibitory. Further inhibition from the pattern generating network for swimming modulates sensory pathways during locomotion.

*Boothby KM, Roberts A (1992) The stopping response of Xenopus laevis embryos: pharmacology and intracellular physiology of rhythmic spinal neurones and hindbrain neurones. J Exp Biol 169:65-86.*

*Perrins R, Walford A, Roberts A (2002) Sensory activation and role of inhibitory reticulospinal neurons that stop swimming in hatchling frog tadpoles. J Neurosci 22:4229-4240.*

*Soffe SR, Roberts A, Li WC (2009) Defining the excitatory neurons that drive the locomotor rhythm in a simple vertebrate: insights into the origin of reticulospinal control. J Physiol 587:4829-4844.*

# Variability in the encoding of low- and high-frequency whisker vibrations in the barrel cortex of the awake rat.

Sina Sieler[1], Maik C. Stüttgen[2], Cornelius Schwarz[2], Andreas K. Engel[1], Christiane Vahle-Hinz[1]

[1]University Medical Center Hamburg-Eppendorf, Dept. of Neurophysiology and Pathophysiology, 20246 Hamburg
[2]Dept. Cognitive Neurology, Hertie-Institute Clinical Brain Research, 72076 Tübingen, Germany

The whisker-to-barrel system of rats and mice is widely used to study coding of surface textures by the somatosensory system during tactile perception and discrimination. It is not yet known how high-frequency whisker vibrations resulting from contact with various textures are encoded in the barrel cortex. Precise control of stimulus presentation over long periods, as required for detailed analysis of relevant stimulus parameters, is only achieved when the animal is not moving, for example under anesthesia. General anesthesia however, alters the encoding properties of cortical neurons in an unknown manner. To avoid these anesthetic effects, we recorded responses to whisker stimulation in awake, head-fixed rats.

Rats were chronically implanted with mobile multielectrode arrays that recorded multi-unit activity and local field potentials (LFPs) in the barrel cortex. Rats were habituated to stress-free head fixation and could be recorded in sessions lasting up to 30 min (Stüttgen & Schwarz, 2008). A feedback-controlled electromechanical stimulator or a piezoelectric stimulator was used for 1-s vibrations of a single whisker at 20, 65, 130, 230, 420, and 620 Hz applied pseudorandom in rostrocaudal direction. Fifty responses to each kind of stimulus were analyzed. Peristimulus time histograms were generated for spikes responses; LFPs were averaged. The phase-locking to the cycles of the sinusoidal whisker movements was analyzed in phase histograms generated from time stamps of LFPs and spikes.

Whisker vibration elicited tightly correlated LFP and spike activities in the awake barrel cortex and sequences of 1:1 stimulus locking were present throughout the 1-s stimulus epoch for the entire range of frequencies tested. However, we observed different types of responses: while nearly all units showed a prominent on-response, in most units this was followed by significantly elevated firing rates as compared to prestimulus period. In contrast, other units showed sustained firing rates that were lower than prestimulus activity. Regardless of the type of response, spikes occurred phase-locked to the vibratory cycles. In addition, many units responded to stimulation offset, either with increased or decreased firing rates.

We further tested whether trial-to-trial variations in evoked responses were due to changes in activity just before the stimulus came on. To this end, the correlation was examined between spike rates during prestimulus and response epochs on a single trial basis. Only for those units that showed an elevated firing rate after the on-response a positive correlation was found.

The results show that low- and high-frequency components of whisker movements are encoded in a temporally precise manner in the awake barrel cortex for up to a second, and thus, are features available for texture discrimination. Specific stimulus parameters are represented by particular neuronal responses and the variability in response strength may be modulated by cortical state changes that are reflected in the prestimulus discharge rate.

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# Poster Topic

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T21-14C Passive biomechanical properties and spike-movement transfer in an insect limb joint
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# A Novel Giant, Non-Cholinergic Neuron in the Ventrolateral Striatum: Implications For Functional Specificity and Selective Vulnerability

Lydia Lebenheim[1], Christian Derst[1], Torsten Weiß[1], Clemens Gruber[1], Daniel Scott Zahm[2]

[1]Charité, Institut für Integrative Neuroanatomie, Philippstraße 12, 10115 Berlin, Germany

[2]St Louis University, School of Medicine, Pharmacological and Physiological Science, 1402 S Grand Blvd,Saint Louis, MO 63104-1083, USA

The striatum is the main input structure of the basal ganglia. Dorsal striatum is divided into histochemically distinct patches and a surrounding matrix. Focusing on the matrix, cortical afferents are topographically organized with separate zones of vibrissal, forelimb, and hindlimb inputs, which altogether occupy the dorsolateral quadrant of the dorsal striatum. In contrast, the ventrolateral striatum is related to the orofacial cortex and involved in orofacial movements.

More than ninety percent of the neurons of the rodent striatum comprise two types of medium spiny neuron, the GABAergic projection neurons of the direct and indirect pathways. In addition to these output neurons the striatum uses several types of interneurons. There are the giant cholinergic interneurons and three types of GABAergic cells, fast spiking (FS) parvalbumin positive interneurons, low-threshold spiking (LSN) somatostatin-, neuropeptide Y-, and nitric oxide synthase-positive interneurons and calretinin-positive interneurons (for review see Kreitzer, 2009, Ann Rev Neurosci 32,127-147).

Here we describe a novel giant, non-cholinergic neuron specifically localized in the ventrolateral striatum. Morphological analysis of rodent and primate brain sections using in-situ-hybridization and immunocytochemical single and double staining disclosed that the novel giant neuron is GABAergic, contains parvalbumin, and, unlike other parvalbumin neurons in the striatum, robustly expresses the Kv3.3 potassium channel protein. Patch clamp experiments confirmed Kv3-like electrophysiological properties and the non-cholinergic nature of this cell type. The ventrolateral striatum is apparently involved in orofacial movement disorders like tardive dyskinesia. The exclusive localization of this novel giant neuron within the ventrolateral striatum may contribute to the selective vulnerability of this area, which results in dyskinetic orofacial movements.

# Axonal calcium imaging reveals spatiotemporal clustering of parallel fiber activation *in vivo*

Christian D Wilms[1], Michael Häusser[1]

[1]University College London, Wolfson Institute for Biomedical Research, Gower Street, WC1E 6BT London, United Kingdom

Parallel fibers, the predominant structure of cerebellar granule cell axons, represent the main excitatory input to Purkinje cells, with ~175,000 parallel fibers synapsing on each Purkinje cell. Work in slices has shown that key aspects of parallel fiber function – such as their ability to trigger synaptic plasticity, dendritic spikes, and modulate action potential output – depend on the spatial pattern of their activation across the Purkinje cell dendritic tree. To understand how they contribute to information processing and memory storage by the cerebellar cortex it is thus necessary to determine the spatial pattern of parallel fiber activity triggered by sensory stimulation.

We addressed this question using *in vivo* two-photon imaging of presynaptic calcium transients across multiple individual parallel fibers of folium Crus II in lightly anaesthetized mice. Six adjoining regions (~60 µm in diameter) in the granule cell layer were bulk electroporated with the calcium indicator dye Oregon Green BAPTA-1. The resulting labeling of parallel fibers was sparse enough to allow discrimination of individual fibers, while allowing simultaneous imaging of up to 21 fibers within a ~60 µm field of view.

Spontaneous activity of individual fibers was low (0.51 ± 0.42 Hz; range 0.06 - 1.6 Hz; n = 28), consistent with patch-clamp recordings from GCs in vivo. Sensory stimulation (air puffs to the perioral region) triggered large calcium transients in single parallel fibers, presumably corresponding to axonal action potentials. High-speed linescans (1kHz) could resolve short bursts of action potentials underlying most sensory evoked responses, consistent with previous in vivo recordings from single granule cells. In total only a small percentage of stained fibers responded to any given sensory stimulus.

Population imaging of parallel fibers revealed that parallel fibers activated by sensory stimuli had a strong tendency to be aggregated ($p < 0.005$). Bootstrap analysis of responses to trains of stimuli revealed that ~30% of the imaged fiber pairs were tightly coupled – suggesting activation by the same mossy fiber. An additional ~30% of the pairs – despite responding to the same stimulus – were independent of each other. This opens the possibility that coupling of responses in spatial clusters of parallel fibers can be adjusted dynamically.

Given our knowledge on Purkinje cell function, parallel fiber clustering is expected to be a major determinant of cerebellar information processing, opening new possibilities for pattern recognition as well as learning and memory.

# CHRONIC DBS OF THE ENTOPEDUNCULAR NUCLEUS OR THE CM-PF COMPLEX IN THE RAT 6-HYDROXYDOPAMINE PARKINSON MODEL IMPROVE LEVODOPA-INDUCED DYSKINESIAS

Mesbah Alam[1], Kerstin Schwabe[1], Joachim Kurt Krauss[1], Hans-Holger Capelle[1]

[1]MHH, Neurosergery, Carl Neubergstr.1, 30625 Hannover, Germany[1]

Objective: Deep brain stimulation (DBS) is used in Parkinson's disease (PD) with levodopa-induced dyskinesias and fluctuations. The subthalamic nucleus (STN) and the globus pallidus internus (GPi) are standard targets in men. More recently, also the centermedian-parafascicular complex (CM-Pf) has been discussed as a possible target. We studied the effect of chronic DBS of the CM -Pf and the entopeduncular nucleus (EPN, the rat equivalent to the human GPi on levodopa-induced dyskinesias and local field potentials (LFPs) in the 6-OHDA rat model of PD.

Methods: Unilateral nigrostriatal dopamine lesions were induced by injection of 6-OHDA in the medial forebrain bundle of female Sprague -Dawley rats (n=14). Subsequently, these rats were rendered dyskinetic by regular intraperitoneal injections of levodopa. Ipsilateral to the lesion, bipolar electrodes for stimulation or recording of LFPs were implanted in the EPN, the CM-Pf and the dorsolateral striatum. After recovery individual thresholds for side effects were determined. Thereafter, EPN, CM-Pf or sham DBS was applied for five days (130Hz, 80µs) and the effect of DBS evaluated on levodopa -induced dyskinesias. Additionally, LFPs were recorded in the striatum before and after the onset of levodopa-induced dyskinesias.

Results: Both, EPN and CM-Pf DBS improved dyskinesias. In the striatum the LFP activity in the beta frequency band was reduced after injection of levodopa, while EPN and CM-Pf stimulation had no effect on beta oscillation.

Conclusions: Our results suggest that DBS of the EPN and CM-Pf exerts its therapeutic effect on levodopa-induced dyskinesias in this PD model independent from its effect on beta activity.

# Decision-making between two grasp types modulated by different reward values in Area AIP and F5 of macaque monkey

Benjamin Wellner[1], Alexandra Wellner[2], Hansjoerg Scherberger[3]

[1]German Primate Center, Neurobiology, Kellnerweg 4, 37077 Goettingen, Germany

In a natural environment, primates often have various options or alternatives to grasp objects. To better understand the decision making process for hand grasping, we investigated grip-type selection in the anterior intraparietal area (AIP) and the ventral premotor cortex (area F5) of macaque monkeys, which both generate early hand grasping instructions.

Macaque monkeys were trained to grasp a handle with one of two possible instructed grip types, or to freely choose between the two grip types to receive a reward. Trials started after the monkey placed both hands on resting positions and fixated a red fixation LED (fixation period). After 600 to 1000 ms, a second LED (cue period) was shown to instruct the monkey about the grip type (cue period). In the instructed task, either a green or a white LED appeared, indicating to perform a power or a precision grip. In the free-choice task, both LEDs lit up, indicating that the monkey was free to choose between the two grip types. After that, the monkey had to memorize the instruction for about one second (memory period). By switching off the fixation LED, the animal was instructed to reach and grasp the target (movement period) in order to receive a liquid reward. During free choice trials, a baiting system modified the reward expectancy to let the animal either select both grip types equally often, or to select predominantly power grips or precision grips. All trials were presented randomly interleaved and in total darkness.

Using permanently implanted electrodes in AIP and F5, we recorded single - and multi -unit spiking activity simultaneously from 128 channels while the animal performed both tasks. From 202 single neurons recorded in 4 recording sessions in a first animal, 70% of the cells were significantly tuned for grip type in at least one task period: cue, memory, or movement. The percentage of significantly tuned neurons exceeded 60% during the memory period. Furthermore, there were only little significant differences between free choice and instructed trials. Some neurons showed clear modulation with respect to altered reward expectations. Analysis of simultaneously recorded multi-unit activity from the same channels showed similar results.

These data suggest that the coding of grasp types in AIP and F5 is quite comparable during instructed and free-choice tasks, and its modulation with reward expectation could indicate a leading role of AIP and F5 for the decision making process for hand grasping. The ability to predict intended grasp actions from AIP and F5 even in free-choice tasks might furthermore be useful for future neural prosthetic applications.

# DECODING THE BEHAVIOR OF LARGE POPULATIONS OF MOTORNEURONS IN HUMANS

Silvia Muceli[1], Francesco Negro[1,2], Wigand Poppendieck[3], Thomas Doerge[3], Dario Farina[1,2]

[1]Center for Sensory-Motor Interaction, Department of Health Science and Technology, Aalborg University, Aalborg, Denmark, 9220

[2]Department of Neurorehabilitation Engineering, Bernstein Center for Computational Neuroscience, Georg-August University of Göttingen, Göttingen, Germany, 37075

[3]Fraunhofer Institute for Biomedical Engineering, Department Medical Engineering & Neuroprosthetics, St. Ingbert, Germany, 66386

The in vivo analysis of motor unit (MU) electrical activities from electromyographic (EMG) recordings provides a window into the outflow of motor neurons to muscles because of the association between action potentials discharged by each motor neuron and action potentials generated by the innervated muscle fibers. Although methods for recording MU activities in humans have been available for long time [1], a classic limitation of these methods is that they allow the concurrent analysis on only a very small fraction of the active motorneurons. This is due to the high spatial selectivity of indwelling electrodes (wires or needles). To circumvent this limitation, we have developed high-density systems for EMG recordings, consisting of several electrodes closely located to form a spatial sampling of the muscle fiber electrical activity. These systems are either invasive or non-invasive. Invasive high-density electrodes that we developed are based on the thin-film technology [2],[3], that allows depositing electrode contacts on a small wire (thickness of 10 μm). A current system developed for in vivo human studies consists of 16 electrodes in a single micro-wire that can be inserted into the muscle with a needle [3]. Non-invasive systems consist in high-density grids of surface EMG electrodes that can contain hundreds of electrodes at a distance of a few millimeters between each other, forming a regular or irregular spatial sampling of the surface electrical potential generated by the muscle fibers.

Signals recorded by high-density EMG systems, either invasively or non-invasively, are processed with decomposition algorithms that identify the sources from the interference patterns. Current algorithms are based either on template matching (e.g., [4]), with optimal solutions of overlapping action potentials [5], or blind source separation techniques [6].

With these methods, it is possible for the first time to record from large populations of motorneurons and thus answer open questions on motorneuron and motor unit physiology. We present here an example of this analysis by showing concurrently recorded high-density intramuscular and surface EMG activity from one subject during various contraction paradigms: i) 10 s isometric contractions of the abductor digiti minimi muscle up to 50 % of the maximal force (MVC) with 5 % MVC intervals, ii) ramped contractions up to the same force levels as in (i), iii) 1 min sustained contractions at 5- 10 % MVC, iv) a contraction with a freely varying force profile. Intramuscular EMG was recorded with the thin-film multichannel electrode described in [3] and surface EMG signals were detected with 13×5 electrode grids (Department of Neurorehabilitation Engineering, University of Göttingen and Politecnico di Torino, Italy) with 2.5 mm interelectrode spacing. Intramuscular and surface signals were decomposed using the methods described in [4] and [6],[7]. In this example, we show that more than 50 MUs can be identified concurrently during natural tasks. For each MU, it is possible to estimate, from the decoded spike train, the mean and peak discharge rate, coefficient of variation of interspike intervals and correlation measures (e.g., common drive index, common input strength).

In conclusion, the proposed methodologies allow in vivo investigation in humans of a large proportion of the motor units active during voluntary contractions.

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# Descending control of turning in the stick insect *Carausius morosus*

Matthias Gruhn[1], Anke Borgmann[1,2], Philipp Rosenbaum[1], Ansgar Büschges[1]

[1]Universität zu Köln, Abt. Tierphysiologie, Biozentrum, Zülpicher Str. 47b, 50674 Köln, Germany

[2]Cellular and Systems Neurobiology Section, Laboratory of Neural Control, NINDS Building 35, 35 Convent Drive, MSC 3700, Bethesda, MD 20892-3700

Descending control from cerebral ganglia is known to play a major role in generating adaptations in the behavior of insects. Despite the substantial knowledge on the neural mechanisms that contribute to the generation of the basic neural locomotor activity patterns, only little is known on which and how descending signals from the brain modify local leg motor networks that are involved in the coordinated activation of the proper leg muscles in adaptive locomotor behavior. We studied this question in the stick insect walking system, in which the necessity for such modifications becomes most apparent during turning, where contralateral legs generate drastically different movement kinematics. We first investigated the motor output of the stick insect leg muscle control system during turning movements of the animal. In order to unravel the role of descending signals on the motor activity generated, we then recorded the activity of leg motoneurons of the main three leg joints in the otherwise deafferented mesothoracic segment, while the front legs perform optomotor response induced turning on the slippery surface.

During inside turning of an ipsilateral front leg, mesothoracic retractor motor neurons showed relatively little, yet rhythmic activity that is mostly in phase with the stance phase of the front leg as monitored by flexor EMG activity (N=10, n=20/28). Protractor motor neurons generally also showed rhythmic activity, generally in antiphase to the retractor activity during the entire walking bout (N=21, n=85/94). During outside turning in the ipsilateral front leg, however, mesothoracic retractor motoneurons showed marked tonic activity with some phasic modulation (n=31/33), while the protractor showed mostly tonic activity with only occasional phasic modulation (n=69/100). Motor neurons, supplying the *levator trochanteris*, which lifts the leg at the coxa-trochanter joint, showed similar activity under both conditions. With a front leg stepping as an inside or as an outside leg, levator activity observed was usually tonic with some phasic modulation riding on top of the activity (N=4, n=16/17). Finally, activity of extensor motoneurons of the femur-tibia joint (N=17) differed again, depending on the function of the leg. When the ipsilateral front leg was stepping as an inside leg, activity of the extensor motoneurons was usually rhythmic, however, with variable phase relationship to the stance phase of the front leg (n=30/39). When the ipsilateral front leg was performing as an outside leg, on the other hand, tibial extensor activity was mostly tonic with occasional phasic modulation (n=31/54).

Our findings demonstrate that motor output in the deafferented mesothoracic ganglion changes depending on the leg function as an inside or outside leg in the context of turning, most likely as a result of changed descending information from more anterior ganglia. There is no indication, however, that the descending information is sufficient to shape the motor activity into an appropriate pattern of coordinated activity between the functional leg joints. Supp. by DFG grants Bu857/9,10.

# Descending unpaired neurons of the suboesophageal ganglion in *Locusta migratoria* and *Manduca sexta* and their sensory input

Jessika Erdmann [1], Hans-Joachim Pflüger[1], Elisabeth Lipke[2], Peter Bräunig[2]

[1]Freie Universität Berlin, Institut für Biologie-Neurobiologie, Königin-Luise Straße 28/30, 14195 Berlin, Germany

[2]RWTH Aachen University, Institute for Biology II, Unit for Developmental Biology and Morphology of Animals, Mies-van-der-Rohe Straße 15, 52074 Aachen, Germany

The suboesophageal ganglion (SOG) is a higher centre for motor coordination in insects. It receives and processes input from the brain and the segmental ganglia of the ventral nerve cord. Octopamine, a major amine in the insect nervous system, is considered to play an important role in initiation and maintenance of motor programs.
Typical efferent unpaired octopaminergic median neurons of the segmental ganglia are lacking within the SOG. Instead, it contains two main populations of octopaminergic intersegmental unpaired median (UM) neurons: ascending UM neurons innervating principal brain neuropils and descending UM neurons projecting towards the thoracic ganglia. Electrophysiological and neuroanatomical evidence suggests that at least some of these neurons descend to the terminal ganglion with broad arborizations in each segmental ganglion. As little is known about their sensory input, we started a comparative electrophysiological study of descending UM neurons of the SOG in the hemimetabolous adult *Locusta migratoria* and holometabolous *Manduca sexta* larvae. We tested possible sensory inputs from thoracic and leg receptors by electrical stimulation of certain nerves of the thoracic ganglia and simultaneous intracellular recordings from single identified descending UM neurons of the SOG. Furthermore, we stimulated nerves of the mouthparts to investigate possible connections between the mouthpart's receptors and these neurons.

# Distance estimation in desert ants, *Cataglyphis fortis* – what role plays ventral optic flow?

Matthias Wittlinger[1], Harald Wolf[1]

[1]Universität Ulm, Institut für Neurobiologie, Albert-Einstein-Allee 11, 89069 Ulm, Germany

Desert ants, *Cataglyphis*, use path integration to return to their nest after a foraging trip. This navigation feat requires continuous updating of a home vector from direction and distance measurements. The directional vector component is provided by a celestial compass (Wehner 2003 J Comp Physiol A 189: 579), while distance estimation is accomplished by a stride integrator (Wittlinger et al. 2006 Science 312: 1965) that accounts for stride length and walking speed. This was demonstrated by examining homing distance after manipulating the ants' leg lengths (Fig.A, „stilts and stumps") and resulting changes in stride lengths.

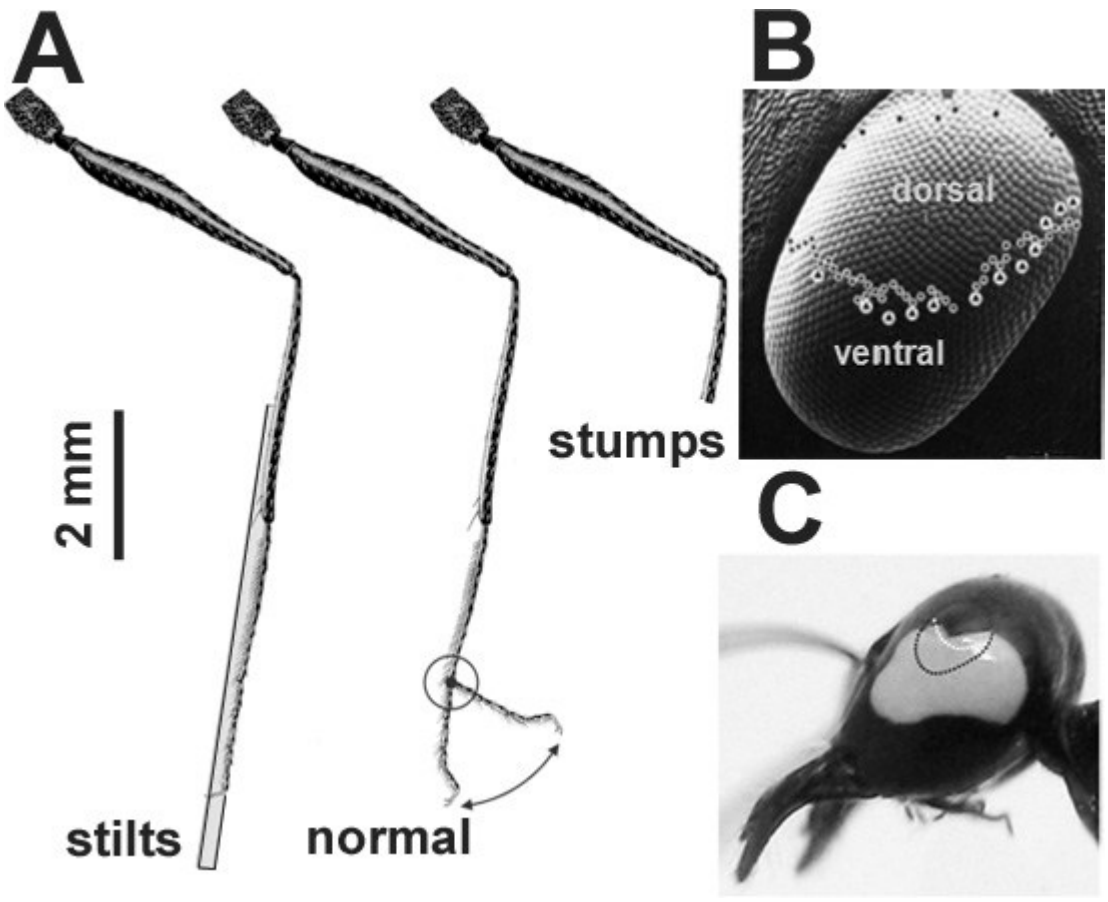
Previous research had indicated that ventral optic flow also feeds into the distance estimator at least to a small extend (Ronacher and Wehner 1995 J Comp Physiol A 177: 21). And indeed, in the "stilts-and-stumps" experiments, slight differences in homing distance were observed compared to predictions derived from altered stride lengths. Over-estimation of homing distance by ants with elongated legs ("stilts") was 18.5% greater, and ants with shortened legs ("stumps") underestimated distance by 15.9%. I.e. ants on stilts ran too far and ants on stumps, too short, all groups searching a bit farther from the nest–feeder distance than would have been expected from the imposed changes in stride lengths (Wittlinger et al. 2007 J Exp Biol: 198).

A possible explanation for these deviations is a discrepancy in the effects the manipulation of leg length has on changes in stride length and changes in body height – with body height above ground determining eye-to-substrate distance, and thus ventral optic flow. Due to the Z-shape of the ant leg, stilted ants may indeed be elevated more, and stumped ants lowered down more, than corresponds to the changes in stride length brought about by the manipulation of leg length, disproportionately increasing ventral optic flow. This line of argument holds true although we tried to minimise ventral optic flow cues in the original experiments by choosing a featureless and visually homogeneous channel set-up.

In the present study we replicated the above stilts-and-stumps experiments. But instead of just minimizing visual cues by providing a featureless set-up, we fully excluded the perception of ventral optic flow by painting over the ventral halves of the ants' eyes (Fig.B, C). In this situation, the ants searched for the nest entrance in exactly the predicted homing distances. As a control, we analyzed the walking behavior (e.g. stride length-to-stride frequency relationships) of normal ants and ants with ventrally covered eyes. No differences in walking behavior were observed.

In summary, the ants' distance estimator can work without any ventral optic flow input, exclusively relying on stride integration. Additional ventral optic flow, if present, might serve a control or calibration function.

Fig.1 Experimental manipulations. A Manipulation of ant legs performed in the present study. In "stilts", attached pig bristles elongated the legs; unmodified legs in "normal", with approximate range of tarsus movement indicated; shortened legs in "stumps". The right hind leg is shown from anterior. B *Cataglyphis* compound eye with dorsal and ventral parts indicated; left is anterior. C *Cataglyphis* ant with painted ventral half of the eye (painted test group).


A
B
C
2 mm
stumps
stilts
normal
dorsal
ventral

# Distribution of tyramine- and octopamine-immunoreactivity in locust muscle.

Bettina Stocker[1], Hans-Joachim Pflüger[1]

[1]Freie Universität Berlin, Neurobiology, Königin-Luise-Str. 28-30, 14195 Berlin, Germany

Using the hemimetabolic insect *Schistocerca gregaria* (desert locust) as a model system, we investigate the influence of neuromodulation on neural networks and behaviour. We focus on the neurohormone/-modulator and monoamine octopamine (OA), which is mainly released by the unique group of insect specific dorsal/ventral unpaired median (DUM/VUM) neurons. However, not solely OA, but also its precursor tyramine (TA) is now known to serve an individual and partly OA-antagonistic function in species like *Drosophila*. Yet still many aspects of TA significance remain unresolved.

Our data elucidate the peripheral distribution of OA and TA with the help of reliable antibodies, proving OA and TA to be present and co-localised in all neuromodulatory varicosities examined on two exemplarily selected skeletal muscles, which both receive innervation by differentially activated DUM neurons. Since amounts of OA and TA within octopaminergic/tyraminergic cells of the brain and thoracic ganglia change dynamically according to the animal's pre-treatment, and thus reflect DUM neuron activity (Kononenko et al., 2009, J Comp Neurol), we examined likewise the periphery under control and tethered flight/stress conditions. A comparison of OA- and TA-IR in single varicosities indeed points to subtle changes in transmitter content due to stress, indicating that these amounts fluctuate activity-dependently at the synapse level.

Supporting the assumption that TA serves only as a precursor of OA, we find that, following TA application, twitch amplitude as well as contraction and relaxation rate of individual muscle twitches increase, but less than in response to OA - an effect which is suppressed by the OA-receptor antagonist epinastine. These facts render specific TA release and the existence of specific TA-receptors at this peripheral location unlikely.

# Electrical Microstimulation in the Superior Colliculus of the Macaque Monkey (Macaca mulatta) Causes Changes of Goal Directed Arm Movements during a Fixation Reach Task

Roland Philipp[1], Klaus-Peter Hoffmann[1]

[1]Faculty of Biology and Biotechnology, Ruhr-University Bochum, Germany

We already know that activity in the intermediate and deep layers of the macaque Superior colliculus (SC) is correlated with the initiation and execution of arm movements (Werner et al., 1997). Recent experiments in our lab showed that electrical microstimulation in the intermediate and deep layers of the SC can elicit arm movements under behaviourally free conditions (Philipp et al., 2006; FENS Abstr. vol 3, A145.16, 2006). This result raised the question whether electrical stimulation also influences the execution of already planned arm movements.

In the following experiments it was investigated whether electrical microstimulation (= 15 µA) alters the start of an intended arm movement. To decide which one is the decisive factor, the start or the end of electrical microstimulation, the stimulation protocol – stimulus duration and onset with respect to the visual go-cue – was varied. We further investigated if and how electrical microstimulation influences the endpoint of the hand on the screen.

Eye- and head-position of the head unrestrained monkey was measured by a search coil system. Arm trajectories were recorded by the miniBird-system. During experimental conditions small electrical currents (200 Hz, 100 - 600 ms duration) were applied to the SC while the monkey waited for the visual go-cue. For each experiment electrical currents were chosen just low enough not to evoke any saccade while the monkey was fixating the visual target on the screen. In each experimental condition electrical stimulation was applied at different time points (300, 400, 500 and 600 ms prior to the visual go-cue).

We found that microstimulation (1) influences the reaction times (RT) of planned arm movements. The earlier the electric current was applied the shorter the resulting RTs with respect to the onset of the visual go-cue (arm starts even before visual go-cue onset). This indicates that the monkey uses the information provided by the electric current to initiate the arm movement independent of the onset of the visual go-cue (replacement of the paradigm inherent cue). (2) Electrical microstimulation started to be most effective in depths below 2.5 mm with a median depth of 3.4 mm which matches the depth distribution of reach neurons. (3) Applied currents influenced the end point of goal directed reach movements to visual targets. They seem to pull the monkey's hand away from the actual reach target such that it remains short of the target in the direction where the monkey “wants” to look due to the stimulation site in the collicular motor map. This observation is in line with the fact that most visual receptive fields were mapped at smaller eccentricities than the reach target. There seems to be a tendency for more scattered final hand positions on the screen caused by electrical microstimulation with longer stimulation durations.

Electrical microstimulation applied prior to the execution of goal directed arm movements (i.e. within the movement preparation period) revealed that RTs as well as reach movement endpoints can be manipulated. This clearly shows that the SC has direct access to circuits for the execution of arm movements. The assumption that the SC contributes to a network providing the PMd with the necessary information needed for monitoring the planned or ongoing arm movement is reasonable. Thus, the SC might be involved in detecting and correcting inaccuracies in the actual motor plan of visually guided arm movements.

# Feature Selection Techniques: A Comparative Study

David Hofmann[1,2], Janne Hahne[3,5], Armin Biess[1,2], Bernhard Graimann[5], J. Michael Herrmann[1,2,4]

[1]Department for Nonlinear Dynamics, Max Planck Institute for Dynamics and Self-Organization, Bunsenstr. 10, 37073 Göttingen, Germany
[2]Bernstein Focus Neurotechnology, Göttingen, Germany
[3]Computer Science Department, Technische Universität Berlin, Berlin, Germany
[4]School of Informatics, University of Edinburgh, Edinburgh, United Kingdom
[5]Otto Bock HealthCare, Duderstadt, Germany

For the purpose of controlling a transradial hand prosthesis we address the question what recording sites and features of myoelectric surface signal are critical in the classification of hand postures. We employ information theoretic and heuristic approaches and compare their performance in terms of classification accuracy.

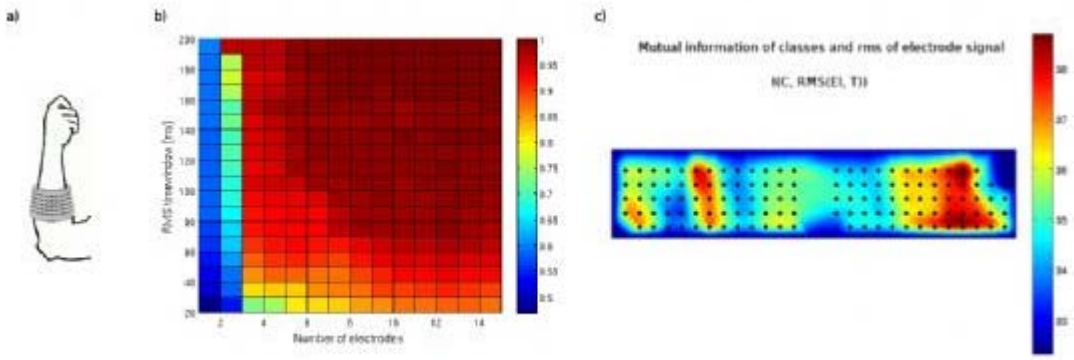
Methods: We recorded myoelectric signals from 126 monopolar channels with two high density sEMG arrays, see fig. 1a. The data were obtained from able-bodied subjects performing repeatedly eight different static contractions (hand open and close, wrist flexion, extension, abduction, adduction, pronation and supination). For each electrode $i$ the mutual information $I(C, rms(El_i, T))$ of the root mean square (rms) with time window length $T$ of its signal and the classes (hand postures) is calculated. Furthermore, we compute the pairwise mutual information $I(rms(El_i, T), rms(El_j, T))$. Using these measures, we construct an electrode selection algorithm and compare its performance in terms of classification accuracy with the electrode selection by intrinsic best feature measure of random forests and the heuristic electrode selection method sequential floating forward selection (SFFS) as well as highest power selection. For classification linear discriminant analysis (LDA) and RF are employed.

Results: Generally we find a trade-off between the number of electrodes and the length of the time window underlying the classification. Functionality of the controlled prosthesis requires recording times below 100ms such that in dependence on the data processing at least 4 to 8 electrodes are needed in order to reach a reasonable accuracy of the classification.

The performance of SFFS is comparable to the mutual information algorithm for the relevant values of the parameter $T$ (fig. 1b). The RF algorithm on the other hand results in a slightly reduced classification accuracy, but offers an approach to a feature selection which is computationally less costly then the other approaches.

Conclusion: This study is part of an exploratory data analysis that aims at optimizing the number and spatial position of electrodes for the control of complex prostheses. Practically, considerably higher numbers of electrodes are needed in order to achieve a redundant and thus more robust classification of hand postures in the presence of noise and non-stationarities.

Acknowledgment: We thank W. Paulus (Department of Clinical Neurophysiology, University of Göttingen) for providing the biosignal amplifier for this study. The project is supported by grant #01GQ0811 within the National Bernstein Network Computational Neuroscience.



# Functional organization of the primary motor cortex in congenital and chronic acquired paraplegia

Magdalini Tozakidou[1], Maria Blatow[2], Michael Akbar[3], Ernst Nennig[2], Julia Reinhardt[1], Christoph Stippich[1]

[1]University Hospital Basle, Switzerland, Department of Diagnostic and Interventional Neuroradiology, Petersgraben 4, 4031 Basel, Switzerland

[2]Department of Neuroradiology, University of Heidelberg, Medical Center, Germany

[3]Department of Orthopedic Surgery, University of Heidelberg, Medical Center, Germany

Somatotopic organization of primary motor cortex (M1) is assumed to be essentially achieved following an ontogenetic program. After brain development body representations may to a certain degree vary in size and localization or be refined or dislocated depending on environmental factors or bodily changes. In this context efferent and afferent feedback connectivity of M1 seems to play a pivotal role and was often assessed in animal experiments by disruption of M1 efferent connections. Investigations of patients with acquired paraplegia after posttraumatic spinal cord injury (SCI) yielded additional information demonstrating preserved somatotopy and a characteristic pattern of M1 reorganization with a spatial shift of lower extremity representations. We had the unique opportunity to study individuals with myelomeningocele (MMC), who were born paraplegic due to a neural tube defect. Thus, we set out to test the hypothesis that the organizing principle of M1 somatotopy was independent of efferent connectivity. Using functional magnetic resonance imaging (fMRI) with active motor tasks for tongue and hands as well as motor imagery for the feet we performed somatotopic mapping of M1 in 10 MMC patients, 13 SCI patients and 14 healthy controls. Intriguingly, MMC patients displayed intact general somototopy including correct localization of the lower extremity, corroborating the initial hypothesis. As compared to SCI patients they did not show the ventral shift pattern of reorganization of the foot representation. Instead, their foot representation was located more lateral along the motor homunculus where one would expect representations of the thigh or hip, suggesting that refinement of lower extremity representations had not taken place in these patients. This study not only examines for the first time M1 organization in MMC patients, but also confirms the corticospinal connectivity-independent basis of motor somatotopy and potentially leads the way to new conceptions in neuroprosthetics.

# Functional recovery of aimed limb movements following partial amputation in the locust *Schistocerca gregaria*

Paul Kevin Gunderson[1], Angus McKnight[2], Tom Matheson[1,2]

[1]Department of Biology, University of Leicester, University Road, LE1 7RH Leicester, United Kingdom
[2]Department of Zoology, University of Cambridge, Downing Street, Cambridge, CB2 3EJ, United Kingdom

The ability to produce correctly aimed limb movements is vital to a range of behaviours including locomotion, reaching and scratching. Any animal can suffer limb damage or limb loss through natural causes, but this is particularly common in insects which must undergo a number of risky moults to grow. Limb damage or limb loss can have profound consequences for an individual unless functionality of the damaged limb is regained. We show that aimed scratching movements of an insect (the locust *Schistocerca gregaria*) undergo an observable recovery of precision following partial loss of the distal tibia of one hind leg.

We used quantitative video analyses to measure scratching movements of locusts with either intact legs or one damaged hind leg, made in response to tactile stimulation of the ipsilateral wing. Adult locusts that had lost part of one tibia through natural causes earlier in development were able to aim the remaining stump appropriately at both anterior and posterior target sites. To investigate the time course of this plasticity we amputated the distal half of the tibia in adult animals 1 week after the imaginal moult and followed changes in their scratching movements aimed at both anterior and posterior targets over the following nine days. We show that both anteriorally and posteriorally directed scratches returned to functionally normal movements within nine days.

To investigate the mechanisms that bring about this recovery in behaviour we analysed the femur-tibia (FT) and thoraco-coxo-trochanteral (TCT) joint angles throughout the course of these scratches. These results were used to establish which joint was subject to the greatest change during the return to limb functionality. We show that there was little change in the mean angle of the FT joint during recovery, but that the mean TCT joint angle decreased by 22.5% in anterior scratches and 41.3% in posterior scratches.

Our data provide evidence for plasticity in the ability to perform aimed scratches following limb damage. This permits an insect to regain functional use of the limb. Angular changes at the thoraco-coxo-trochanteral joints play an important part in this compensatory plasticity.

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# Generalization patterns during reach adaptation to target jump

Stephanie Westendorff[1], Bahareh Taghizadeh[1], Alexander Gail[1,2]

[1]German Primate Center, Sensorimotor Group, Kellnerweg 4, 37077 Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen, Germany

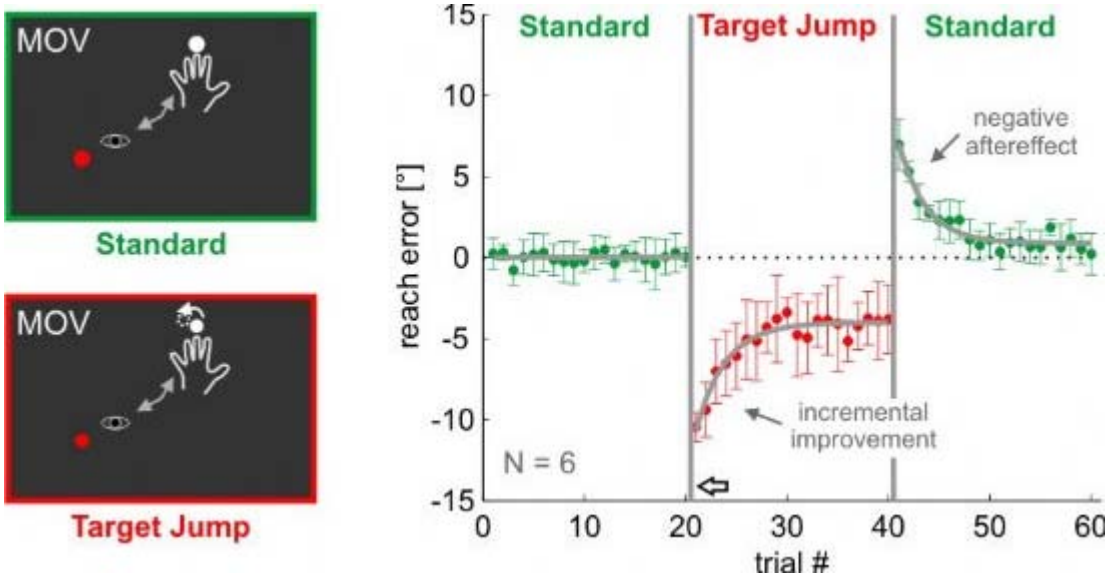
Adaptivity of sensorimotor systems is often investigated with visuomotor rotation tasks. In typical visuomotor rotation tasks, adaptation is mostly induced by an artificial mismatch between visual and proprioceptive sensory input. This mismatch requires realignment of the sensory map (location map) as well as of the sensorimotor map (displacement map). Target-jump paradigms, i.e. tasks with sudden displacement of the movement target during movement initiation or execution, do not induce cross-modal mismatch. Therefore they allow isolated adaptation of the displacement map. But they do not induce adaptation when subject are aware of the displacement. Here we present and characterize a novel target-jump paradigm, in which reach movements reliably get adapted to target displacements.

Subjects had to make center-out reaches to a peripheral target. Simultaneously they had to make a saccade to a neutral target. Saccadic suppression during the eye movement allowed us to displace the reach target during hand movement initiation or execution, without subjects noticing directional displacement.

With this novel design we could reliable induce adaptation. Subjects incrementally adapted to the target jump, with average learning rates similar to shifting prism adaptation, and showed negative aftereffects.

To develop models of the adaptation process it is important to understand how the adaptation effect generalizes to neighboring reach targets. Subjects were first adapted to the target displacement, and then probed at different positions along the circumference of the adapted target. We tested whether the induced reach error, i.e. the difference between reach endpoints with and without perturbation transfers to untrained targets as a positional offset or a directional offset. Positional offset is characterized by reach endpoints which are shifted relative to the original position in a direction parallel to the direction of the jump. Directional offset is characterized by reach endpoints which are rotated relative to the original direction from the starting point to the reach endpoint.

Our results suggest that movement endpoints to novel targets are best described by a positional offset. In contrast to a widely held assumption this suggests for the target jump paradigm that direction and amplitude do not adapt independently from each other. The reach error at the probe targets decreases with increasing angular distance from the adapted target. Notably, the generalization pattern is asymmetric around the learned movement direction with larger adaptation effects in the direction of the target jump than opposite. Models of generalization should take this generalization pattern into account to provide a veridical description of human behavior.



# Generation and investigation of animal specific Hill-type muscle models of the stick insect.

Marcus Blümel[1], Christoph Guschlbauer[1], Silvia Gruhn[1], Sott Hooper[2], Ansgar Büschges[1]

[1]Universität zu Köln, Tierphysiologie, Zülpicher Str. 47b, 50674 Köln, Germany
[2]Department of Biological sciences, Ohio University, Athens, OH 45701, USA

Understanding neuronal mechanisms of behavior generation and control require, at least at some point, a thorough understanding of how motor patterns are translated into movements. It is well known that neuro-mechanical transformation and muscular force generation are complex and for the most part non-intuitive. Apart from the most simple muscles and motor tasks, computer models are necessary to estimate how a motor pattern interacts with the real world.
The work we present here consists of three parts. First we present and investigate a 10 parameter Hill-type muscle model of the stick insect extensor tibiae muscle, which is animal specific. Further we explain what type of experiments and prior knowledge is needed to accumulate sufficient data to model individual muscles.
In the second part of this work we show that building individual models provides the chance to investigate inter-animal correlations of model parameters. As the individual modeling had been accomplished for extensor muscles of 10 animals, we were able to identify two pairs of significantly correlated parameters.
In the final part we investigate the model accuracy by performing three different types of simulations: A) Isometric simulations with constant frequency stimulation, B) isometric simulations with three different natural, physiological pulse patterns and finally C) isotonic contraction simulations for the same patterns used in B.
In order to study how much improvement can be made by building individual models, we compared the simulation RMS error of different simulation configurations. Besides the default, "muscle specific" configuration, we set up three other configurations which include combinations of averaged parameter values. One of them, the all-averaged configuration, contains the averaged parameters of 9 stick insect extensor muscle models. This approach enables us estimate how much error is introduced by common procedures like averaging maximum muscle force or averaging force-length and force-velocity curve. We can show that muscle specific modeling can decrease the error of our model in average by 4-7%.

# Influence of glial- and muscle-derived matrix molecules on axon growth of cultured mouse embryonic motoneurons

Rebecca Conrad[1], Alice Klausmeyer[1], Teresa Sczepan[1], Andreas Faissner[2], Stefan Wiese[1]

[1]Department of Cellmorphology and Molecular Neurobiology, Group of molecular Cellbiology, Universitätsstr. 150, 44780 Bochum, Germany[2]Department of Cellmorphology and Molecular Neurobiology

Mechanisms controlling neuronal survival and regeneration play an important role during development, after birth and under lesion conditions. Isolated embryonic mouse motoneurons (E12.5) have been a useful tool for studying such basic mechanisms. These motoneurons depend on extracellular matrix (ECM) molecules exhibiting either repellent or attractive guidance cues which are potent mediators of axonal growth and guidance, survival and regeneration. ECM proteoglycans and glycoproteins were identified as components of the glial scar acting as a growth barrier for regenerating axons. By using the water lysis method we generated a three dimensional matrix composed of different ECM molecules depending on the cells used for their production. Matrix was produced by the astrocyte cell lines A7, Neu7 the immortalized Schwann cell line IMS32 and the myoblast precursor cell line C2C12 (MB) and differentiated myotubes (MT). Chondroitin sulfate proteoglycans (CSPGs) like phosphacan or glycoproteins like fibronectin or tenascin were identified in the matrices produced by the different cell lines. These matrices were used as a substrate for cultured embryonic motoneurons to investigate axon growth, survival effects and signalling events. CSPGs and glycoproteins are known to influence axonal growth and guidance in the CNS in many different ways and were potent mediators of intracellular signaling cascades that promote axon growth of neurons. Our studies revealed that cell -derived matrix molecules exhibit neurite outgrowth promoting properties towards cultured embryonic motoneurons.

# Interplay of Local and Global Co-ordination in Stick Insect Walking - an Evolutionary Robotics Approach

Arndt von Twickel[1], Katja Hellekes[2], Frank Pasemann[1], Ansgar Büschges[2]

[1]Institute of Cognitive Science, Dept. Neurocybernetics, University of Osnabrück, Albrechtstraße 28, 49069 Osnabrück, Germany
[2]Dept. of Animal Physiology, Zoological Institute, University of Cologne, 50674 Cologne, Germany
[3] Corresponding author: arndt.von.twickel@uni-osnabrueck.de

Whereas co-ordination of six-legged walking in stick insects has been understood well on the behavioral level [1] knowledge about its neural basis remains limited due to the complexity of the interactions between biomechanical and neural systems. This is despite recent progress via experimental [2,3] and simulation studies [4,5,6] dealing with intra- and inter-leg couplings. Complementary to the behavioral and neural approaches we have previously applied the artificial life approach to evolutionary robotics: We developed neural controllers in a physical 3D simulation under diverse environmental conditions to learn about constraints and opportunities of embodied single leg control [7]. Here we present the results of an extension of this approach to the modular evolution of six-legged walking control. Minimal evolved single leg controllers and controllers derived from biological data are coupled via inter-segmental synaptic connections. Applying different constraints on the coupling structure during evolution, e.g. limiting the connectivity structure to structures found in biology, results in diverse yet robust hexapod controllers. The interplay of local and global co-ordination in these controllers is discussed in the context of neural and behavioral findings in the stick insect. Analysis of the controllers e.g. reveals that global co-ordination may replace local co- ordination. This is consistent with biological findings in the stick insect which indicate that, depending on the context, front-, middle- and hindlegs process local sensory information in different ways. This is ascribed to different inter-segmental coupling influences [8].

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# Intersegmental, Task and Sensory Dependencies for Reinforcement of Movement in an Insect Walking System

Katja Hellekes[1], Eric Blincow[2], Ansgar Büschges[1]

[1]Dept. of Animal Physiol., Biocenter, University of Cologne, Zülpicher Str.47b, 50674 Cologne, Germany

[2]Department of Biology, University of Saskatchewan,112 Science Place, Saskatoon, SK S7N 5E2, Canada

The motor output for a stepping leg results from the interaction of descending signals from the brain, intersegmental signals from neighboring legs and activity of sensorimotor networks of the leg muscle control system. One important mechanism by which sensory feedback contributes to the generation of the stepping motor output is by reinforcement of movement (reviews in: Clarac et al. 2000, TINS; Büschges 2005, J. Neurophysiol.). In stick insects flexion signals from the femur-tibia (FTi-) joint, reported by the femoral chordotonal organ (fCO), are known to reinforce stance phase motor output of the FTi-joint when the locomotor system is active (Bässler 1988, JEB): flexor motoneuron (MN) activity is promoted and extensor MN activity is inhibited, representing a reflex reversal ("active reaction",AR). While it is clear that the generation of the AR depends on the behavioral state, it is not known, which detailed behavioral and sensory constraints matter for its generation, i.e. whether intersegmental signals, or the locomotor behavior generated, e.g. forward (fw) or backward (bw) walking or other local sensory feedback signals are relevant.

We addressed this issue for the FTi-joint control network in stick insects (Carausius morosus) by stimulating the fCO with ramp-and-hold stimuli (range: 110°- 30°) in front (fl), middle (ml) or hind (hl) leg, while recording the activity of corresponding tibial MN`s and muscles in animals that were walking on a slippery surface with all or a subset of their remaining legs (Gruhn et al. 2006, J.Neurosci. Methods). fw or bw walking was induced by tactile stimulation of abdomen or antennae, respectively. To investigate the role of local position and load information the fCO was stimulated in the hl with it being fixed at different positions and with and without campaniform sensilla (CS) being intact: 1. We found that generation of the AR depended on stepping activity of ipsilateral neighboring legs: in the ml AR was generated in 62% (N=6) with ipsilateral fl and the contralateral legs present and stepping, and in 58% (N=5) when only the ipsilateral fl was present and stepping. However, its probability was reduced to 4% (N=7), when only contralateral legs were present and stepping. 2. We found that the generation of the AR depended on the walking direction: In fl of fw walking animals the AR was generated in 67% (N=7), but not during bw walking (N=7;14%). 3. We found that the generation of the AR was segment specific: contrary to fl (see above), in ml the AR was generated for both walking directions (N=7; fw 49% and bw 37%), while its probability slightly exceeds chance level in hl, neither in fw nor in bw walking (N=7, 22%). 4. We found that the generation of the AR depended weakly on position signals from the coxa of the same leg, but indeed on load signals from the leg (cf. Akay & Büschges, 2006).

Our results indicate that intersegmental signals from rostral stepping legs and task-specific modifications play a prime role in generating adaptive modifications of local sensorimotor processing. Supp. by DFG grant Bu857/9,10.

# Intracellular recording of motoneuron activity during phonotactic walking in female crickets

Fabienne Dupuy[1], Berthold Hedwig[1]

[1]University of Cambridge, Department of zoology, Downing Street, CB2 3EJ Cambridge, United Kingdom

Female crickets recognize the song of a conspecific male and walk toward the singing male using auditory information to orientate (Hedwig, 2006). Perception of the song occurs via the tympanal organ located in the front leg. The auditory activity is forwarded to the prothoracic ganglion and via the ascending neuron 1 (AN1) then reachs the brain where the song recognition occurs. The motor activity for walking is generated by a pattern generator which activates motoneurons located in the thoracic ganglia. This activity can be modulated by acoustic information when females steer towards a sound pattern. During phonotaxis the lateral movement of a frontleg is clearly affected (Baden and Hedwig, 2008). For steering behaviour the auditory information has to be integrated to allow the changes in leg movements which control the walking direction. Extracellular recording of muscles show that the slow extensor tibia neuron responds more to ipsilateral sounds and the fast flexor tibia motoneurons are more excited by contralateral sound. No overlap of arborisation pattern of motoneurons and auditory neurons occurs in the prothoracic ganglion (Baden and Hedwig, 2008). The integration of auditory information into locomotor activity of the legs is still not understood. To investigate it we analyse the auditory related input received by the motoneurons during phonotaxis. Intracellular activity is recorded in a tethered female cricket *Grillus bimaculatus* walking on a trackball while the animals steer towards a sound pattern. Preliminary recordings of front leg extensor tibia motorneurons and of flexor tibia motorneurons in phonotactically walking crickets indicate that the pattern of neuronal activity is different when the animals steer to the left or right side.

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# Intrinsic and network properties of a highly synchronous hindbrain motor nucleus

Boris Philippe Chagnaud[1], Michelle J. Zee[1], Robert Baker[2], Andrew H. Bass[1]

[1]Cornell University, Neurobiology and Behavior, Tower road, 14850 Ithaca, USA[2]Department of Physiology and Neuroscience, New York University Langone Medical Center, New York, NY 10016, USA

Synchronous, high frequency firing is basic to neuronal function, so far mainly shown at cortical and subcortical levels. Here, intracellular single cell analyses identified intrinsic and network properties determining synchronous, ultrafast-gamma firing in a hindbrain motor nucleus dedicated to vocalization in fish. Individual, neurobiotin-filled vocal motoneurons exhibited modest dendritic arbors of 3-4 main branches extending bilaterally throughout, and contained mainly within, paired midline motor nuclei. Motoneurons lacked spontaneous activity, only firing action potentials at frequencies matched to excitatory vocal pacemaker input resulting in highly synchronous motoneuron activity and a pulsatile nerve volley directly determining natural call frequency. Intracellular current injection revealed low somato-dendritic, voltage-dependent excitability with rapid accommodation of action potential firing rate. Electrotonic coupling was demonstrated in collision tests of antidromic motoneuron activation via the vocal nerve and intracellularly evoked action potentials. Action potentials showed a strong after-hyperpolarization during vocal activity, with intracellular chloride injections revealing a background membrane hyperpolarization blocked by extracellular bicuculline, a GABAA receptor antagonist. Together with immunocytochemical evidence for dense GABAergic input throughout both vocal motor nuclei, the results support prominent roles for synchronizing inhibition, synchronous excitatory input and electrotonic coupling in determining vocal oscillatory output in the ultrafast-gamma frequency range. Axonal recordings further showed differential recruitment of variably sized vocal motoneurons without disrupting motoneuron synchronicity, but accounting for natural variation in call amplitude.

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# Locust leg afferents and their influence on descending neurons of the suboesophageal ganglion

Elisabeth Lipke[1], Peter Bräunig[1]

[1]RWTH Aachen University, Institute for Biology II, Unit for Developmental Biology and Morphology of Animals, Mies-van-der-Rohe Straße 15, 52056 Aachen, Germany

The suboesophageal ganglion (SOG) of hexapods represents a major centre for overall motor coordination. Therefore it is reasonable to assume that afferent signals from thoracic motor circuits feed back on neurons in the SOG. We have already shown for descending dorsal unpaired median (DUM) neurons of the SOG that their activity is, amongst others, influenced by sensory information of the legs. In addition to these DUM neurons, there are descending interneurons (DIN), showing an increase of spike activity, when certain leg nerves are stimulated. To clarify the origin of the sensory information affecting these interneurons, we started to investigate the influence of typical sense organs of leg, like the femoral chordotonal organ, the subgenual organ, or the multipolar sensilla of the femur-tibia-joint. Interestingly none of these well-studied proprioceptors is able to cause an effect in these DIN's of the SOG. The same surprising result was already obtained while studying descending DUM neurons of the SOG. Using intracellular recordings in combination with stimulation of nerves or sense organs we have got first hints as to where in the leg this feedback originates, that is which leg afferents are responsible for these effects, and how this sensory information is relayed to the neurons of the SOG.

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# Mapping the spatial structure of LFP activity in motor cortex

Sarah Wirtssohn[1], Thomas Brochier[1], Michael Denker[2], Sonja Grün[2,3], Alexa Riehle[1]

[1]Mediterranean Institute of Cognitive Neuroscience (INCM), CNRS - Univ. Aix-Marseille 2, Marseille, France

[2]RIKEN Brain Science Institute, Wako City, Japan

[3]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Jülich, Germany

Local field potentials (LFPs) represent mainly the averaged synaptic activity in the vicinity of the recording electrode with contributions from spike afterpotentials or intrinsic trans-membrane current changes. In primary motor (MI) and premotor (PM) cortical areas, LFP activity has two main properties. (i) LFPs are characterized by large movement-related potentials (MRPs) both before movement onset and during movement execution [1]. (ii) During an instructed delay, LFPs show oscillatory activity mostly restricted to the beta-range (15–30Hz), considered to be mainly related to movement preparation or attentional processes [2]. And (iii), occasionally oscillations in the high gamma band (45-150Hz) can be observed during movement execution [3]. However, little is known about the spatio-temporal distribution of LFP signals across the surface of motor cortex in relation to different task requirements. Here, we analyze the similarities and differences of MRPs and beta and gamma oscillations in the spatial domain, recorded during a complex reaching and grasping task in relation to two different grips and two load forces.

Two monkeys are trained to press a switch with one hand, and then to grasp and pull an object using either a Side Grip or a Precision Grip. The object is either heavy or light. The non-active arm is fixed, to assure that only one hand is used. The trial starts by the monkey pressing a switch. A center point is presented, followed by a cue providing the instruction about the grip to be used. After a delay of 1s, a second cue is presented, serving as GO signal and providing the additional information about the object load. The monkey has then to release the switch, grip the object, pull it and hold it in a narrow position window for 500ms to receive a food reward. The four trial types are randomized. Massively parallel neuronal activity (LFPs and spiking activity) was recorded by using a 100 electrode Utah array, chronically implanted at the MI/PMd border.

Our data show that the amplitude and particularly the shape of the MRPs depend strongly both on grip type and electrode position. To quantify the spatial diversity of these temporal profiles, we characterized distinct features in the MRP, such as the occurrence of individual components, their peak amplitudes and their peak latencies in respect to movement onset, depending on the grip type and electrode location. Furthermore, the strength of gamma oscillations during movement execution depends also on electrode location and grip type. In contrast, as revealed by a time-resolved spectral analysis, beta oscillations during the preparatory phase do not show a strong dependence on the cortical location, but vary in strength for the two grip types. Our results, combined with the analysis presented in [4], suggest that neural networks underlying movement execution, expressed by the MRP and high gamma oscillations, seem to be more local than those underlying movement preparation, expressed by oscillatory LFP activity in the beta range.

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# Mapping the synchronization structure of LFP activity in motor cortex

Michael Denker[1], Sarah Wirtssohn[2], Thomas Brochier[2], Alexa Riehle[2], Sonja Grün[1,3]

[1]RIKEN Brain Science Institute, Laboratory for Statistical Neuroscience, Wako-shi, Japan
[2]Mediterranean Institute of Cognitive Neuroscience (INCM), CNRS - Univ. Aix-Marseille 2, Marseille, France
[3]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Jülich, Germany

Modulations of the local field potential (LFP) are commonly attributed to the synchronization of inputs received by neurons in the vicinity of the recording electrode. Moreover, the LFP is shown to relate to firing rates and the degree of correlation exhibited by nearby neurons [1], establishing the LFP as an excellent monitor of the coordinated neuronal population activity. In primary motor (MI) and premotor (PM) cortical areas, during an instructed delay LFPs show oscillatory activity mostly restricted to the beta-range (15–30Hz). These oscillations are commonly considered to be related to internal processes such as movement preparation or attention [2]. However, although it is demonstrated that beta activity exhibits in general a wave-like propagation across the motor cortical surface [3], little is known about fine-scale changes in the spatial structure of synchronization of LFP signals in relation to the particular task. Here, we map the network defined by the strength of mutual interaction between preparation-related beta activity across different recording sites in relation to two different grip types that characterize the motor task.

In short, two monkeys were trained to press a switch with one hand, and then to pull an object using either a Side Grip or a Precision Grip. The object is either heavy or light. To allow the monkey to prepare the upcoming movement, the grip type was revealed to the monkey preceding a delay period of 1s before the GO signal. Massively parallel neuronal activity (LFPs and spiking activity) was recorded by using a 100 electrode Utah array, chronically implanted at the MI/PMd border. Details of the experiment can be found in [4].

We find that although beta oscillations during the preparatory phase exhibit differences in spectral power content depending on the two grip types, they do not show a strong dependence on the cortical location. To investigate whether beta activity is a homogeneous signal across MI and PMd or not, we computed the spatial pattern of spectral coherency between activity recorded at different electrodes. To further evaluate the degree of coupling between the recording sites we employed methods from phase synchronization analysis as a complementary approach. Based on these two analyses, we inferred a connectivity graph that reveals the spatial structure of interactions. Furthermore, the phase analysis allowed us to describe the parameters of LFP wave propagation as a function of the task. These results are qualitatively compared to the map of features, such as peak latencies, extracted from movement-related potentials observed during movement execution (see [4]) in order to assess the degree of spatial and behavioral specificity inherent to beta oscillations.

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# MEASURES OF CORRELATION BETWEEN MOTOR UNIT SPIKE TRAINS IN HUMANS

Francesco Negro[1], Dario Farina[1,2]

[1]Department of Neurorehabilitation Engineering, Bernstein Center for Computational Neuroscience, Georg-August University of Göttingen, Göttingen, Germany

[2]Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Aalborg, Denmark

During sustained contractions, motorneurons receive both common and independent synaptic inputs from presynaptic neurons and supraspinal centers. The common inputs slightly increase the probability that pairs of motorneurons discharge action potentials almost concurrently. The correlation between motorneuron spike trains, which can be measured with motor unit recordings in vivo, is believed to be proportional to the strength of the common input signal. Therefore, this measure is often used for obtaining information about the connectivity between the motorneuron pool and spinal or cortical networks. However, correlation between spike trains is known to be influenced not only by the amount of common input but also by the filtering applied [1] and by the characteristics of the input signals [2, 3]. In this study, we analyze the methods for the estimation of correlation in the motorneuron output, in order to clarify the meaning of different indexes of correlation used in human motor unit studies, and we propose the use of more robust indexes. The experiments were conducted on 8 healthy men (age: 25.7 ± 2.3 yrs). Intramuscular EMG was recorded during an isometric contraction (100 s duration) of the abductor digiti minimi muscle at a force level for which the degree of interference in the signal was sufficient to allow accurate signal decomposition (3-5 motor units). Simulations were performed using a model of populations of motoneurons that received common and independent inputs (Gaussian noise filtered in the frequency band 0-100 Hz). The model was an extension of that described by Cisi & Kohn [4]. The common drive index (CDI) with Hann windows of 150 ms and 400 ms, common input strength (CIS), synchronous impulse frequency (SIF), and coherence function were calculated for the decomposed motor unit spike trains. The CDI was different when the two low-pass filters were applied to the motor unit spike trains ($P<0.05$), however the values of coherence in the bandwidth of the filters was not influenced by the filter type. Correlations were also calculated as a function of discharge rate. There was a positive linear association between CIS and the geometric mean of discharge rate of the pair of motor units used for the calculation in 7 subjects (average $R^2 = 0.12 \pm 0.06$, $P<0.05$) and between the common drive index calculated using the 150 ms filter and the geometric discharge rates for 6 subjects (average $R^2 = 0.06 \pm 0.05$, $P<0.05$). However, both the SIP index and the common drive index (400 ms) showed no association with the discharge rate in all subjects ($P>0.05$). Moreover, all the correlation indexes analyzed increased with the number of motor units used in the calculation. The computer model assisted the interpretation of the experimental results, showing that a saturation level of the estimated correlation value is expected when 4-5 motor units are used for the calculation (Fig. 1). In conclusion, the estimation of correlation between spike trains of motor units depends on the discharge rate (up to a saturation limit) and the pre-filtering applied. Robust indexes of correlation can be obtained from recordings of populations of motor units [5, 6] and coherence values.

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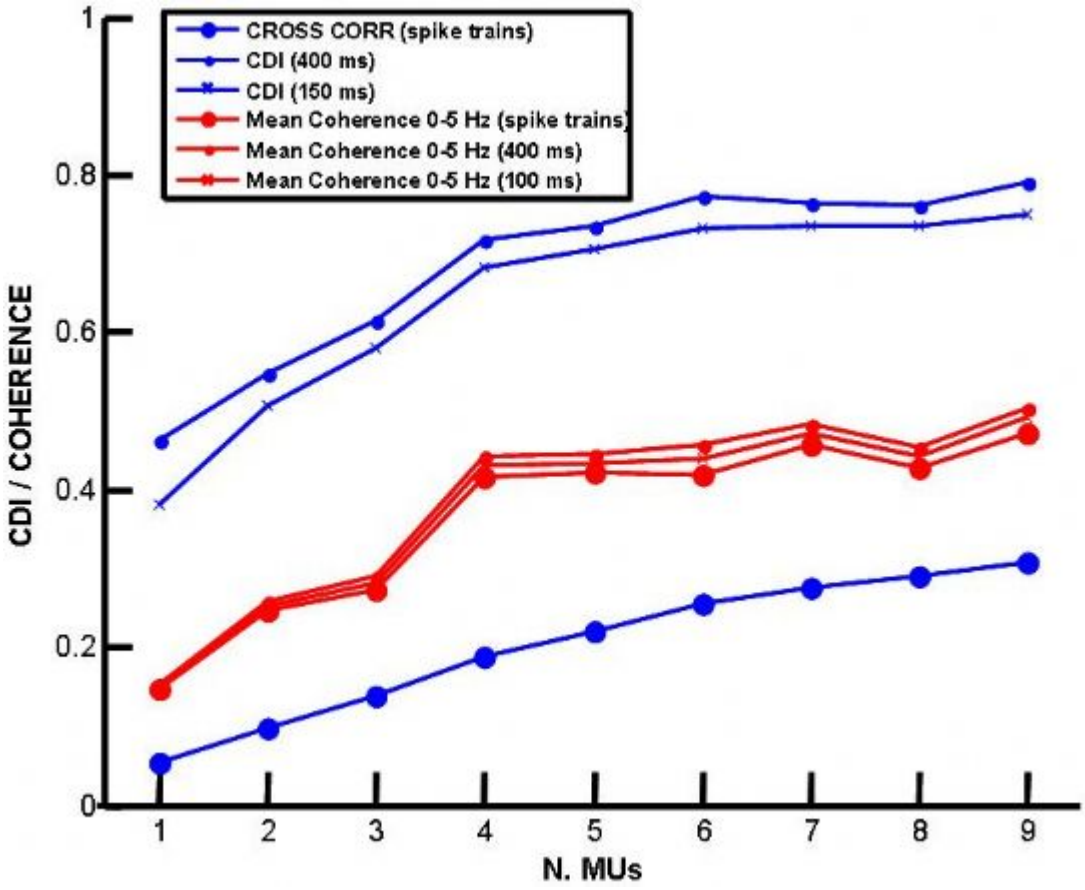
Fig 1 Association between the estimated correlation level vs the size of the population of motor units used for the calculation. Results are shown for the simulation data and three main indexes of correlation: peak of the cross correlation between spike trains, CDI with two low-pass filters, and coherence.


CROSS CORR (spike trains)
CDI (400 ms)
CDI (150 ms)
Mean Coherence 0-5 Hz (spike trains)
Mean Coherence 0-5 Hz (400 ms)
Mean Coherence 0-5 Hz (100 ms)
CDI / COHERENCE
0
0.2
0.4
0.6
0.8
1
1
2
3
4
5
6
7
8
9
N. MUs

# Motor imagination combined with peripheral stimulation increases cortical excitability

Natalie Mrachacz-Kersting[1], Signe Rom Kristensen[1], Imran Khan Niazi[1], Kim Dremstrup[1], Dario Farina[1, 2]

[1]Center for Sensory-Motor Interaction, Aalborg University,Fredrik Bajers Vej 7D3, 9220 Aalborg, Denmark

[2]Department of Neurorehabilitation Engineering, Bernstein Center for Computational Neuroscience, Georg-August University of Göttingen, Göttingen, Germany

The plasticity of the human motor cortex plays an important role in functional recovery after stroke and it is known that repetition of a simple motor task can reorganize the motor cortex [Classen J et al. J Neurophysiol. 1998 Feb;79:1117-23]. However, little is known on the cortical function during movement execution in stroke patients, thus the outcome of classic therapies is scarcely predictable. This study investigated a novel approach for changing the excitability of the cortical projections to the tibialis anterior (TA) by pairing motor imagination and peripheral nerve stimulation. It was hypothesized that the concomitance between the cognitive process of movement (motor imagination) and the ascending volley due to the peripheral electrical stimulation would increase cortical excitability.

In three subjects, the movement-related cortical potentials (MRCPs) were measured at the location Cz while they performed 50 imaginary ankle dorsiflexions with their right foot, with resting intervals of random duration in the range 5-8 s. A visual interface provided the cue for the subjects on when to start the motor imagination. From the average MRCP, the preparation, execution and post-execution phases were identified. During the following interventions, subjects were again asked to imagine a dorsiflexion movement of their right foot with visual cue for the onset of the motor imagination. During each motor imagination, a single electrical stimulus was applied to the deep branch of the common peroneal nerve at motor threshold. The timing of the peripheral stimulation was such that the afferent volley arrived at the cortex during either the preparation phase (intervention 1, INT1), the execution phase (intervention 2, INT2) or post the execution phase (intervention 3, INT3). The three interventions were randomized with >3 days between each session. Alterations in the excitability of the cortical projections to the TA were assessed by transcranial magnetic stimulation pre and post the intervention. A focal figure of eight double cone coil (110 mm diameter) was placed with the centre of the coil overlying Cz. The 'hot-spot' was identified and marked using Brainsighttm (version 1.5. Rouge Research Inc.), a stereotatic image guidance system. The rest threshold (RTh) was established according to standard procedures. Subsequently an input-output curve was generated with at least 12 stimuli at each intensity (90, 100, 110, 120 and 130% of RTh).

Figure 1A displays the change in MEP amplitude for one representative subject prior to and following the three interventions. The intensity of the TMS was set to 120% RTh. The MEP amplitude increased only following the second intervention with respect to baseline, i.e. when the peripheral stimulus was timed to arrive at the peak negativity. The change in MEP amplitude with increases in TMS intensity is shown in Figure 1B. Across all three subjects only INT2 resulted in consistent MEP amplitude increases across all stimulus intensities.

The results support the hypothesis that the combination of motor imagination and peripheral stimulation increases cortical excitability. Moreover, the increase in cortical excitability was found to depend on the timing of the arrival of the electrical stimulation with respect to the cognitive state. These observations indicate the potential use of combined motor imagination and electrical stimulation as a new neuro-rehabilitation strategy.

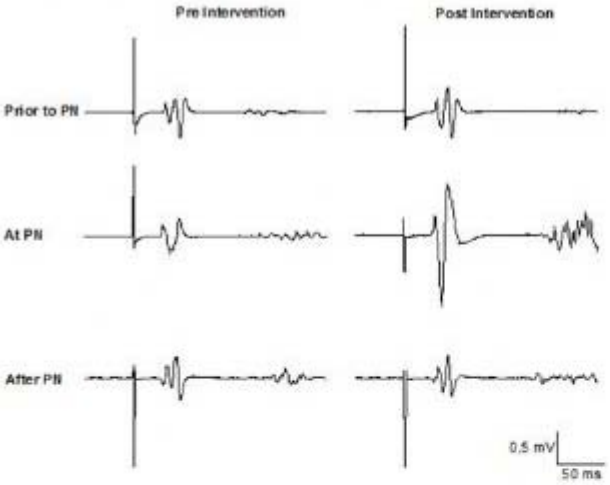



Figure 1A: *Effect of the three different interventions on the size of the raw TA MEP amplitude at rest for one subject.*

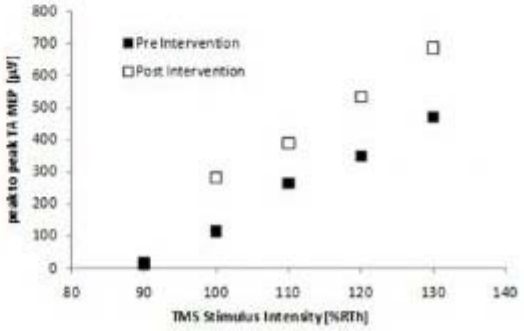



Figure 1B: *Effect of TMS intensity on the amplitude of the TA MEP for one subject prior to and following INT2.*

# NEW POLYMERIC CARRIER ENHANCES BRAIN AVAILABILITY OF DOMPERIDONE

Christiane Knoth[1], Mirjam Hemmelmann[2], Matthias Barz[2], Christoph Hiemke[1], Frank Rösch[3], Ulrich Schmitt[1], Rudolf Zentel[2]

[1]University Medical Center Mainz, Dept. of Psychiatry and Psychotherapy, Untere Zahlbacher Str. 8, 55131 Mainz, Germany
[2]Institute of Organic Chemistry, Johannes Gutenberg University Mainz, Duesbergweg 10-14, 55099 Mainz, Germany
[3]Institute of Nuclear Chemistry, Johannes Gutenberg University Mainz, Fritz-Straßmann-Weg 2, 55128 Mainz, Germany

## INTRODUCTION

Macromolecular carrier systems like HPMA-copolymers1 for pharmaceuticals have been well examined in the recent years in the field of drug targeting, mainly for cancer therapy. In our approach we investigated the potential of transporting drugs across the blood-brain barrier (BBB) into the brain. Domperidone, a known D2-receptor antagonist, is devoid of CNS-effects because it rarely crosses the BBB [2,3]. We examined whether domperidone enclosed in micelles formed by an amphiphilic poly(N-(2-hydroxypropyl) methacrylamide)-block poly(lauryl methacrylate)-copolymer4 (pHPMA-co-pLMA) with an average molecular weight of 21 kDa is able to mediate D2-related effects on locomotor behaviour in mice on the rotarod which is a functional test for central nervous action. FVB/N mice treated with domperidone (50mg/kg) alone or domperidone embedded in pHPMA-co-pLMA micelles (PolyDOM) and subsequently P-glycoprotein knockout mice treated with domperidone were tested on the rotarod 0.5 hours after intra-peritoneal (i.p.) injection after having been adapted to the test for one week by 5 daily trials.

## RESULTS AND DISCUSSION

Rotarod performance was impaired in every treatment group 0.5 h after i.p. injection compared to saline injected control animals. But only in mice treated with PolyDOM rotarod behaviour was significantly affected ($F(3; 38)=6.680$, $p<0.001$) while P-gp ko mice showed barely a trend towards impairment ($p=0.063$). Between wild type mice receiving domperidone injection and controls no difference was detected. These results indicate enhancement of drug entry into the mouse brain mediated through transport by pHPMA-LMA. Unexpectedly P-gp function seemed unlikely to be affected by the polymer 0.5h after injection but after 2h, thus domperidone is probably not only transported by this efflux transporter but also through other mechanisms at the BBB like e.g. the multidrug-resistance related protein (MRP) or the breast-cancer-resistance-protein (BCRA). Since pHPMA-co-pLMA has already been found to be non-toxic in multidrug resistant MDCKII cells and even able to penetrate into the nucleus4 this macromolecular structure seems to be a promising agent to enhance drug transport into the brain. Further investigations are needed to elucidate the underlying mechanisms.

# Object discrimination at the neuronal level How object features are encoded by the weakly electric fish, Gnathonemus petersii

Simone Gertz[1], Jacob Engelmann[2], Gerhard von der Emde[1]

[1]Institut of Zoology, Neuroethology/Sensory Ecology, Endenicher Allee 11-13, 53115 Bonn, Germany
[2]AG Active Sensing, University of Bielefeld, Germany

The weakly electric fish Gnathonemus petersii explores its environment by active electrolocation, during which they emit electric signals and perceive the resulting electrical field with epidermal electroreceptors. Depending on their electrical properties, objects project "electric images" onto the sensory surface of the fish. Analyses of these images enable the fish to detect nearby objects and discriminate between different object parameters. The focus of this study is to understand this capability at the neuronal level, i.e. to find out how information about objects is processed in the first central stage of the ascending electrosensory pathway, the electrosensory lateral line lobe (ELL). Thus, we relate the sensory input caused by an object's "electric image" to the neuronal response. The curarized fish was stimulated with an artificial electrical signal to replicate the natural electric field surrounding the fish. We recorded extracellularly from single neurons while presenting either a plastic or metal cube (8x8x8mm³) within the receptive field (RF) of the unit and determined discharge rates and first spike latencies. With both object types, we determined the RF organization of E(xcitatory)- and I(nhibitory)-units. Our data show that E- and I-units are sensitive to amplitude modulations due to material properties of the presented object, and that the receptive fields are complex and large. We further propose that at the level of the ELL, both spike rate and first spike latency are used for coding and that integration of several inputs in higher brain areas is needed to completely process the electric image of an object. In a theoretical approach, we show that the ability to differentiate object impedances improves when input of several neurons is integrated. In the future, we want to test these findings in subsequent experiments by multicellular recordings.

# Plasticity in the HVC of the Bengalese finches is crucial for song syntax stability

Alexander Hanuschkin[1], Markus Diesmann[2,3,4,5], Abigail Morrison[1,2,3]

[1]Functional Neural Circuits Group, Faculty of Biology, Albert-Ludwig University of Freiburg, Germany
[2]Bernstein Center Freiburg, Albert-Ludwig University of Freiburg, Germany
[3] RIKEN Brain Science Institute, Wako City, Japan
[4] RIKEN Computational Science Research Program, Wako City, Japan
[5] Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM6), Research Center Juelich, Germany

The high vocal center (HVC) of the Bengalese finch relies on reafferent signals from the auditory system to generate variable squences of syllables (song syntax) [1]. This leads to the hypothesis that song syntax memory is stored in the efferent connections from the auditory areas to the HVC nuclei. Deafening experiements in the Bengalese finch reveal a gradual loss of the song syntax over the course of a week [2,3]. This finding cannot be explained by a loss of song syntax memory, because the bird is able to reproduce the original syntax once auditory feedback is restored [4].
In a computational study we investigate the change of song syntax in the presence and absence of auditory feedback and with altered auditory feedback. Changes in song syntax are quantified by the sequencing stereotype S* and the average transition entropy [3]. The gradual loss of syntax can only be reproduced if plasticity in the HVC is assumed. We discover the gradual loss of syntax is accounted for by the hypothesis that the song syntax is imprinted on the network structure through repetition. Similarly, an alternative hypothesis that an efference copy of the HVC motor pattern is learnt can also account for the data. The figure illustrates our simulation results. Before 4000s the song syntax is stably produced in the presence of auditory feedback (S*=0.75+/0.02 with S*=0.75 for perfect syntax). Between 4000s and 8000s the auditory feedback is suppressed. The resulting gradual decrease in sequencing stereotype can be fitted by a power law. After 8000s auditory feedback is restored and the original song syntax is immediately reestablished (S* =0.73+/0.02). The plasticity in HVC is behaviorally relevant for the Bengalese finch in order to keep the song syntax stable in the presence of natural auditory feedback perturbations.

Acknowledgments
Partially funded by DIP F1.2, BMBF Grant 01GQ0420 to BCCN Freiburg, EU Grant 15879 (FACETS), EU Grant 269921 (BrainScaleS), Helmholtz Alliance on Systems Biology (Germany), NextGeneration Supercomputer Project of MEXT (Japan), Neurex, and the Junior Professor Program of BadenWürttemberg. The authors would like to thank Jun Nishikawa and Kentaro Katahira for stimulating and fruitful discussions. All simulations are performed using NEST [5]. The computations are conducted on the high performance computer cluster of the CNPSN group at RIKEN BSI, Wako, Japan.

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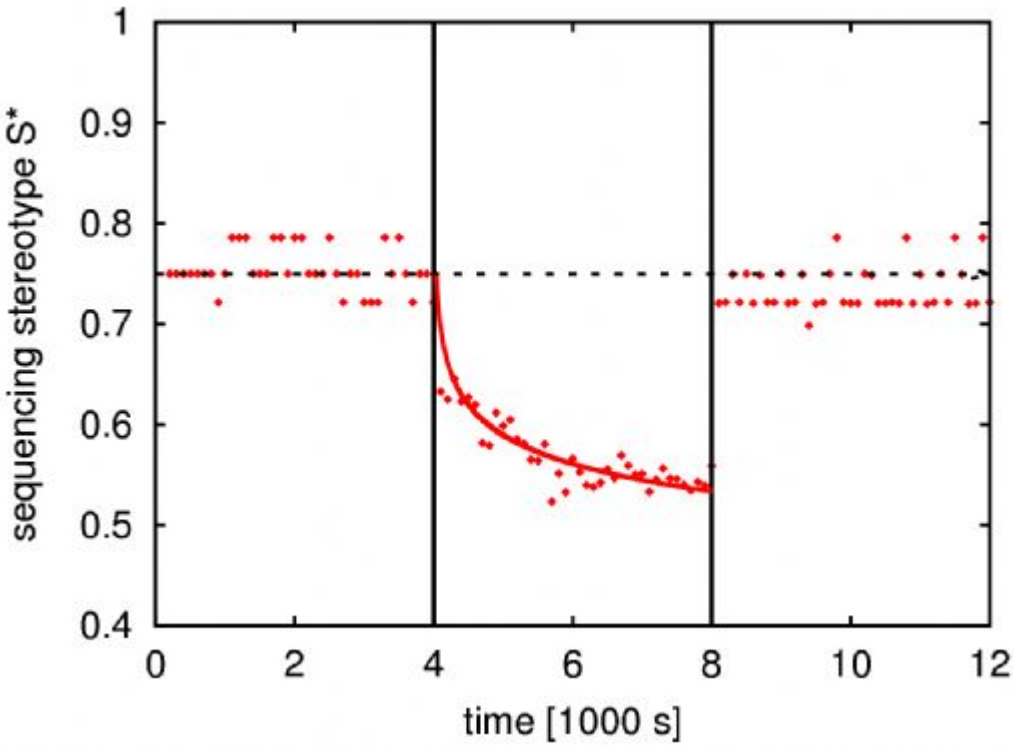
sequencing stereotype S*
1
0.9
0.8
0.7
0.6
0.5
0.4
0
2
4
6
8
10
12
time [1000 s]

# PTP-NP/Phogrin expression alters during the spinal cord development

Teresa Sczepan[1], Alice Klausmeyer[1], Rouven Hecht[1], Rebecca Conrad[1], Stefan Wiese[1]

[1]Department of Cell Morphology and Molecular Neurobiology, Group of Molecular Cell Biology, Universitätsstraße 150, 44780 Bochum, Germany

PTP-NP/Phogrin (phosphotyrosine phosphatase – neural and pancreatic) was first detected as a dense core secretory granule transmembrane protein-tyrosine phosphatase homologue in neuroendocrine cells of the pancreatic islets of Langerhans and the brain (Wasmeier and Hutton, 1996). PTP-NP, also called IAR (islet cell-antigen related PTP) is a 1004 amino acid transmembrane protein with a luminal, transmembrane and cytoplasmic part and 74 % identity to IA-2 which is a known auto-antigen in diabetes mellitus. PTP-NP/phogrin belongs to a family of receptor phosphotyrosine phosphatases with lack in usual phosphatase activity due to an amino acid exchange that leads to an inactivation of the conventional phosphatase domain. Two sorting signals reveal that PTP-NP/Phogrin is localised in secretory vesicles. The exact function of PTP-NP/Phogrin is still unknown but it seems that PTP -NP/Phogrin is involved in exocytosis of insulin -producing cells (Jiang et al., 2006). Besides the islet cells of the pancreas PTP -NP/Phogrin is expressed during early mouse development in the neural tube and endocrine cells of the adult brain. Here we show that PTP -NP/Phogrin is expressed during development of the spinal cord and differs between the different developmental stages. Highest PTP-NP/Phogrin expression levels coincide with phases of neurite extension in the spinal cord. Cultured embryonic mouse motoneurons and PC12 cells show expression of PTP-NP2/Phogrin in the cytoplasm with highest levels in the growth cones.

# Ready for takeoff – motor control of flight start in winged stick insects (Insecta: Phasmatodea)

Rebecca Klug[1], Uta J. Grimm[2], Adrian Giersch[2], Reinhold Hustert[2]

[1]Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Abteilung Morphologie und Systematik, Berliner Straße 28, 37073 Göttingen, Germany

[2]Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Abteilung Neurobiologie, Berliner Straße 28, 37073 Göttingen, Germany

Insects can take off for flight in several manners: i) by rapid leg extension or jumping; ii) by using the wings only, and iii) by dropping from an elevated substrate into air. We studied flight starts and corresponding motor programs of winged stick insects (40 % of the about 3000 described phasmid species) which fly with two active wings. The strategies differ for the flight start of moderately and well flying representatives of Phasmatodea. A larger phasmid with a heavy abdomen, *Sipyloidea sipylus*, was compared to a lightweight and strong flyer, *Pseudophasma acanthonota*, with a slender stick-like body shape.

These phasmids were filmed using a high speed camera during takeoffs from vertical and horizontal platforms. The wing stroke patterns were compared to myograms from major flight muscles during free flight starts and during tethered flight. Myogram-driven LED-flashes were used to mark in single frames of the highspeed video recordings when muscle potentials (EJPs) to a specific flight muscle appear during the flight cycle of the wings.

*Sipyloidea sipylus* starts from horizontal substrates without changing leg position during its first wing beat. It unfolds and elevates the wings maximally but the first downstroke terminates early at a horizontal level before touching the substrate. That mainly helps to raise the long and heavy abdomen and also prevents the wings from damage by hitting the substrate. The motor program of wing elevator and depressor muscles corresponds to this specific flight start. During the second and complete wing stroke the animal stretches all legs which then loose substrate contact and all further downstrokes of the wings end 30-450 below the horizontal level of the wing-carrying metathorax of *Sipyloidea sipylus*.

*Pseudophasma acanthonota* can take off from a horizontal platform with its first wing beat since the first downstroke already raises the complete body into air and into the typical flight position. Both wings perform their full downstroke (30-45 degrees below the horizontal) without touching the substrate. That is mainly due to the relatively short and light abdomen in this well flying species.

Takeoffs from the preferred resting posture, vertical hanging in stick mimicry, need a different strategy to get into a good free flight position. In *Sipyloidea sipylus* during the first wing stroke the legs loose hold on one side and the downstroke stops early just as in horizontal takeoffs (the wings might get entangled in plant structures of the substrate). With the body being vertical in air, the animals begin a hovering and turning manoeuvre to the side where the legs were released first. The wings move in a horizontal plane which is almost perpendicular to the almost vertical body. At the upper and lower stroke reversals of the wings these become strongly twisted nearly in an s-shape. The animal slowly ascends and then transits to normal forward flight with a normal 45 degree pitch of the body.

These takeoff patterns seem to be adaptations to two-winged flight with an elongated body.

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# Recruitment of V2a interneurons during swimming in juvenile zebrafish.

Jessica Ausborn[1], Riyadh Mahmood[1], Abdel El Manira[1]

[1]Karolinska Institute, Department of Neuroscience, Retzius väg 8, 17177 Stockholm, Sweden

Central pattern generators in the spinal cord produce a locomotor pattern that is expressed as a coordinated activity of motoneurons controlling muscle contractions. The natural variability in speed of locomotion requires an orderly recruitment of motoneurons from those innervating slow to those innervating fast muscle fibers.
In juvenile/adult zebrafish we showed that motoneurons are organized in four pools with specific topographic locations and are incrementally recruited to produce swimming at different frequencies. The recruitment threshold is set by a combination of specific intrinsic properties and the synaptic drive they receive.
In this study we sought to examine if a similar topographic organization also applies to premotor interneurons and if this can explain the topographic gradient in synaptic drive in motoneurons. An important group of excitatory premotor interneurons in zebrafish are Chx10-expressing V2a interneurons, also called circumferential descending (CiD) interneurons that make monosynaptic connections with motoneurons. To determine if the pattern of recruitment of V2a interneurons mirrors that of motoneurons, we used an in-vitro brainstem-spinal cord preparation from juvenile/adult zebrafish in which locomotor activity was induced by electrical stimulation of descending excitatory axons from the brainstem while whole-cell patch clamp recordings were made from GFP-labeled V2a neurons.
Our results show that, in contrast to motoneurons, no topographic organization was apparent for the recruitment of V2a interneurons at different swimming frequencies. The amplitude of membrane potential oscillations during swimming did not show any relationship with position, soma size or input resistance of these interneurons. In addition, although V2a interneurons exhibit a broad spectrum of firing patterns and intrinsic properties we weren't able to identify distinct subgroups like for motoneurons.
To determine the timing of the recruited (spiking) and not-recruited (non-spiking) V2a interneurons, phase analysis in relation to motoneuron activity was performed. The activity of the spiking V2a interneurons always preceded that of the motoneurons, while the membrane potential oscillation peak of non-spiking V2a interneurons occurred after.
This suggests that the recruitment of motoneurons is accomplished by a successive synchronization of premotor excitatory V2a interneurons into the active part of the network.
Since the recruitment of V2a interneurons does not show a topographic distribution we conclude that a simple topographically organized connectivity pattern cannot account for the recruitment pattern of motoneuron pools at different swimming frequencies.

# Reduced intracortical inhibition and facilitation in the primary motor tongue representation in stuttering

Nicole E Neef[1], Walter Paulus[1], Andreas Neef[2], Alexander Wolff von Gudenberg[3], Martin Sommer[1]

[1]Georg-August-University, Clinical Neurophysiology, Robert-Koch-Str. 40, 37075 Göttingen, Germany
[2]BFNT, Göttingen, Germany
[3]Institut der Kasseler Stottertherapie, Bad Emstal, Germany

This study aimed at detecting neurophysiological changes in the primary motor tongue representation of the left and right hemisphere in adults with persistent stuttering.
In 12 patients and in 14 control subjects, we examined motor threshold, motor-evoked potential (MEP) input-output curves, short-term intracortical inhibition (SICI) and intracortical facilitation (ICF) using transcranial magnetic stimulation (TMS).
In control subjects a significant inhibition of the MEP-amplitude at short inter-stimulus interval (ISI) as well as a significant facilitation of the MEP-amplitude at long ISIs was evident. Patients with persistent stuttering showed an inhibition at ISI 3ms, no inhibition at ISI 2ms and a reduced facilitation at ISI 10 and 15 ms. Furthermore, MEP input-output curve was steeper in patients with persistent stuttering. Motor thresholds did not differ between groups.
In persistent stuttering intracortical excitability of the primary motor tongue representation is altered with a deviant time course for inhibitory activity and reduced paired-pulse facilitation.
These results specify changes in intracortical networks probably mediated by altered GABAergic regulations and deviant subcortical-cortical loops in persistent stuttering. Thus, a better understanding of disease mechanisms and a potential role in understanding pharmacological treatment response emerges by using TMS in persistent stuttering.

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# Representation of categorical perceptual decisions in monkey prefrontal and premotor cortices.

Katharina Merten[1], Andreas Nieder[1]

[1]University of Tübingen, Animal Physiology, Institute of Neurobiology, Auf der Morgenstelle 28, 72076 Tübingen, Germany

Different types of sensory information stimulate our sense organs and the intensity is reflected by early sensory cortices. However, the key point of sensory detection tasks is that repeated presentations of the same near-threshold stimulus sometimes fail to produce a percept of the stimulus. One challenging question is how sensory information is ultimately transformed into a categorical decision about the stimulus in higher brain areas. Another challenge is to study the coding of the decision independently from the motor preparation for the report of the decision. We designed a rule-based sensory detection task that allowed a clear dissociation of a decision about the presence of a visual stimulus from motor preparation and investigated the formation of abstract decisions with electrophysiological recordings at the level of the prefrontal (PFC) and premotor (MPC) association cortices.
Two monkeys (Macaca mulatta) were required to detect the presence or absence of a brief visual stimulus at different luminance levels around perceptual threshold. Three different visual objects were used to ensure that the monkeys relied on the mere presence of the stimuli while ignoring object properties. Importantly, during the decision period the monkeys did not yet know how to respond to the presence or absence of a stimulus. Three seconds after the stimulus presentation period, a color cue signified the rule of how the animal needed to respond. If the monkeys decided that a stimulus was present, a red rule-cue required them to release a lever whereas they continued to hold the lever after a blue cue. Conversely, if the monkeys did not detect a stimulus, the red cue indicated to keep holding the lever whereas the blue cue required a lever release.
A preliminary analysis of the data suggests that about 11% of PFC and 16% of MPC neurons modulate their firing rate depending on the abstract decision about the sensory percept. This activity reliably predicted the monkeys' upcoming report about the presence or absence of the stimulus, even before the subject can prepare for a motor response. About 60% of active neurons in both areas did not modulate their firing rate with increasing stimulus intensity. The remaining neurons slightly increased or decreased the firing rate dependent on the stimulus intensity, yet this modulation was up to 6-fold smaller compared to the change in firing rate because of the decision. These data indicate that neurons in the PFC and PMC represent simple decisions about sensory stimuli independent of motor preparation.

# Retinotopic encoding of reach-to-grasp movements in the macaque premotor area F5

Sebastian J. Lehmann[1], Hansjoerg Scherberger[2]

[1]Institute of Neuroinformatics, Uni/ETH Zurich
[2]DPZ Goettingen, Neurobiology, Kellnerweg 4, 37077 Goettingen, Germany

Generating appropriate hand movements for the manipulation of a large variety of objects is a complex task that our brain achieves with seemingly no effort. The mechanisms of how the brain solves this problem – generating precise motor commands depending on sensory information about the object to be grasped – is by far not well understood. The ventral premotor cortex (area F5) in the macaque brain has been shown to encode hand grasping movements, in particular the shape of fingers, in order to execute different grip types. In the present work we investigated the influence of different spatial factors on the grasp encoding in area F5 while monkeys performed a delayed grasping task. We systematically varied the initial hand, eye, and target position in space to study the influence of these signals on the coding of grasp type in F5.

Macaque monkeys were trained to grasp a handle with one of two grip types (either a precision grip or a power grip) while the position of the handle and the eye fixation light were systematically varied in three vertical and three horizontal positions with 11 cm spacing. All task conditions were presented randomly interleaved, and eye position was monitored with an optical eye tracker. Grasping trials consisted of four epochs (fixation, cue, memory, and movement) during which the monkey had to maintain eye fixation of a red LED in the dark. To initiate the task, the animal placed its left hand at the start position and fixated the red LED. In the following cue epoch (1000ms duration), the handle position was revealed (target illumination) and the grasp type instructed by the color cue of a second LED (green: power grip; orange: precision grip). After another delay of about one second during which the animal could plan but not execute the movement, a 'go' signal was presented that required the animal to execute the grasp movement while maintaining eye fixation. Every correct trial was rewarded with a small amount of fluid.

Preliminary results from one animal revealed the existence of F5 neurons that
are modulated by reach target position as well as the eye fixation position. Quantitative analysis of the gain and preferred direction of the reach target and fixation point influence revealed that many cells in F5 encode the spatial grasp target position in retinal coordinates, that is relative to the eye fixation position. Furthermore, most of these cells are also modulated by the grip type (power vs precision grip). At the population level, the reach representation tends to be stronger at the beginning of the planning epoch, while the grasp representation seems to be dominating at the time of movement execution.

These results indicate that F5 plays an important role for the coordination of reaching and grasping movements already in the planning epoch, which reflects the fact that hand and arm movements are functionally strongly coupled and interdependent.

# Stepping patterns in free walking adult stick insects

Martyna Grabowska[1], Elzbieta Godlewska[1], Ansgar Büschges[2], Joachim Schmidt[2], Silvia Daun-Gruhn[1]

[1] Emmy Noether Research Group of Computational Biology,
[2] Department of Animal Physiology, Institute of Zoology,
University of Cologne,
Zülpicher Str. 47b, 50674 Cologne, Germany

Since Graham (1972) analysed the walking patterns of the stick insect *Carausius morosus*, it is common believe that adult and juvenile stick insects express specific walking patterns. Free walking juveniles showed mostly a tripod gait on horizontal surfaces but were also able to switch into a tetrapod gait. Under the same conditions adult stick insects walked exclusively in a tetrapod gait.
The purpose of our study was to investigate whether Graham's finding holds under different conditions. Therefore we analysed the walking behaviour of seven adult animals on a plane surface with a 15° downward slope. The animals were filmed from above with 60 frames per second, which allowed us to exactly determine the position of each leg at any time. Altogether, we evaluated 34 walks of the seven animals.
In contrast to Graham, we observed that under these conditions adult stick insects used both, the tripod as well as the tetrapod gait. In fact, they could even switch between both gaits during one walk.
Sometimes stick insects used their forelegs to perform some kind of searching movement. Usually these movements made it impossible to determine the walking pattern because of their irregularity (=undefined pattern, 35%). In order to detect regularities in the undefined patterns, we ignored the irregular stepping of the forelegs and were then able to observe stereotypic movements of the middle and hind legs. We are going to refer to these new regular patterns (only middle and hind leg steps) as “brackets” and “stairs”. Comparing the bracket and stairs patterns with the stepping patterns of the middle and hind legs during a tripod and tetrapod gait, it became apparent that the bracket pattern is a subset of the tripod pattern, and that the stairs pattern is a subset of the tetrapod pattern. Using this new analytic method, 60% of the undefined patterns (including foreleg steps) could be classified as stairs patterns and 20% of the undefined patterns as bracket patterns.
Furthermore, we observed that the stepping frequency was constantly higher in the case of the tripod gait and the bracket pattern than in the case of the tetrapod gait and the stairs pattern. The duration of the swing phase in contrast remained similar in these four different patterns. We conclude that for higher velocities stick insects choose tripod gait or bracket pattern over tetrapod gait or stairs pattern.
Now the question arises whether the velocity or the slope of the surface has a greater influence on the walking pattern of the stick insect. To find an answer to this question, we are going to analyse the stepping patterns of adult stick insects on a horizontal surface as well as on a surface with a 15° upward slope and compare those results with the ones presented above.

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# The *Drosophila* femoral chordotonal organ: a detector for substrate vibrations?

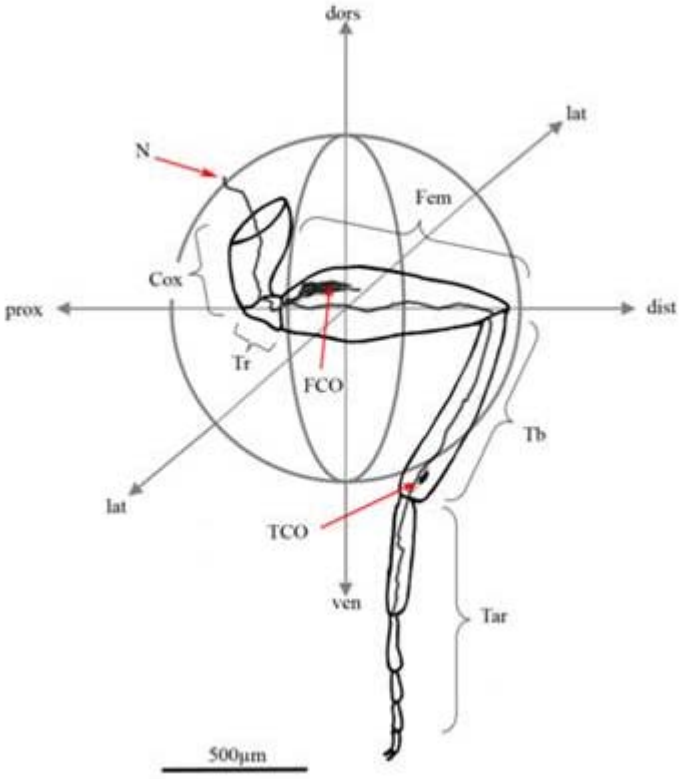
Robert Jago Wiek[1], Martin C. Göpfert[1]

[1]Johann-Friedrich-Blumenbach Institute for zoology and anthropology Department of Cellular Neurobiology, Goettingen Germany
[2]GGNB doctoral program: "Sensory and Motor Neuroscience"

In the peripheral nervous system of insects scolopidia form mechanoreceptive Chordotonal organs. Femoral chordotonal organs (FCO) monitor the position and movement of the tibia in the *New Zeeland tree weeta* and in the stick insect *Cuniculina Impigra*. The tibial subgenual organ detects substrate vibrations in orthopterans and hemipterans. In orthopterans the leg scolopidial tympanal organ is the equivalence of the ear.

Little is known about the *Drosophila* FCO morphology and its functional properties are yet unknown. Here we show that functional properties of the *Drosophila* FCO can be dissected by use of the Gal4 -UAS system in combination with in vivo $Ca^{2+}$-imaging technique. As sinusoidal stimulation elicits changes in $[Ca^{2+}]$, one can assume that so called substrate vibrations can be detected by the *Drosophila* FCO.

As previous investigations of FCOs in different insect species extended the knowledge about locomotor control and walking behavior, the underlying genetic machinery was not observed. The genetic accessibility of the model organism *Drosophila m.* holds the tools to investigate the function of the FCO in a much broader approach.



# The medial nidopallium of pigeons plays a central role in a serial reaction time task

Sascha Helduser[1], Onur Gunturkun[1,2]

[1]Ruhr-Universität Bochum, Institut für kognitive Neurowissenschaften, AE Biopsychologie, Universitätsstr. 150, D-44801 Bochum, Germany

[2]International Graduate School of Neuroscience, Ruhr-Universität, Bochum

The ability to learn and execute sequential motor actions plays an important role in both human and animal behaviour. The learning and production of motor sequences has been extensively studied in the song system of oscine birds. The song system comprises two interacting pathways. A motor pathway produces learned song in a stereotyped manner. A basal-ganglia forebrain circuit, the anterior frontal pathway (AFP) plays a crucial role in the learning process in juvenile birds. In addition, it is supposed to be required for song maintenance in adults. The motor pathway feeds information about ongoing motor activity into the AFP. The AFP, in turn, can influence the motor output via the lateral magnocellular nucleus of the anterior nidopallium (LMAN) that projects to the motor pathway.

Adding evidence to the hypothesis that the song system developed from pre-existing pathways controlling general body movements, parts of the medial nidopallium of the pigeon have been shown to comprise similar connection patterns as the oscine LMAN. Hence, we hypothesize that the medial nidopallium of the pigeon has a similar function in the acquisition and production of motor sequences as the LMAN.

In the present study, we investigated the role of the medial nidopallium of the pigeon in the production of a well learned motor sequence. Therefore, a 5 choice serial reaction time task (SRTT) was applied. Therein, pigeons were trained to peck five keys on a touch screen in a fixed sequence. After overtraining the pigeons in the sequence, the region of the nidopallium assumed to be equivalent to LMAN was inactivated by injections of tetrodotoxin via chronically implanted microcannulae during test sessions.

The reaction times of the pigeons were not affected by the inactivation of the medial nidopallium. However, the error rates increased. In particular, perseverative responses, i.e. the repeated pecking on one key, as well as the skipping of one position in the sequence occurred significantly more often. Thus, our results indicate that the medial nidopallium of pigeons, the putative homologue of the oscine LMAN, plays an important role in the execution of sequential motor actions.

# The spatiotemporal evolution of cathodal stimulation induced after-effects: differential adaptation in primary and secondary motor areas

Sein Schmidt[1], & Robert Fleischmann[1], Kerstin Irlbacher[1], Stephan A. Brandt[1]

[1]Charite Berlin, Neurology, Chariteplatz 1, 10117 Berlin, Germany

Introduction:
The human motor system comprises heavily interconnected primary and secondary motor areas (Freund and Hummelsheim, 1985;Chouinard and Paus, 2006). Functional primary motor cortex inhibition, be it physiologically or pathologically, causes substantial local as well as remote changes in cortical excitability (Nelles, et al., 1999;Johansen-Berg, et al., 2002;Dancause, 2006). A recent model suggests ipsilateral secondary motor areas to be employed first with secondary recruitment of contralateral areas occurs if ipsilateral compensation does not suffice (Swayne, et al., 2008). Functional inhibition can be induced by non-invasive brain stimulation, e.g. transcranial direct current stimulation (tDCS) (Vines, et al., 2006;Nitsche, et al., 2008;Avanzino, et al., 2008),and primary as well as the dorsal premotor cortex (dPMC) can be probed directly with navigated transcranial magnetic stimulation (nTMS) (Teitti, et al., 2008;Fleischmann, et al., 2010). This allows us to investigate the spatial and temporal evolution of the motor network excitability modifications induced by tDCS. Although studies of segregated intra- and interhemispheric interactions after tDCS has been described to some extent elsewhere(Gilio, et al., 2003;Schambra, et al., 2003;Lang, et al., 2004;Nitsche, et al., 2005), no study has addressed a coherent model based understanding of distant effects.

Objective:
This study aims at testing a hierarchical model of neurophysiological adaptation processes in the ipsilateral dPMC and contralateral M1 (cM1) induced by cathodal tDCS.

Methods:
In a double-blinded, randomized and sham controlled design, we applied cathodal as well as sham tDCS to the primary motor cortex of the dominant hemisphere of 16 healthy subjects. Finger-tapping is used to quantify functional iM1 impairment. In comparison to the functional impairment, the impairment of corticospinal, intracortical and interhemispherical excitability is investigated in ipsi- and contra-interventional primary and secondary motor areas in 10 minute bins over 80 minutes post tDCS.

Results:
We find that cathodal tDCS induces immediate functional iM1 impairment associated with reduced iM1 excitability. The cM1 and dPMC show only mild increase or no modification of the excitability, respectively. iM1 function returns to sham corrected baseline levels after 40 minutes with a concomitant turnover of its activity from decrease to increase. At his stage, cM1 activity is normalized while the dPMC, however, exhibits a substantial increase in temporal proximity to normalized iM1 function.

Discussion:
We find a spatially and temporally discrete adaption process in all regions studied. Cathodal tDCS induced functional iM1 inhibition is associated with decreased measures of excitability as shown previously (Nitsche, et al., 2008). We provide novel evidence that enhanced cM1 activity is not essential for normalization of iM1 function and a strong late effect in the ipsilesional dorsal premotor cortex subsequent to iM1 modifications that we argue might be a homeostatic process. These effects are in line with the hierarchical model developed by Swayne and colleagues in stroke patients (Swayne, et al., 2008) and supports the notion of a dynamic approach to understanding the aftereffects induced by tDCS. Perceptively, these findings can help us better understand strategies of compensation for electrophysiological disturbances in the brain as well as to possibly develop recovery stage adapted tDCS protocols of recovery maximization.

# Whole-body kinematics and 3D targeting of foot contacts in unrestrained climbing stick insects (*Carausius morosus*)

Leslie Michael Theunissen[1], Volker Dürr[1,2]

[1]Bielefeld University, Department of Biological Cybernetics, Universitätsstr. 25, 33615 Bielefeld, Germany [2]Cognitive Interaction Technology - Center of Excellence (CITEC), Bielefeld University, Germany

Stick insects appropriately coordinate their legs when moving in a complex environment. The six legs are coordinated not only temporally, but also spatially. Appropriate touch-down of each foot is crucial for stable climbing in the canopy. Cruse (1979) found that stick insects adjust the touch-down position of a hind leg according to the current position of the ipsilateral middle leg. This "targeting mechanism" was also found for the middle legs that touch-down in close vicinity to the front legs. As yet, these findings concern coordination on a horizontal platform, whereas in their natural environment, stick insects need to coordinate their legs in a three-dimensionally structured canopy.

Here, we analysed three-dimensional targeting of unrestrained climbing stick insects on a parcours with steps of different height. Three step heights and an additional flat walkway (as control) were used to reveal differences between normal walking and different levels of climbing complexity. The walking behaviour of stick insects across the low step (8 mm) was close to normal walking. A step height of 24 mm could be climbed by means of a single higher foot step. In the highest condition (48 mm) stick insects typically required several foot contacts on the vertical aspect of the step. Apart from their legs, stick insects adjusted their body posture also by use of thoracic joints.

A motion capture system yielded three-dimensional positions of retro-reflective markers attached to the stick insect legs, thorax and head. The movement of each body segment and each one of the six legs, was reconstructed by means of kinematic calculations based on marker positions and anatomical measurements.

The measured touch-down positions of hind and middle legs were expressed relative to the positions of the corresponding anterior legs. For this, both external, world-centred coordinates and relative, body-centred coordinates were used. The touch-down positions of the middle legs were located posteriorly and medially to the concurrent front leg position. The median Euclidian distance for steps during horizontal walking is 10.1 mm (SD ±7.1 mm). Euclidean distances of foot contacts on vertical surfaces of the setup were in a similar range (11.3 ±7.5 mm). Detailed analysis of the x-, y- and z-coordinates revealed a similar "targeting mechanism" during climbing as proposed by Cruse (1979) for level walking.

In comparison, targeting in hind legs was more precise, exhibiting a median Euclidian distance of 4.5 mm (SD ±7.3 mm) to the concurrent position of the ipsilateral middle leg. On vertical surfaces, the Euclidian distances were slightly larger than during level walking (vertical: 6.2 ±6.9 mm; horizontal: 4.2 ±6.7 mm). In both middle and hind legs, the likelihood distributions of relative touch-down positions were more widespread for contacts onto vertical surfaces than for contacts onto horizontal surfaces. Finally, the x-, y- and z-coordinates of the body-centred touch-down positions of hind legs were similar during horizontal walking and climbing. This suggests that, indeed, targeting takes place in three-dimensional space.

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# Working memory in the leg muscle control system of the stick insect Cuniculina impigra

Eva Berg[1], Ansgar Büschges[1], Joachim Schmidt[1]

[1]Biocenter Cologne, Animal Physiology, Zülpicherstr. 47 b, 50674 Cologne, Germany

Walking stick insects that do not find a foothold perform searching movements (SMs) with the respective leg. SMs are rather stereotypic, cyclic movements. Here we explore changes in space and time in the SM pattern upon a one-time disturbance by an obstacle.

Experiments were performed on stick insects (Cuniculina impigra) with a single intact foreleg that was restrained to move in the vertical plane. A stick was moved into the leg's path of movement such that it was touched one time by the distal tibia. After touching, the leg continued with SMs. Muscle activity was monitored by EMG recordings from the levator and depressor trochanteris.

Upon disturbance, animals shifted their SMs towards the position where the disturbance had occurred and the amplitude of SMs decreased significantly. These changes were independent of where in the leg's trajectory the stick was touched, e.g. in its upper or lower portion. Animals performed altered SMs for several seconds, during which the movements slowly shifted back to the original position with increasing amplitudes .The ratio between integrals of levator and depressor trochanteris muscle activity changed upon disturbance and was related to the direction of shift of SMs. Duty cycles and phase relationships of levator and depressor trochanteris did not change clearly.
This directional motor response that outlasts the short stimulus for some time might indicate some sort of short term working memory that provides information about the disturbance as such and its position.
In addition, we investigated the necessity of several leg sense organs for the successful execution of the directional motor response by ablation experiments. Animals still performed normally when the tibia was replaced by a peg leg (no sensory information from tibial hair fields and tibial campaniform sensilla) but were impaired when the trochanteral hair field BF1, which mainly detects upward movements of the femur, was ablated.

# Passive biomechanical properties and spike-movement transfer in an insect limb joint

Jan Marek Ache[1,2], Tom Aagaard Nielsen[2], Ansgar Büschges[1], Tom Matheson[2]

[1]University of Cologne, Zoological Institute, Biocenter Cologne, Zülpicher Str. 47b, 50674 Cologne, Germany
[2]University of Leicester, Department of Biology, University Road, Leicester LE1 7RH, UK

To understand the control of movement it is necessary to take into account not only the neuronal control signals but also the biomechanical properties of the body. To improve our understanding of aimed limb movements in an insect, we analyzed the passive biomechanical properties of the locust's metathoracic femur-tibia (FT) joint with respect to the influence of the extensor and flexor tibiae muscles, the effect of passive stretch and compression of the two muscles, and the activation history of the fast extensor tibiae motoneuron (FETi). We also stimulated FETi to investigate the interplay between these passive properties and active movements.
The resting angle of the FT joint was first measured in isolated hind legs of 17 locusts. In intact isolated legs, the tibia was slightly flexed in the absence of motor activity. Ablation of the extensor tibiae muscle caused the tibia to move to an almost fully flexed resting position in all cases. In contrast, ablation of the antagonist flexor tibiae muscle (leaving the extensor intact), had no marked effect on the resting angle of the FT joint. This angle is therefore determined and dominated by passive properties of the extensor tibiae muscle. Imposed flexion and extension of the tibia had a significant effect on the subsequent resting angle of the joint. The tibia was significantly more flexed after a preceding full flexion and more extended after a full extension. These imposed movements of the tibia had a larger effect on the resting angle than did ablation of the flexor tibiae muscle.
A quantitative analysis of the spike-movement transfer of FETi was carried out in 7 animals. For constant FT start angles, the spike-movement transfer was constant in all animals. This held true for different stimulus protocols, including single-pulse stimulation, 5 FETi spikes elicited at 6.5Hz, and 5 FETi spikes elicited at 20Hz. A hierarchical Bayesian model which explicitly incorporated measurement uncertainty and variability in the time-varying joint angle was applied to the single-pulse stimulation data. This showed that the within-animal variability was ten-fold smaller than the between-animal variability.
The resting angle of the FT joint depended on the recent history of activation of the muscles acting on it, and is therefore better described as a varying *resting state*. The resting state was analyzed in detail by measuring how the FT angle after an active movement depended on the starting position before the movement and on the pattern of stimulation applied to FETi to generate the movement. The start angle predicted very reliably the end angle, regardless of stimulation protocol or animal. Different stimulus protocols resulted in different offsets in resting angle after the movement. These were fairly constant across animals.
Our results add to an increasing body of evidence showing that passive biomechanical properties of insect limbs shape and modify the motor output rather than being simple mediators between neural activity and movements. The highly reliable spike-movement transfer shows that the nervous system can control movements with high accuracy due to very low noise induced by the biomechanical properties.

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# Poster Topic

## T22: Homeostatic and Neuroendocrine Systems, Stress Response

T22-1A Brain endothelial Tak1 mediates the induction of fever
*Dirk Ridder, Ming-Fei Lang, Sergei Salinin, Markus Schwaninger*

T22-2A Chemosensation and neuroendocrine signaling in the murine and porcine GI-tract
*Désirée Haid, Nicole Hass, Patricia Widmayer, Heinz Breer*

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T22-5A Estrogen Receptor a in Kisspeptin Neurons Controls the Timing and Completion of Puberty
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*Sandra Ammersdörfer, Sarah Galinski, Karl-Heinz Esser*

T22-4B Interaction between chemosensory and neuroendocrine cells in the gastric mucosa
*Julia Eberle, Nicole Hass, Patricia Widmayer, Heinz Breer*

T22-1C Investigating the cellular dichotomy of the hypothalamic orexin system.
*Cornelia Schöne, Denis Burdakov*

T22-2C Multimodal Imaging of Neuro-metabolic Coupling
Following Single or Multiple Spreading Depolarisations
*Delphine Feuerstein, Markus Gramer, Heiko Backes, Tetsuya Kumagai, Michael Sué, Stefan Vollmar, Rudolf Graf*

T22-3C Nutrient sensing elements on the GI tract: correlation with the energy status
*Patricia Widmayer, Désirée Haid, Markus Küper, Michael Kramer, Heinz Breer*

T22-4C The neuropeptide SIFamide in *Drosophila melanogaster*: in search of its function
*Abud Farca Luna, Simon Kobbenbring, Karina Schäfer, Angelika Schulz, Michael Gertig, André Fiala*

# Brain endothelial Tak1 mediates the induction of fever

Dirk Ridder[1], Ming-Fei Lang[1], Sergei Salinin[1], Markus Schwaninger[1]

[1]University of Heidelberg, Institute of Pharmacology, Im Neuenheimer Feld 366, 69120 Heidelberg, Germany

Fever is a ubiquitous response among animals against different kinds of tissue damage. Fever and sickness behavior require the induction of cyclooxygenase-2 (COX-2) and increased prostaglandin E2 (PGE2) production. COX-2 induction depends on the transcription factors NF-κB and AP-1. The cells producing COX-2 and PGE2 upon immune stimuli are mainly thought to be endothelial cells, but so far genetic evidence is lacking. In this study we have generated a Cre-mouse line to delete transforming growth factor-ß-activated kinase 1 (Tak1), a master regulator of the transcription factors NF-κB and AP-1, specifically in the blood-brain barrier. Mice lacking Tak1 in brain endothelial cells show a blunted fever response and reduced sickness behavior upon intravenous stimulation with the endogenous pyrogen IL-1ß. We could also show that COX-2 induction upon stimulation with IL-1ß in brain endothelial cells in vitro depends on Tak1, although Tak1 inhibition does not affect the activation of NF-κB upon stimulation with IL-1ß. In conclusion we could show that Tak1 in brain endothelial cells is necessary for the induction of fever and sickness behavior and that this effect is most likely mediated by activation of p38 MAPK and AP-1.

# Chemosensation and neuroendocrine signaling in the murine and porcine GI-tract

Désirée Haid[1], Nicole Hass[1], Patricia Widmayer[1], Heinz Breer[1]

[1]University of Hohenheim, Institute of Physiology 230a, August-von-Hartmann Straße 3, 70599 Stuttgart, Germany

To assure an appropriate nutrient supply, it is of vital importance to adjust food intake controlled by hunger and satiety, but also digestive processes such as gastric secretion and motility according to the composition of the ingested food. These adaptive reactions are supposed to be mediated via neuronal or endocrine systems and require chemosensory systems in the gastrointestinal mucosa capable to sense constituents of the diet and to convey the chemosensory information onto neuronal or endocrine cells. Little is known about cellular and molecular basis of chemosensation and neuroendocrine signaling in the GI-tract. In this study, attempts were made to identify putative chemosensory cells in the gastric mucosa and to determine the topographic distribution, and their spatial relationship with neuronal and endocrine systems. In comparative studies we focused on the gastrointestinal tract of mice and pig. Rodents provide a variety of advantages for experimental analyses, however, their gastrointestinal physiology differs significantly from that of human. In contrast, the pig is considered as most appropriate for studying gastrointestinal processes in man. Therefore, the pig is accepted as a suitable model for studying aspects of gastrointestinal physiology with particular attention to relevant disorders in human. Thus it is of great interest to understand the chemosensation of swine and to uncover if there are substantial differences to the rodent gastrointestinal system. Investigation of the murine and porcine gastric mucosa has led to the identification of substantial cell populations which express gustatory transduction elements such as T1R3, TRPM5 or PLCß2. Based on the expression of gustatory signaling elements, we hypothesize that these cells represent candidate chemosensors in the GI -tract. Closer inspection revealed that the morphology and topographic distribution of putative chemosensory cells in the gastric mucosa varied significantly between mice and pig. In the stomach of mice candidate chemosensory cells are segregated at the boundary between the esophagus and stomach, as well as between the stomach compartments fundus and corpus. The palisade-like arrangement of putative chemosensory cells at these strategic positions, i.e. at the gastric entrance and at the transition from the storage compartment fundus to the digestive compartment corpus, suggests an important role in an early monitoring of the ingested food. In the corresponding regions of the pig stomach no such compact cell cluster was observed. Furthermore, in contrast to the murine stomach, where putative chemosensory cells were often located in close proximity to endocrine cells, in the porcine stomach candidate chemosensory cells themselves appear to be endocrine cells; they thus may simultaneously operate as “sensor” and “effector” cells. Moreover recent experiments revealed that in the porcine gastric mucosa even neuronal fibers of the enteric nervous system were found in close vicinity to cells expressing TRPM5. As neuronal fibers obviously have no direct access to the gastrointestinal lumen it is conceivable that they interact with chemosensory cells. It is possible that there is a direct physical contact between cells and fibers, forming afferent synapses, or convey chemosensory information via paracrine messengers onto afferent nerve fibers.

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# Chronic restraint stress differentially affects the functional integrity of the parvalbumin and cholecystokinin interneurons in the hippocampus of adult male rats

Wen Hu[1], Mingyue Zhang[2], Boldizsar Czeh[1,4], Weiqi Zhang[2], Gabriele Flugge[1,3]

[1]Germany Primate Center, Clinical Neurobiology Laboratory, Kellnerweg 4, D-37077 Gottingen, Germany
[2]Laboratory of Molecular Psychiatry, Department of Psychiatry, Westfalische Wilhelms University, Munster, Germany
[3]DFG Research Center Molecular Physiology of the Brain (CMPB), University of Gottingen, Germany
[4]Current address: Molecular Neurobiology, Max-Planck-Institute of Psychiatry Munich, Germany

Chronic stress is known to induce alterations in neuronal networks in many brain areas, and is therefore a risk factor for the development of psychiatric disorders. Previous studies have well documented alterations in physiological functions of hippocampal excitatory neurons following chronic stress, but little is known about an impact of stress on the GABAergic network. Here we studied effects of chronic restraint stress on hippocampal GABAergic neurotransmission and network function focusing on two perisomatic interneurons, the parvalbumin (PV) and the cholecystokinin (CCK)-positive neurons. Using an established protocol, male rats were daily restrained for 21 days and whole -cell voltage clamp was performed at CA1 pyramidal neurons in acute hippocampal slices.

Chronic restraint stress resulted in Ca2+-dependent increased sIPSCs in CA1 pyramidal neurons. In addition, rhythmic IPSCs originating from the PV interneurons were impaired in coherence. In contrast, the temporal precision in CCK interneurons remained unaffected. Concomitantly, chronic stress reduced the number of PV-immunoreactive cells without significantly affecting the number of CCK-immunoreactive cells. However, chronic restraint stress also caused a dysfunction in endocannabinoid mediated modulation of GABAergic transmission. Depolarization induced suppression of inhibition (DSI), a form of short term synaptic plasticity which is specifically present at CCK interneuron terminals, was impaired by the stress.

We therefore propose that chronic stress differentially modulates the functional integrity of PV and CCK cells and results in an imbalance in perisomatic inhibition mediated by these two interneuron subtypes. These stress-induced dysfunctions in the inhibitory network may in turn impair rhythmic oscillations and thus lead to cognitive deficits that are common in stress-related psychiatric disorders.

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# Direct action of insulin on steroidogenic factor 1 positive neurons in the ventromedial hypothalamus

Simon Hess[1], Tim Klöckener[2,3], Brigitte Hampel[2,3], Moritz Paehler[1,2], Jens C. Brüning[2,3], Peter Kloppenburg[1,2]

[1]University of Cologne, Biocenter Cologne, Institute for Zoology, Zülpicher Strasse 47b, 50674 Cologne, Germany

[2]Center for Molecular Medicine Cologne (CMMC) and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50674 Cologne, Germany

[3]Institute for Genetics, Department of Mouse Genetics and Metabolism and Max Planck Institute for the Biology of Aging, University of Cologne, 50674 Cologne, Germany

During the last decade it has been demonstrated that insulin directly acts on the brain in order to control food intake and energy expenditure (Brüning et al., 2000). However, most of the studies addressed the role of insulin in the arcuate nucleus of the hypothalamus (ARC). Insulin receptors are also expressed in other brain areas involved in the control of energy homeostasis, among them the ventromedial hypothalamus (VMH). Lesions of the VMH lead to obesity underlining the crucial role of the VMH in controlling feeding and body weight (Marshall and Mayer, 1956). Recent studies have shown that mice lacking the steroidogenic factor (SF-1) suffer from a failure to appropriately develop adrenal glands and gonads, and also have an abnormally developed VMH (Ikeda et al., 1995; Shinoda et al., 1995). These mice, when rescued from lethality by adrenal transplantation, developed massive obesity resulting from both hyperphagia and reduced energy expenditure (Majdic et al., 2002).

While it has already been demonstrated, that the adipocyte-derived hormone leptin has a direct effect on SF-1 positive neurons in the VMH (Dhillon et al., 2006), the the role of insulin in SF-1 neurons has not been defined, yet.

Here, we show that insulin activates PI3-kinase signaling in SF-1 positive neurons. To assess the role of insulin on the spontaneous activity of SF-1 neurons we used the non-invasive perforated patch technique, which insured integrity of intracellular components. We could demonstrate that insulin directly hyperpolarized a major subpopulation of SF-1 neurons leading to a reduction in firing frequency. This effect was largely dependent on the activation of ATP-dependent potassium channels (KATP) suggesting a similar mechanism of insulin‘s action as in POMC neurons of the ARC (Plum et al., 2006b; Spanswick et al., 2000). In contrast, insulin failed to activate PI3-kinase and to reduce the firing frequency in SF-1 neurons where the insulin receptor was specifically deleted. Taken together, our results suggest that insulin plays an important role in this brain region for the control of feeding and body weight.

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channels in hypothalamic neurons of lean, but not obese rats. Nat Neurosci 3, 757-758 (2000)

# Estrogen Receptor a in Kisspeptin Neurons Controls the Timing and Completion of Puberty

Christian Mayer[1], Maricedes Acosta-Martinez[2], Sharon L. Dubois[2], Andrew Wolfe[2], Sally Radovick[3], Jon E. Levine[2], Ulrich Boehm[1]

[1]Institute for Neural Signal Transduction, Center for Molecular Neurobiology, Hamburg,Germany, [2]Department of Neurobiology & Physiology, Northwestern University, Evanston, IL, USA, [3]Division of Pediatric Endocrinology, Johns Hopkins University, Baltimore, MD, USA

Puberty onset is mediated by increased neurosecretion of gonadotropin releasing hormone (GnRH). Accelerated GnRH release depends upon stimulation by kisspeptin neurons. A role for estradiol-17ß (E2) and estrogen receptor a (ERa) in regulating kisspeptin neurons, and hence puberty, has been hypothesized. Here we demonstrate that ERa in kisspeptin neurons controls both the timing and completion of puberty in female mice. A kisspeptin cell-specific knockout of ERa (KERKO) reveals these two important functions during pubertal maturation. First, female KERKO mice exhibit advanced onset of puberty characterized by precocious luteinizing hormone (LH) secretion and premature vaginal opening. Our findings demonstrate that ERa signaling in kisspeptin neurons mediates a prepubertal "brake" that inhibits GnRH release during the juvenile period. Second, female KERKO mice do not develop normal ovulatory cyclicity. Thus, despite the early onset of puberty in these animals, they fail to complete the pubertal process and remain arrested in an acyclic mid-pubertal state. This disruption of puberty in KERKO mice is associated with profoundly diminished hypothalamic kisspeptin expression, suggesting that ERa signaling in kisspeptin neurons normally directs a progressive up-regulation of kisspeptin expression that is in turn critical for the establishment and maintenance of adult GnRH secretory patterns. Activation of ERa signaling in kisspeptin neurons is thus essential in females for gating the onset of puberty as well as ensuring its completion.

# High fat induced obesity impairs intrinsic properties of anorexigenic POMC neurons in the hypothalamus

Andreas Pippow[1], Moritz Paehler[1,2], Lars Paeger[1,2], Simon Hess[1,2], Tim Klöckener[2,3], Merly Vogt[2,3], Christophe Pouzat[4], Jens C. Brüning[2,3], Peter Kloppenburg[1,2]

[1]University of Cologne, Biocenter Cologne, Institute for Zoology, Zülpicher Strasse 47b, 50674 Cologne, Germany

[2]Center for Molecular Medicine Cologne (CMMC) and Cologne Excellence Cluster on Cellular Stress Responses in Aging-Associated Diseases (CECAD), University of Cologne, 50674 Cologne, Germany

[3]Institute for Genetics, Department of Mouse Genetics and Metabolism and Max Planck Institute for the Biology of Aging, University of Cologne, 50674 Cologne, Germany

[4]Laboratoire de Physiologie Cerebrale, CNRS UMR 8118, UFR biomedicale de l'Université René Descartes, Paris V, 45 Rue de Saints Peres, 75006 Paris, France

Energy homeostasis is tightly controlled by neuronal circuits mainly located in the hypothalamus to adapt food intake and energy expenditure according to the availability of fuel sources in the periphery of the body. Two populations of neurons within the arcuate nucleus of the hypothalamus, which express proopiomelanocortin (POMC) or neuropeptide Y/agouti-related protein (NPY/AgRP), are crucial for the regulation of body weight. Our long-term goal is to understand in detail diet and age associated changes in this hypothalamic circuit. Here, we compared intrinsic electrophysiological characteristics and synaptic input of POMC neurons between 15 to 20 weeks old mice on a normal diet (Normal Chow Diet: NCD) and 15 to 20 weeks old mice that were fed a High Fat Diet (HFD). We found several physiologically important parameters that were significantly different between these experimental groups, including membrane potential, firing frequency, voltage activated $Ca^{2+}$ currents, $Ca^{2+}$ resting level, $Ca^{2+}$ handling properties and inhibitory synaptic input.

Perforated patch-clamp recordings showed that the resting membrane potential of POMC neurons was significantly hyperpolarized in mice on a high fat diet. The hyperpolarization was accompanied by a significant decrease of the firing frequency, and the number of neurons with no spontaneous action potential firing was significantly increased. Furthermore, whole-cell voltage-clamp recordings revealed that the frequency of inhibitory postsynaptic currents (IPSCs) was significantly increased, while the excitatory input remained unchanged. Application of $GABA_A$ receptor blockers eliminated IPSCs completely, but neither $GABA_A$ nor $GABA_B$ receptor blockers (or a combination of both) restored the membrane potential and firing rate to control levels. The latter results indicate that the high fat diet induced hyperpolarization and decrease in firing rate could be caused by altered intrinsic properties.

To identify the neuronal properties and mechanisms that mediate the high fat diet induced changes in membrane potential and firing rate, we have started a detailed analysis of diet-induced changes in ionic conductances. So far, we found that the high fat diet induced a significant increase in voltage activated $Ca^{2+}$ current ($I_{Ca}$), and changes in $Ca^{2+}$ handling properties. The current amplitude and current density of $I_{Ca}$ was increased without a change in the voltage dependence of $I_{Ca}$. These results are in line with previous work that showed PI3K promoted trafficking of voltage dependent $Ca^{2+}$ channels to the plasma membrane (Viard et al. 2004). In addition to increasing the voltage dependent $Ca^{2+}$ influx, the high fat diet increased the resting level for free intracellular $Ca^{2+}$ and reduced the $Ca^{2+}$ binding ratio and the $Ca^{2+}$ extrusion rate. Our working hypothesis, which we currently verify, is that the increased intracellular $Ca^{2+}$ level activates $Ca^{2+}$ dependent $K^+$ currents, which hyperpolarize the membrane potential and decrease the firing frequency. In addition, we have first evidence that the $Ca^{2+}$ handling of mitochondria is modulated by HFD. Experiments in which $Ca^{2+}$ release from mitochondria was induced by carbonyl cyanide p(trifluoromethoxy)phenylhydrazone (FCCP), indicated a reduced $Ca^{2+}$ storage capacity in mitochondria in POMC neurons during high fat diet.

# hSMP, a novel mechanism for CA1 pyramidal neurons to homeostatically regulate their activity

Xiaojie Huang[1], Oliver M. Schlüter[1]

[1]European Neuroscience Institute Göttingen, Molecular Neurobiology , Grisebachstr. 5, 37077 Göttingen , Germany

Hebbian forms of plasticity cause synapse specific changes in synaptic weights to encode information in neuronal networks. These long-term changes in synaptic strength are considered to be the cellular correlate of memory. NMDA receptor-dependent long-term potentiation (LTP) is a well studied form of Hebbian plasticity which has been discovered in different brain regions including the hippocampus. But this unconstrained one-directional plasticity also reveals a severe problem in maintaining network stability. To balance this problem, neurons have the ability to homeostatically regulate their activity on a network level so to keep the system at a predefined set point. Both changes in the intrinsic excitability and a homogenous adjustment of the synaptic weights (synaptic scaling) contribute to this adjustment, without altering the synapse specific codes. Recently a new form of homeostatic synapse-driven membrane plasticity (hSMP) has been described in nucleus accumbens (NAc) medium spiny neurons (MSN). Here, we show that chronic inhibition of the NMDA receptors by APV increased the intrinsic excitability of CA1 hippocampal pyramidal neurons of organotypic slice cultures. In contrast to MSN, the chronic enhancement of NMDAR function with the co-agonist DCS, failed to induce hSMP in hippocampal slices. We will demonstrate results, investigating the role of different NMDAR subunits in hSMP, using a combination of pharamacological and genetic tools to alter the NMDAR subunits in a specific way.

# Immobilization stress in a bat model: Effects on behaviour, cortisol level and neuromorphology of the amygdala in the Short-tailed fruit bat, Carollia perspicillata.

Sandra Ammersdörfer[1], Sarah Galinski[1], Karl-Heinz Esser[1,2]

[1]Auditory Neuroethology and Neurobiology, Institute of Zoology, University of Veterinary Medicine Hannover, Germany
[2] Center for Systems Neuroscience Hannover, Germany

The mammalian amygdala plays a crucial role in the processing of emotions, eliciting of emotional reactions and in mediating the stress response. Stress is defined as any type of threat to the organism, which requires compensatory responses for the maintenance of homeostasis. These stress responses include for example changes in the endocrine system, caused by activation of the hypothalamo-pituitary-adrenal system and the release of glucocorticoids. The amygdala is highly sensitive to stress hormones and shows morphological changes for example in clinical disorders related to stress (e.g. depression, post-traumatic stress disorder). Our study focuses on the central nucleus, the major output nucleus of the amygdala, which seems to be important for analysing the emotional relevance of sensory information. Via amygdaloid projections to the brainstem and the hypothalamus, the output nucleus mediates stress reactions and impairs the autonomic nervous as well as the neuroendocrine system. Therefore, we expect neuromorphological alterations in the central nucleus of the amygdala in response to aversive experiences (e.g. immobilization stress).
Besides studying the effects of immobilization stress on the neuromorphology of the central nucleus, we further investigated corresponding effects on the hormonal as well as the behavioural level.
For the neuroanatomical study part, the brains of the stressed bats were prepared, embedded and serially sectioned on a cryostat (frontal sections of 40 µm thickness). Staining was achieved with cresyl violet and a modified silver impregnation. Brains of control animals were treated equally. As a prerequisite for our analysis on the cellular level, a parcellation of the amygdala of C. perspicillata was established. Slices of controls and bats subjected to immobilization were microscopically analyzed to reveal stress-induced neuronal plasticity within the central nucleus of the amygdala.
For verifying, whether the selected stress regime is suitable for the Short-tailed fruit bat or not, the cortisol concentration (a reliable physiological parameter for stress) of the animals' faeces was measured pre- and post-stress. Faecal sampling was chosen to avoid the necessity of handling of the animals, which can produce a stressful situation itself. Another advantage of faecal sampling is that each individual can be used as its own control (pre- vs. post-stress), which excludes the impact of inter-individual variations in the hormone level.
The endocrinological verification yielded evident proof of the applicability of our stress regime in the bat.
Based on this evidence, we considered whether stress effects can also be observed behaviourally. To answer this question, the behaviour of the animals was observed in a custom-made plus maze for bats. This in rodents well-validated behavioural experimental design (in rodents: elevated plus maze) relies on the approach-avoidance conflict of animals to novel stimuli. A plus maze consists of four arms (two open and two enclosed ones) and serves as a test for anxiety, which is supposed to correlate with the stress level of an animal. The performance of each individual in the maze was observed, recorded and analysed and will be described in detail on the poster.

# Interaction between chemosensory and neuroendocrine cells in the gastric mucosa

Julia Eberle[1], Nicole Hass[1], Patricia Widmayer[1], Heinz Breer[1]

[1]University of Hohenheim, Institute of Physiology 230a, August-von-Hartmann Straße 3, 70599 Stuttgart, Germany

Sensing constituents of the luminal content in the stomach is of vital importance since various gastric processes, such as secretion of acid and digestive enzymes, as well as the release of peptide hormones, are precisely adjusted. According to the ingested food, these adaptive reactions are mediated either by neuronal or by endocrine mechanisms, however it is yet not known how the appropriate information regarding the composition of the gastric chyme is acquired by the neural and endocrine cells. Based on the expression of gustatory receptors and transduction elements, candidate chemosensory cells have recently been identified in the mucosa of the gastrointestinal tract. It is hypothesized that these cells may sense constituents of the luminal chyme and transduce the stimulus into a cellular response; it is conceivable that the chemosensory response is subsequently conveyed onto neuronal or endocrine cells. If the strategy for interactions between sensory cells and effector cells is reminiscent of the lingual gustatory system, paracrine mechanisms may be involved. One of the prerequisites for a paracrine communication is an adjacent localization of chemosensory "input" cells and endocrine "output" cells or neuronal afferent fibers. To scrutinize this hypothesis we set out to visualize the relative spatial distribution of candidate chemosensory cells and hormone-producing cells in the gastric mucosa via immunohistochemistry. It was found that two different populations of endocrine cells were positioned in close vicinity to sensory cells. One population produced the hormone ghrelin, known as an important short-term regulator of appetite and food intake, the other population produced serotonin, which acts on a wide range of GI functions including peristalsis, nausea and vomiting as well as gastric nociception. Due to the adjacent localization it is possible that a functional interplay occurs between the candidate chemosensory cells operating as "sensor" cells and the adjacent endocrine cells operating as "effector" cells. Furthermore, based on immunohistochemical findings it is possible that candidate chemosensory cells may also interact with fibers of the enteric nervous system. Complex networks of fibers visualized via immunoreactivity of the neuronal marker PGP9.5 were seen throughout the gastric mucosa, some of them in close contact with putative chemosensory cells. This spatial arrangement of candidate chemosensory cells and candidate effector cells suggests a direct probably paracrine interplay between these cell types.

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# Investigating the cellular dichotomy of the hypothalamic orexin system.

Cornelia Schöne[1], Denis Burdakov[1]

[1]University of Cambridge, Dept. of Pharmacology, Tennis Court Road, CB21PD Cambridge, United Kingdom

Orexin neurons are part of the brain circuits regulating arousal and appetite. Recently, it was suggested that orexin neurons could be divided into two populations, depending on their electrophysiological characteristics. These H-type (hyperpolarising) and D-type (depolarising) neurons have overlapping but distinct distributions in lateral and dorsomedial / perifornical regions of the hypothalamus and respond differently to changes in extracellular glucose levels. These differences in postsynaptic properties suggest that the cells may have different physiological roles. Here we report further electrophysiological and morphological characteristics supporting the idea of two distinct subtypes in the orexin neuron population. Synaptic currents in EGFP-labelled orexin neurons were investigated by performing whole-cell patch-clamp recordings of miniature postsynaptic currents in acute mouse brain slice preparations. Our experiments indicate that H-type neurons have stronger excitatory synaptic inputs compared to D-type neurons. Conversely, D-type neurons have stronger inhibitory inputs. Furthermore H-type orexin neurons showed larger dendritic trees compared to D-type cells. The consequences of these results for arousal and appetite regulation in the brain will be target of future investigations.

# Multimodal Imaging of Neuro-metabolic Coupling Following Single or Multiple Spreading Depolarisations

Delphine Feuerstein[1], Markus Gramer[1], Heiko Backes[1], Tetsuya Kumagai[1], Michael Sué[1], Stefan Vollmar[1], Rudolf Graf[1]

[1]MPI für neurologische Forschung, Multimodal Imaging, Gleueler strasse 50, 50931 Köln, Germany[1]

Spreading depolarisations (SDs) have been suggested as a mechanism for secondary neuronal damage following occlusion of the middle cerebral artery in the rat. Recent experimental and clinical studies have also shown that the frequent occurrence of SDs in brain injury patients lead to an inverse hemodynamic response [1, 2, 3], a decrease in tissue oxygen availability [4] and a drop in brain glucose concentrations down to levels critical for cell viability [5]. To test whether temporal clusters of repetitive SD events jeopardize physiological neuro-metabolic coupling, we used a multimodal monitoring approach combining a) laser speckle flowmetry (LSF) for cerebral blood flow, b) positron emission tomography (PET) of $^{18}$F-2-fluoro-2-deoxy-D-glucose (FDG) for cerebral metabolic rate of glucose, and c) rapid-sampling microdialysis (rsMD) for dynamic extracellular concentrations of glucose.

Male Wistar rats were positioned in a custom-built LSF/PET holder for simultaneous laser speckle contrast imaging and FDG-PET measurement. The temporoparietal cortex of the animals was exposed to laser illumination (785 nm, 70 mW) through thinned skull and dura mater. A microdialysis probe (15 kDa, 0.6 mm OD) was inserted through a small craniectomy/durectomy and perfused at 1.6 µL/min with artificial cerebro-spinal fluid. The dialysate was assayed for glucose and lactate every 15 seconds using rsMD as described previously [6]. After baseline measurements of rsMD and LSF, FDG was injected and PET images were acquired for 90 min. SDs were induced 20 min after FDG injection by either a needle prick or by continuous epidural application of potassium chloride (3M).

SD waves were detected by LSF as hyperaemic waves propagating over the surface of the brain at a rate of 2 to 5 mm/min. Using region of interest analysis, the number, amplitude and duration of the hemodynamic waves were correlated to the PET and rsMD data. Locally, the FDG uptake increased with the number of SDs propagating in each region of interest and the rsMD glucose levels progressively decreased as SDs repeated. The first SD wave usually caused the most dramatic metabolic changes, with a prolonged oligemia following the transient hyperaemic wave, as well as a decrease in glucose concentration by 48 ± 11 µM compared to a mean of 18 ± 9 µM drop for subsequent waves. Thus, multimodal imaging is a powerful tool to study SD-related or SD-induced pathophysiological processes in a temporal and spatial domain.

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# Nutrient sensing elements on the GI tract: correlation with the energy status

Patricia Widmayer[1], Désirée Haid[1], Markus Küper[2], Michael Kramer[2], Heinz Breer[1]

[1]University of Hohenheim, Institute of Physiology 230a, August-von-Hartmann Straße 3, 70599 Stuttgart, Germany

[2]University Hospital Tübingen, Department of General, Visceral and Transplant Surgery, 72076 Tübingen, Germany

Dysfunction of the gastrointestinal (GI) tract is of eminent etiological relevance for obesity. Peptide hormones originating from the GI tract influence food intake and energy balance. Typical the release of hormones from the GI-tract correlates with the amount and the composition of ingested food, however, the underlying mechanisms are not fully understood. It can be assumed that chemosensation in the GI tract plays a central role in efficiently monitoring the nutritive constituents of the ingested food. Consistent with a chemosensory assessment of the luminal chyme, distinct cell types have been identified in the gastric mucosa which express elements of the gustatory transduction cascade, including the T1R3 subunit of the amino acid and carbohydrate receptors, the effector enzyme PLCß2 and the ion channel TRPM5. In order to unravel a possible effect of the nutritional status on the chemosensory system in the GI tract, studies were performed analyzing an obese mouse line (ob/ob-mice). We observed different expression levels of chemosensory signaling elements compared to normal-weight mice suggesting a correlation with the energy status. A similar correlation was found in analyses of gastric tissue samples from morbidly obese and normal-weight patients. In both cases elevated expression levels for gustducin, PLCß2 and TRPM5 and a reduced expression level for T1R3 was observed in obese tissue samples. Moreover, for TRPM5 also the number of TRPM5-positive cells was significantly increased in obese gastric tissue samples. Comparative studies analyzing mice kept at different alimentary paradigms indicated that high-macronutrient diet also led to altered expression levels of taste transduction elements in the gastric mucosa. These results suggest that both the energy status and the nutrient composition of ingested food have a profound impact on candidate chemosensory cells in the GI tract.

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# The neuropeptide SIFamide in *Drosophila melanogaster*: in search of its function

Abud Farca Luna[1], Simon Kobbenbring[1], Karina Schäfer[1], Angelika Schulz[1], Michael Gertig[1], André Fiala[1]

[1]Georg-August-Universität Göttingen, Johann-Friedrich-Blumenbach-Institute, Department of Molecular Neurobiology of Behavior, c/o ENI, Grisebachstr. 5, 37077 Göttingen, Germany.

SIFamide is a neuropeptide present and highly conserved in different arthropods such as crustaceans and insects. The location of the neuropeptide in the nervous system of arthropods is also conserved since in many insect species SIF-amide has been found to be present in two pairs of cells located in the pars intercerebralis. These cells project axons to the larval ventral nerve cord and ramify throughout different structures of the adult brain like the optic lobes and antennal lobes. However, the function of SIFamide is not well understood. We are searching for possible roles of SIFamide in *Drosophila melanogaster*. To this end we are using different behavioral and physiological assays. Taking advantage of the genetic tools available in *Drosophila* we ablated SIFamidergic neurons using the Gal4-UAS system. Those animals were tested in comparison with appropriate control strains for courtship behavior and circadian locomotor activity. For testing effects of SIFamide on odor processing within the antennal lobe the calcium sensor cameleon 2.1 was expressed in the olfactory sensory neurons or in olfactory projection neurons, respectively. Different odors (3-octanol, amyl acetate, methyl cyclohexanol, benzaldehyde and cis-vaccinylacetate) were presented to the animals and odor-evoked calcium activity was monitored before, during and after application of SIFamide. Preliminary results provide no indication for a detectable influence of SIF-amide on odor-evoked calcium activity in the *Drosophila* antennal lobes. Also, no effect of ablating SIFamidergic neurons on courtship behavior could be observed. However, in the absence of SIF -amidergic neurons daily locomotor activity increases approximately two times during the dark phase and during the late light phase, suggesting a possible link between the SIFamidergic system in insects and the overall activity state of the animals.

# Poster Topic

## T23: Neural Networks and Rhythm Generators

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*Robert Heinz Günter, Dirk Feldmeyer*

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*Annika Mix, Klaus Funke*

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*Wiebke Nissen, Inga H Deakin, Riam Kanso, Markus H Schwab, Klaus-Armin Nave, David M Bannerman, Paul J Harrison, Ole Paulsen, Karri Lamsa*

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T23-16C The role of TGF-ß2 on the development of neuronal networks
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T23-17C The spatial phase relationship between oscillations and spikes varies during maturation of the rat prefrontal cortex
*Nicole Cichon, Michael Denker, Marco D. Brockmann, Ileana L. Hanganu-Opatz, Sonja Gruen*

T23-18C Unravelling the Central Pattern Generator for Cricket Singing
*Stefan Schöneich, Berthold Hedwig*

# Acetylcholine differently modulates neuronal activity in layer 6a of the barrel cortex

Robert Heinz Günter[1], Dirk Feldmeyer[2,3]

[1]Institute of Neuroscience and Medicine, INM-2, Research Centre Jülich, D-52425 Jülich, Germany
[2]Department of Psychiatry and Psychotherapy, RWTH Aachen University, D-52074 Aachen, Germany
[3]Jülich-Aachen Research Alliance-Brain, Translational Brain Medicine

Neocortical acetylcholine (ACh) release is known to enhance signal processing by increasing the amplitude and signal-to-noise ratio of sensory responses. The intracortical ACh concentration specifically increases during wakefulness and periods of sustained attention. Several studies showed that ACh enhances the excitability of pyramidal cells through activation of postsynaptic muscarinic ACh receptors (AChRs).
We used acute brain slices of rat barrel cortex to test the effect of ACh application in layer 6a (L6a) pyramidal neurons and interneurons. Neurons were filled with biocytin during patch-clamp recording to allow a subsequent morphological identification.
Pyramidal cells in layer 2/3 and 5 of the barrel cortex are depolarised in the presence of ACh while layer 4 (L4) spiny neurons are hyperpolarised. Similarly to layer 2/3 and 5, L6a pyramidal neurons exhibit also a depolarising response to ACh application. However, there were at least three clearly distinct types of ACh responses in this heterogeneous group of L6a neurons: The first group of L6a pyramidal cells showed only a subthreshold depolarisation (3-20 mV) in the presence of ACh that did not initiate action potential (AP) firing. Maximum depolarisation was achieved at 100 µM ACh and apparently mediated by muscarinic AChRs.
In a second subpopulation of L6a pyramidal neurons 100 µM ACh elicited a superthreshold depolarisation with a regular spiking (RS) pattern of 4-6 Hz. At lower concentrations (1-30 µM) ACh increased the frequency AP firing evoked by somatic current injections; however the firing pattern remained regular.
In a third subpopulation ACh induced a change from a RS pattern to a repetitive firing of AP bursts. The superthreshold response to ACh (100 µM) was characterised by repetitive oscillatory (8-10 Hz) bursting of APs with an intraburst frequency of 130-160 Hz (4-6 APs per burst). This repetetive bursting effect was reversible and concentration dependent.

In contrast to excitatory neurons the resting membrane potential of fast spiking and non-fast spiking interneurons in layer 6a hyperpolarised with a maximum amplitude of up to 6 mV in presence of ACh. This suggests a reduction of the inhibitory tone in this layer. Thus, the observed changes in the dynamics of AP firing patterns could be the result of altered synaptic interactions between excitatory and inhibitory L6a neurons. The characterisation of these interactions and the underlying synaptic microcircuitry in layer 6a will be the aim of further examinations.

# Age-dependent Differencies in the Effect of Intermittent Theta Burst Stimulation via Transcranial Magnetic Stimulation on the Expression of Proteins Related to Cortical Inhibitory and Excitatory Activity in Young Rats

Annika Mix[1], Klaus Funke[1]

[1]Ruhr University Bochum, Neurophysiology, Universitätsstr. 150, 44801 Bochum, Germany

We could recently show that intermittent Theta Burst Stimulation (iTBS) of the rat brain via repetitive transcranial magnetic stimulation (rTMS) induced a reduction of the activity of certain interneurons in adult rats [1,2]. This was mainly indicated by reduced expression of glutamic acid decarboxylase 67kDa (GAD67) and parvalbumin (PV) following iTBS application. Now, we tested whether these effects are similar in young animals of different age in which development and integration of inhibitory neurons into the cortical network may still be under way.

Conscious rats between 23 and 90 days of age were treated with three blocks of either verum or sham iTBS (50Hz/5Hz, 3x600 stimuli, 15 minutes break) and sacrificed for immunohistochemical analysis 90min later. Protein expression analysis focussed on GAD67, GAD65, the calcium binding proteins PV, calbindin and calretinin, Neuropeptide Y and c-Fos in the frontal, motor, somatosensory and visual cortex.

The described effects of iTBS on the inhibitory cortical network, like reduction in the expression of mainly parvalbumin and GAD67 [1,2], did not occur before day 35-40 of postnatal development. Interestingly, iTBS-mediated changes in c-Fos expression already occurred in the youngest group of animals indicating that iTBS could induce cortical network activity similarly as in adult animals but without modulating the activity of the PV-expressing interneurons.

We conclude that the functional development of the inhibitory network of PV-positive cells is not fully completed in young rats until the age when iTBS-induced depression of PV-expression occurred. Although the number of PV-positive cells has already reached the adult level at postnatal age of 21 days [3], synaptic integration and synaptic plasticity mechanisms might still be deviant from the adult state before day 35-40. This study not only gives rise to questions of functional neuronal circuit development, it also indicates that the contribution of the inhibitory systems to the rTMS-induced after-effects may be age-dependent.

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# Altered Hippocampal Gamma Oscillations and GABAergic Inhibition in Mice Over-Expressing the Schizophrenia Candidate Gene Neuregulin-1

Wiebke Nissen[1], Inga H Deakin[2], Riam Kanso[3], Markus H Schwab[5], Klaus-Armin Nave[5], David M Bannerman[4], Paul J Harrison[2], Ole Paulsen[3,6], Karri Lamsa[1]

[1]University of Oxford, Department of Pharmacology, Mansfield Rd, OX1 3QT Oxford, United Kingdom
[2]University of Oxford, Department of Psychiatry, Oxford, United Kingdom
[3]University of Oxford, Department of Physiology, Anatomy and Genetics, Oxford, United Kingdom
[4]University of Oxford, Department of Experimental Psychology, Oxford, United Kingdom
[5]Department of Neurogenetics, Max-Planck Institute of Experimental Medicine, Goettingen, Germany
[6]Department of Physiology, Development and Neuroscience, University of Cambridge, Cambridge, United Kingdom

Neuregulin 1 (NRG1) is a pleiotropic growth factor with diverse functions in neurodevelopment and synaptic plasticity. NRG1 has been genetically linked to a risk of developing schizophrenia in humans, and the expression of the type I isoform of NRG1 is selectively increased in the disorder, particularly in the hippocampus and frontal cortex. Behavioural phenotyping of transgenic mice over-expressing type I NRG1 (NRG1tyI) has revealed an (age-emergent) impairment of hippocampal-dependent spatial working memory. To further explore this, we investigate the role of increased NRG1tyI expression on hippocampal network functioning in NRG1tyI mice. Interestingly, gamma-frequency oscillations and underlying GABAergic inhibition were found to be altered in NRG1tyI mice. The peak frequency, but not the power, of *in vitro* cholinergically-induced gamma oscillations in the CA3 area was significantly reduced in the transgenic mice (mean frequency Â± SEM: 22.0 Â± 0.6 Hz, n=32 slices) compared to wild-type littermates (26.5 Â± 0.5 Hz, n=44 slices, $p<0.001$). Action potential firing of CA3 principal cells was similarly phase-locked to field potential gamma cycles in both genotypes. However, recordings of synaptic inputs onto CA3 pyramidal cells revealed significantly slower inhibitory currents (half amplitude decay time in NRG1tyI: 12.5 Â± 0.5 ms, n=8 cells vs wt: 10.3 Â± 0.2 ms, n=13 cells, $p<0.001$) during gamma oscillations whereas excitatory current kinetics were unchanged. The data suggest altered functioning of local inhibitory GABAergic circuits that target pyramidal cells and generate the rhythmic inhibition on which gamma oscillations depend. Because parvalbumin-positive (PV+) interneurons are thought to be key players in the generation and maintenance of gamma oscillations, and are a likely primary target of NRG1 signalling, we next crossbred NRG1tyI mice with PV-Cre mice and utilised an optical stimulation approach to enable the selective study of PV+ interneurons in CA3 hippocampal slices. Using this technique we are currently studying the functional properties of PV+ cell inhibitory axons onto CA3 pyramidal cells in NRG1tyI x PV-Cre mice and PV-Cre littermates.

# ATP-activated P2X and P2Y receptors differentially modulate gamma network oscillations in the rat hippocampus

Zin-Juan Klaft[1], Steffen B. Schulz[1,2], Anton R. Rösler[1], Uwe Heinemann[1], Zoltan Gerevich[1]

[1]Charité Universitätsmedizin Berlin, Institute for Neurophysiology, Oudenarder Str. 16, 13347 Berlin, Germany

[2]equal contribution

ATP is the primary energy source within cells and also an extracellular signalling molecule released e.g. by neurons and glial cells. Functional ionotropic (P2X) and metabotropic (P2Y) ATP receptors have been described in the hippocampus. Here we investigate the role of ATP and its receptors on oscillatory neuronal network activity in acute hippocampal slices of the rat. Gamma oscillations (30-50 Hz) were induced in the CA3 region by using either acetylcholine (ACh) or kainic acid (KA). Exogenous ATP reduced the power of KA-induced gamma oscillations solely by the activation of adenosine receptors after its degradation. In contrast, exogenous ATP attenuated ACh-induced oscillations through both adenosine and ATP receptors with adenosine receptors accounting for ~55% and ATP thorugh P2 receptors for ~45% of suppression, respectively. This modulation could also be induced by endogenous ATP since inhibition of the degradation of ATP had an inhibitory effect on oscillation power. By means of specific antagonists it could be revealed that ionotropic P2X1-3 receptors inhibited ACh-induced gamma power whereas the metabotropic P2Y1 receptor increased it. By using ATP-sensitive electrochemical biosensors, we also measured the level of extracellular ATP in different layers of the CA3 region during the development of oscillations. We found that the ATP concentration declined by ~0.6 µM during the generation of ACh-induced gamma oscillations exclusively in the pyramidal cell layer. KA-induced gamma oscillations did not show changes in extracellular ATP levels. In conclusion, our results suggest that endogenously released ATP finely modulates the power of cholinergically induced gamma oscillations in the CA3 region of the hippocampus by activating P2X and P2Y receptors.

# Auditory effects in mouse visual cortex are linked to general anesthesia level

Rüdiger Land[1], Gerhard Engler[2], Andrej Kral[1], Andreas K Engel[2]

[1]Medizinische Hochschule Hannover, Institut für Audioneurotechnologie (VIANNA), 30625 Hannover, Germany

[1]Universitätsklinikum Hamburg-Eppendorf, Institut für Neurophysiologie und Pathophysiologie, 20246 Hamburg, Germany

General anesthesia pharmacologically alters cortical network dynamics. This change is reflected in anesthesia-dependent cortical rhythms like burst- suppression during deep anesthesia. In addition to these prominent changes in ongoing cortical activity, sensory stimulation elicits anesthesia concentration dependent responses in sensory cortices. Using multi-channel recordings from mouse visual cortex and subiculum, we examined responses to visual and auditory stimulation during continuously changing levels of anesthesia. During all levels, from light to deep anesthesia, visual cortex can be activated with visual stimulation. During deep burst-suppression, latency of visual evoked potentials is not changed in comparison to light anesthesia, although the responses differ qualitatively by inducing burst activation. Interestingly, auditory stimulation significantly suppresses ongoing activity in V1 during light anesthesia, however at increased anesthesia levels reversely elicits long latency burst activation in V1. Only at high levels of anesthesia (with burst- suppression), subiculum is activated by visual as well as auditory stimuli. Increase in anesthesia concentration is linked to an increase in correlation between multiunit signals, which reaches a maximum with the onset of burst-suppression. The maximum indicates the switch in network properties, in which V1 and subiculum become unspecifically responsive to visual and auditory stimulation. These findings support the idea that general anesthesia level greatly influences the effects of sensory stimulation in primary sensory cortex by modulating functional network connectivity. The findings also provide evidence that the 'unimodal' character of mouse primary visual cortex is dependent on anesthesia level.

# Bifurcations of network states in the hippocampal area CA3 – a modelling study

Ekaterina A. Zhuchkova[1], Anastasia I. Lavrova[2,3], Susanne Schreiber[1,2], Lutz Schimansky-Geier[2,3]

[1]Humboldt University of Berlin, Institute for Theoretical Biology, Invalidenstraße 43, 10115 Berlin, Germany
[2]Bernstein Center for Computational Neuroscience, Philippstraße 13, 10115 Berlin, Germany
[3]Humboldt University of Berlin, Department of Physics, Neutonstraße 15, 12489 Berlin, Germany

Electrical network activity in the hippocampus is known to exhibit different rhythms, such as in the theta (4-12 Hz) and gamma (30-80 Hz) frequency bands. Interestingly, the same neuronal network, as the pyramidal cell-interneuron network in area CA3, can exhibit both network states and dynamically switch between them [1,2]. Here, we use mathematical modelling to analyze multiple rhythms and bifurcations in a minimal network of synaptically coupled pyramidal, basket, and O-LM-cells of the CA3 region. In contrast to the scheme previously proposed by Tort et al. [2], we employ simplified models of FitzHugh-Nagumo neurons with adjusted parameters. Excitatory and inhibitory coupling is, however, still represented by realistic synaptic currents. This approach enables us to do a thorough bifurcation analysis and to identify parameters of synaptic connections that can efficiently induce switches in network activity. Also we investigate how multistability determines certain regimes in this system.

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# Brain neurons for auditory processing and phonotaxis in the cricket

Kostas Kostarakos[1], Berthold Hedwig[1]

[1]University of Cambridge, Department of Zoology , Downing street, CB2 3EJ Cambridge, United Kingdom

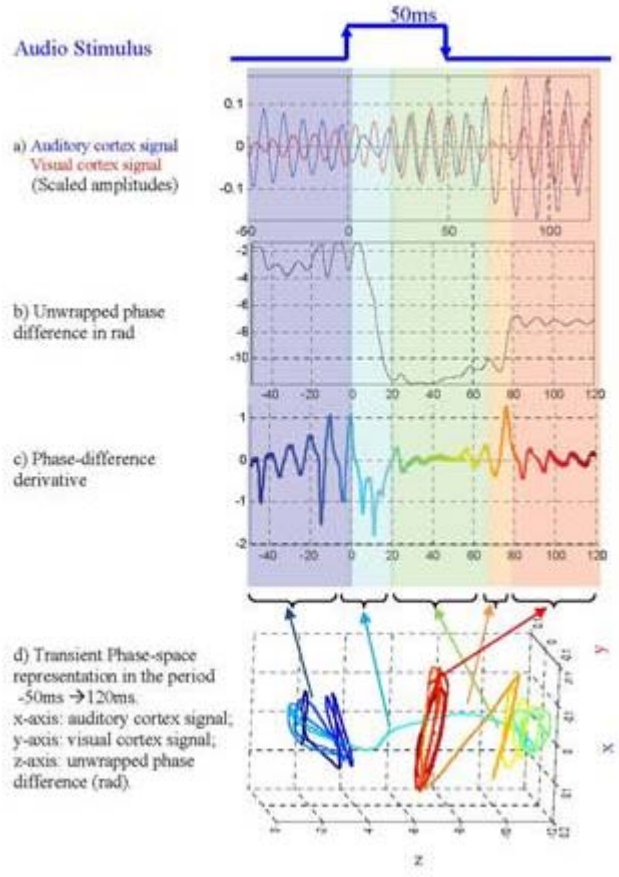
Although cricket phonotaxis has been a model system for auditory processing in insects for almost 50 years, we still have no proper understanding of the auditory circuits in the brain controlling this behaviour. Analysing the structure and activity of brain neurons in crickets which are actively performing phonotaxis appears to be a promising approach. We therefore record auditory brain neurons with glass microelectrodes in tethered females of G. bimaculatus that walk on a trackball and steer towards the male calling song. This allows a functional analysis of the system by (1) identifying the morphology of the neurons, (2) comparing their activity patterns with the auditory steering behaviour and (3) testing the functional significance of neurons for phonotaxis by intracellular current injection. Neurons are stained to reveal their dendritic arborisations and axonal projection areas.
We identified several types of local auditory brain neurons. All of these exhibit dense arborisations around the axonal projections of the ascending neuron AN1 and form a ring-like auditory neuropil in the brain. Some of the local neurons responded with a very short latency of 20 ms and copied the pulse pattern of the male calling song. Other neurons received excitatory and inhibitory inputs and their spike activity depended on the pulse repetition rate. These neurons may be involved in the process of pattern recognition. At least two local neurons projected contralaterally and linked both auditory neuropils in the brain. They may support pattern recognition and steering in split song paradigms. Delay lines in neural networks are thought to filter stimuli of specific temporal structure and may be involved in the recognition of temporal patterns. Interestingly, some of the local neurons failed to respond to the first pulse of the chirps which always caused the strongest response in thoracic interneurons. As these neurons started to spike with the second sound pulse, the long latency of their response may reflect a crucial mechanism of pattern recognition. The activity of these neurons strongly depended on the pause durations between the pulses of the test paradigm. A fundamental question in auditory processing is whether directional information is processed serial or parallel to the process of pattern recognition. At least two neurons responded with a very short latency of 20 ms and similar activity to AN1, and projected to a region where descending premotor interneurons originate. These interneurons forward auditory information with a shorter latency than the temporal sensitive neurons of the pattern recognizing network in the anterior part of the brain. This may indicate processing of directional information in parallel to pattern recognition networks. These fast responses of neurons which project to descending interneurons may explain rapid steering movements of only 50-60 ms towards individual pulses once the pattern of the male song has been recognized. Current experiments focus on identifying the structure and response patterns of the local interneurons to work out underlying mechanisms of temporal filtering that tune the system to the species specific calling song.

# Brain oscillatory dynamical activity of spatially extended cortical neural networks

Abdelhafid Zeghbib[1], Antje Fillbrandt[1], Frank W. Ohl[1,2]

[1]Leibniz Institute for Neurobiology, Magdeburg, Germany
[2]Institute of Biology, Otto-von-Guericke-University, Magdeburg, Germany

Neuronal oscillations of local field potentials (LFPs) recorded from auditory and visual cortex in awake Mongolian gerbils (Meriones unguiculatus) were observed, in our study, in different frequency bands. Phase space representation of these oscillations revealed the existence of many quasi attractors within the auditory-visual system. Periods showing these quasi attractors were interrupted by transient states. Quasi attractors and transient states (Fig. 1) of spatially extended neuronal ensembles, like in auditory -visual system, can serve as neural mechanisms for information transfer between auditory and visual cortex.
After the animals were adapted to stimulus pairs of tone-flash complexes with fixed stimulus onset asynchrony (SOA) of +200ms or -200ms, the neuronal activities of inter-cortical auditory-visual system showed a significant increase of coherence within several frequency bands in the range of 7-200 Hz. Oscillatory coherence in brain dynamics can be an expression of its self -sustained behavior and is also considered to reflect integration of multimodal sensory inputs.
The detailed evolution of attractor orbits can be a signature of brain activities linked to cognitive functions (e.g. learning, perception, and motor control). Hence, pattern extraction from these neuronal dynamical states can help also to formulate and test new audiovisual processing models.



# Cholinergic Modulation of $GABA_A$-receptor mediated inhibition in neocortex

Luise Liebig[1], Harald Hentschke[1]

[1]University of Tübingen, Section of Experimental Anaesthesiology, Schaffhausenstr. 113, 72072 Tübingen, Germany

Neocortical GABAergic interneuron classes differ in terms of sensitivity to the neuromodulator acetylcholine. We asked whether interneurons differing in cholinergic sensitivity are presynaptic to distinct subtypes of the $GABA_A$ receptor. To this end, we studied the efficacies of two $GABA_A$ receptor modulators in the presence and absence of cholinergic excitation. It was hypothesized that modulators which act on $GABA_A$ receptor subtypes targeted by acetylcholine-sensitive interneurons will be more effective in the presence of acetylcholine. By contrast, substances acting on $GABA_A$ receptor subunits which receive input from interneurons not sensitive to acetylcholine should not be affected by a change in the level of cholinergic excitation.

We investigated zolpidem, which acts preferably via $GABA_A$ receptors with the a1 subunit, and diazepam, which is not subunit-selective. Extracellular recordings were performed in organotypic cultures of mouse neocortex which show spontaneous phases of activity (UP states) separated by periods of neuronal quiescence. Zolpidem or diazepam was tested under two different baseline conditions: ACH+, acetylcholine (1 uM) and the acetylcholine esterase inhibitor neostigmine (1 uM) in the bath; ACH-, muscarinic and nicotinic receptor blockers atropine and mecamylamine, respectively, in the bath. Peri-event time histograms (PETH) of action potentials, triggered to the onset of UP states as measured in extracellular field potential recordings or current-clamp recordings, were constructed.

To assess the effect of the cholinergic tone on the efficacy of zolpidem and diazepam, an effect size measure (Hedges' g) was compared between the control condition (ACH+ or ACH-) and co-application of the $GABA_A$ receptor inhibitor. Hedges' g, here abbreviated as $g$, is the difference of the means of two populations divided by their pooled standard deviation; hence a value 0 indicates no difference between the two populations.

Zolpidem showed an inhibitory profile which was independent of the level of cholinergic activation. Under both baseline conditions, Zolpidem unfolded a maximal inhibitory effect 15 ms after the beginning of UP states ($g$ approaching 0.9). After ca. 50 ms post-event, $g$ decreased to 0.4 and remained at this level until the end of the UP states.

By contrast, the effect of diazepam reached its maximum 5 ms after UP state begin ($g$=1) when cholinergic receptors were activated. 75 ms post-event $g$ rapidly decreased to 0.3 and declined gradually until 600 ms into the burst, when diazepam lost its inhibitory effect entirely ($g$=0). With cholinergic receptors blocked, however, diazepam had no more depressant effect in an interval of 75 – 150 ms post-event ($g$=0).

Zolpidem acted independently of the cholinergic tone whereas the efficacy of diazepam could be enhanced by the presence of acetylcholine. We propose that this difference is due to a differential sensitivity of interneuron classes to the cholinergic tone. Zolpidem presumably acts via $GABA_A$ receptors which are postsynaptic to acetylcholine-insensitive GABAergic interneurons. Diazepam may enhance inhibition mainly or additionally via receptors postsynaptic to interneurons which are sensitive to acetylcholine.

# Circadian and Activity-Dependent Regulation of Myelin Genes in Wild Type and SHARP-1 and -2 Double Null-Mutant Mice

Lisa Reinecke[1], Sven P. Wichert[1], Klaus-Armin Nave[1], Moritz J. Rossner[1]

[1]Max-Planck-Institute of Experimental Medicine, Goettingen, Germany
[2]GGNB doctoral program

Living organisms evolved molecular and physiological mechanisms designed to anticipate and cope with the daily recurrent changes of life, which are integrated by a so-called circadian clock. This molecular system operates in any cell, deviates from the 24 hour solar cycle and needs to be constantly reset with the external time, a process called entrainment. The two basic Helix-Loop-Helix (bHLH) transcription factors, SHARP-1 (Dec2) and SHARP-2 (Dec1, Stra13) were shown to be important amplitude modifiers acting as repressors and co-activators of the molecular clockwork in the forebrain of mice. Both factors play redundant roles in the adaptation to altered light/dark cycles, in the control of sleep and wakefulness, and in learning and memory formation.
Preliminary microarray data identified several sleep-wake dysregulated genes in the cortex of Sharp-1 and -2 double mutant (S1/2 $^{-/-}$) mice. Surprisingly, among these were several genes encoding myelin proteins. To corroborate these findings with more precise sampling procedures, we combined laser captured microdissection (LCM) with quantitative RT-PCR to analyze the potential circadian pattern of myelin gene expression in distinct forebrain areas and as well as in central and peripheral nerves.
In the adult wild type hippocampus and cortex, myelin genes follow an immediate early gene (IEG) expression, thus paralleling states of activity. In the corpus callosum however, the regulation is different. In S1/2$^{-/-}$ mice we observe, dependent on the analyzed microarea, an inverse (hippocampus) or dampened circadian (cortex, corpus callosum) amplitude. In the optic nerve, myelin genes do not seem to be regulated whereas in the sciatic nerve we found differences dependent on the time of the day and among genotypes under dark:dark conditions. Synchronised oligodendrocyte-enriched cultures show a circadian-like mRNA expression of various myelin genes for at least two days. We found that myelin genes are inversely regulated in relation to the core clock gene Per2 in vitro.
In summary, our data indicate a novel high plasticity of myelin genes, which we speculate reflects a tissue-specific metabolic / homeostatic integration of neurons and oligodendroglia. In S1/2$^{-/-}$ mice these effects appear to be blunted, potentially due to the lack of the repressive or activating functions of the SHARP proteins.
Recently, several studies have shown that myelin gene expression is reduced in the cortex of patients suffering from psychiatric diseases such as schizophrenia, bipolar disorder and depression. Our results may be relevant for the novel discussed potential function of regulated myelination as a mechanism of plasticity in the adult cortex (Fields, 2005).

# Circadian clock molecules of the cockroach *Leucophaea maderae* and their expression pattern in the cockroach central nervous system.

Achim Werckenthin[1], Christian Derst[2], Monika Stengl[1]

[1]Universität Kassel, Abteilung Tierphysiologie, FB 10 Mathematik und Naturwissenschaften, Heinrich-Plett-Str. 40, 34132 Kassel, Germany
[2]Institut für Integrative Neuroanatomie, Centrum für Anatomie, Charité - Universitätsmedizin Berlin, Philippstr. 12, 10115 Berlin, Germany.

The molecular clockwork of insect circadian pacemaker neurons is best studied in the fruitfly *Drosophila melanogaster*. Several interlocked feedback loops of circadian clock genes were described which lead to about 24 h periods of mRNA concentration rhythms. These oscillations in clock gene transcription then control expression of downstream genes which finally orchestrate physiological and behavioral rhythms. Genome sequencing projects compared the molecular clockworks of several insect species such as the holometabolous bee *Apis mellifera*, the beetle *Tribolium castaneum*, and the hemimetabolous pea aphid *Acyrthosiphon pisum*. These comparative studies showed that the molecular clockwork of circadian genes is rather diverse in the different insect species. In some insects it is closer related to the mammalian clockwork than to the clockwork of *D. melanogaster*.
The cockroach *Leucophaea maderae* is an established model organism for behavioral and neurophysiological analysis of the circadian system. However, almost nothing is known about the molecular clockwork in this species. In addition to *period* (*per*) the circadian genes *timeless* (*tim*) and the *cryptochromes* (drosophila-like *cry1* and the mammalian-like *cry2* ) are of particular interest, because of differences between the various insect species. Using RT-PCR, degenerate primers and RACE, the full length coding sequence of *per* and *cry2* as well as a short sequence of *tim* could be acquired in *L. maderae*. Several attempts to clone *cry1* failed, despite sufficient sequence information for degenerate primers from other insect species. Thus, so far we have no evidence for the expression of this gene in *L. maderae*, in contrast to *A. pisum*, which expresses *cry1* as well as *cry2*.
Based on the cDNA sequences obtained from the cockroach antibodies were generated against LmPER, LmTIM and LmCRY2 to search for circadian rhythms in protein concentration as well as expression patterns in the central nervous system (CNS). Western blots of whole brains (optic lobes, proto-, deuto- and tritocerebrum) homogenates of animals kept in 12:12 hours light-dark cycle using the LmCRY2 antibody showed a higher protein level during the night compared to the day. However, we did not find any consistent cycling of LmTIM and LmPER with the new antibodies. This is in contrast to previous results with another LmPER antibody that suggested two peaks of PER expression in the whole brain: one at dawn and one at dusk. CNS whole-mount PERir stainings showed widespread PER-expression in the central nervous system. It stained mainly nuclei of neurons in the brain as well as in the subesophageal ganglion and the thoracic and abdominal ganglia. We are currently testing whether the lack of PER-cycling in whole brain extracts of the cockroach is caused by phase differences of circadian pacemaker cell groups or due to differentially available epitopes recognized by the different antibodies. [Supported by DFG grant STE531/15-2 and 18-1 to Monika Stengl]

# Connectivity of the Medullary Vocal Pattern Generator in Anuran Amphibians

Silke Maier[1], Anna C. Schneider[1], Wolfgang Walkowiak[1]

[1]Universität zu Köln, Zoologisches Institut; AG Walkowiak, Zülpicher Str. 47b, 50674 Köln, Germany

In anuran amphibians the pretrigeminal nucleus (preV), also known as dorsal tegmental area of the medulla (DTAM), is a crucial part of the vocal pattern generator for the production of advertisement calls[1]. Its relation to vocalization was first noted on the basis of lesion and stimulation experiments: Whereas lesions eliminate generation of all vocal behavior, electrical stimulation produces well-patterned calls. A second semi-independent generator is postulated in the region of the motor nuclei IX-X/XII. First anatomical studies in the aberrant species *Xenopus laevis*, using HRP-WGA and fluorescent amines, supported the hypothesis that the DTAM is involved in vocalization, because it projects into relevant motor areas[2-3]. Besides these projections it has been shown that the DTAM is also connected reciprocally to the ventral striatum, the dorsal infundibular nucleus (IN) and the rostral raphe pars dorsalis (rRpd). A direct projection from DTAM to the preoptic area (POA), which was orignally assumed[2], could not be verified. The POA, especially the anterior part (APO), plays an important role in both reproduction and call production. It is regarded as the vocal pacemaker nucleus[4-5]. Until today, it is not known how the input from the APON into the preV area is organized.

In our current studies we examine the connectivity of the preV in the more common amphibian *Bombina orientalis*. For this purpose, we used biotin and fluorescent tracings. To find the optimal application site for the tracers within the preV, we recorded field potentials elicited by stimulating the POA. The biotin tracings resulted in retrogradely labeled neurons in the striatum, IN, reticular formation and motor nuclei V, IX-X and XII. In contrast, no retrograde labeled cells were detected in the POA or in the thalamus. Anterogradely stained fibers reached POA-regions as well as the IN. The IN is of interest, because it gets input from the POA and is connected with the preV as well. Its ventral part (VIN) possesses a high number of androgen and estrogen concentrating cells, which also implies an involvement in calling behavior[6]. Our fluorescent tracing into the preV showed a rostral projection to the border of the posterior part of the POA. However, it is not clear, if these projections terminate into the POA. Further anterogradely labeled fibers were found in the ventral medulla oblongata, the tegmental nucleus (Teg), the midbrain torus semircularis (TS), the optic tectum, the thalamus, the hypothalamus (Hyp) and the IN. Among those the Teg, the TS, and the Hyp contained stained neurons as well. The investigation highlights a series of candidates for controlling the preV.

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# Consequences of variable circuit architecture on motor rhythm generation and muscles

Nelly Daur[1], Ayanna S. Bryan[1], Veronica J. Garcia[1], Katherine E. Deeg[1], Dirk Bucher[1]

[1]University of Florida, Whitney Laboratory for Marine Bioscience, 9505 Ocean Shore Blvd., 32080 St. Augustine, USA

The triphasic pyloric rhythm in the isolated stomatogastric nervous system of the lobster exhibits stable relative timing between neurons that fire in different phases, both throughout the lifetime of individuals and across individuals. The phase relationships are not dependent on cycle frequency and firing rate of individual neurons. In addition, they are constant despite the fact that one neuron type in the pyloric circuit, the PY neurons, exists in different numbers of copies (3-7) across individuals. Therefore, some of the functional synaptic connections are distributed over different numbers of individual cells. We investigated how these differences in circuit architecture might be compensated to yield similar phase relationships. The core pyloric circuit consists of a group of pacemaker neurons and two types of follower neurons. The single LP neuron and the PY neurons fire in rebound from inhibitory input from the pacemaker neurons. LP rebounds faster than PY and reciprocal inhibitory connections between them are thought to determine their relative timing. Therefore, the PY to LP connections should critically determine the phase of the LP burst end. We found that across individuals with 3, 4, or 5 PY neurons, activity patterns were not statistically different, including phase relationships, spike frequencies, and waveform parameters. We hypothesized that this is due to the presence of long-term compensatory mechanisms and that acute removal of PY neurons should therefore advance the LP neuron off phase. Photoablation experiments showed that advance in LP off phase was significantly correlated with the percentage of PY neurons ablated. However, across individuals, LP off phase advance was variable at any percentage of ablated PY neurons, with some individuals showing no change. This was true even when all PY neurons were ablated, and therefore not due to differences within the population of PY neurons. In addition, we found further evidence that the PY neurons are a fairly homogeneous population in this species. Current injections into any PY neuron during ongoing rhythmic activity consistently changed LP activity, and voltage- and current clamp recordings in the silenced circuit confirmed strong PY to LP connections in all cases. Different fibers of PY innervated muscles always received synaptic input from all neurons present. The inconsistent responses to acute removal of PY neurons raise the possibility that different individuals use different underlying mechanisms for achieving "correct" relative timing of LP, some more dependent on overall strength of PY to LP synaptic input, some more dependent on the intrinsic firing properties of LP.

# Coordinated neuronal activity between hippocampus and neocortex of learning rats

Nadine Becker[1], Matthew W. Jones[1]

[1]University of Bristol, School of Physiology and Pharmacology, University Walk, BS8 1TD Bristol, United Kingdom

Learning and execution of complex behaviours require interactions between networks of neurons that encode, store and share information across numerous brain regions. Systems level analyses of how the brain processes information are therefore central to understanding how behaviour is generated. Spatial learning in rodents constitutes a useful model in which to correlate neurophysiology with cognitive behaviours. We therefore studied neuronal activity during spatial learning tasks and subsequent sleep in rat hippocampal, posterior parietal cortical (PPC) and medial prefrontal cortical (mPFC) networks. While hippocampus and PPC are required for spatial navigation, hippocampus and mPFC are critically involved in working memory and are coherently active during decision making. Accordingly, we expected all three brain regions to communicate differentially during task episodes and learning stages that selectively recruit working memory, decision-making, acquisition and consolidation.

Six adult male rats were chronically implanted with independently movable tetrodes to simultaneously record single unit data and local field potentials (LFP) in the hippocampus, PPC and mPFC. Recordings were made over several weeks while rats were trained on an end-to-end T-maze task involving distinct working memory and decision-making (choice) episodes and control (guided) episodes. Significant LFP coherence in the theta frequency range (6-10 Hz) was evident across all three regions, but was differentially weighted depending on task episode and learning stage. mPFC-hippocampal theta coherence was consistently higher during choice episodes than during guided episodes of the task, independent of the rats' learning stage. In contrast, PPC-hippocampal theta coherence did not differ between guided and choice episodes, but was significantly increased when rats had learned the task as compared to when they performed at chance level. The degree of mPFC-PPC theta coherence depended conjunctively on task episode and learning stage, being higher during choice episodes and increasing with learning. Reversing the task rules abolished the task episode-dependence of theta coherence; furthermore, learning of the new rules was no longer associated with altered coherence patterns. These data may indicate that the rats adopted a different strategy following the rule reversal.

Alongside within-frequency LFP coherence, cross-frequency coupling mediates functional interactions between brain regions. We found that the amplitude of hippocampal gamma frequency (30 -100 Hz) oscillations was modulated by the phase of PPC and mPFC theta oscillations. This modulation was increased when rats had learned the task as compared to when they performed at chance level. Current analyses are relating these LFP effects to single unit firing rates and patterns across the three structures.

In summary, our data indicate that PPC, mPFC and hippocampal interactions are differentially involved in different task episodes and learning. Thus, hippocampal -prefrontal-parietal interactions can be used as a model system to study how the brain integrates and consolidates information during a spatial learning process.

# Cortical Spiral Dynamics in a Gerbil Model of Epilepsy

Kentaroh Takagaki[1], Xiaoying Huang[2], Jian-Young Wu[3], Frank W. Ohl[1, 4]

[1]Leibniz Institut für Neurobiologie Magdeburg, Forschergruppe Neuroprothesen, Magdeburg
[2]Duke University, Department of Neurobiology, Durham, USA
[3]Georgetown University, Department of Physiology, Washington DC, USA
[4]Otto-von-Guericke Universität, Magdeburg

Spiral dynamics can form stable, emergent patterns of activity in many biological systems. For example, these dynamics are thought to contribute critically to the maintenance of fibrillation in the myocardium. Using voltage-sensitive dye imaging in the cortex, we can obtain a spatiotemporal record of such single-trial activity patterns with high sensitivity and sub-millisecond sampling rate[1,2]. We have recently used these methods to demonstrate spiral dynamics in (A) a cortical slice model of epileptiform activity[3], (B) in vivo during pharmacologically-induced epileptiform states[4], and (C) in vivo during sleep-like states[4]. The duration of spiral dynamics is often brief, especially in non-pathological states, but they do coincide with modulations in the frequency and spatial coherence of ongoing activity patterns[4]. This suggests that spiral dynamics may serve to stabilize, organize and modulate population activity on the mesoscopic scale.

Here, we demonstrate the presence of very long, very stable epochs of spiral activity in a naturalistic Mongolian gerbil model of epilepsy (see Figure; single detector trace and frame images from a selected segment). Our findings lead us to speculate that spiral dynamics may be stabilizing the pathological firing patterns in some forms of epilepsy.

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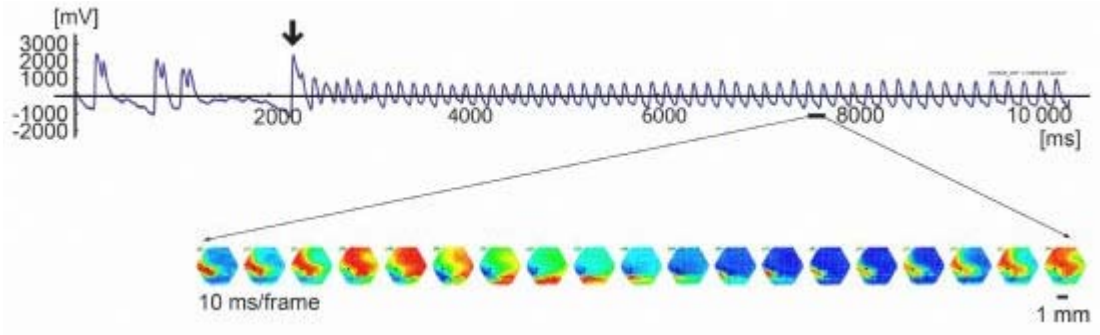

# Development of oscillatory patterns and synchronization within the prefrontal-entorhinal-hippocampal network of the neonatal and juvenile rat

Beatrice Pöschel[1], Ileana L. Hanganu-Opatz[1]

[1]Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, Developmental Neurophysiology, Falkenried 94, 20251 Hamburg, Germany

The adult prefrontal cortex (PFC) and hippocampus (HPC) are critically involved in executive and mnemonic processing. It has been suggested that within the framework of anatomical connections, flexible binding of neuronal populations in oscillatory rhythms represents a mechanism to support these cognitive abilities. However, the contribution of early patterns of oscillatory activity within the prefrontal -hippocampal network to the maturation of such abilities is still unclear. We previously showed that both the PFC and HPC express patterns of oscillatory activity with distinct spatial and temporal organization during the first two postnatal weeks. Moreover, the hippocampal theta activity seems to drive the generation of neonatal prefrontal oscillations. It remains to be elucidated whether the hippocampal drive acts exclusively via direct synaptic projections to the PFC or whether additional contribution of the entorhinal cortex (EC) as an important nodal point of cortico-hippocampal circuits is required.

To decide on the role of the EC within the prefrontal -hippocampal network, we performed extracellular multielectrode recordings in the PFC, hippocampus and EC of postnatal day (P) 0 to 15 rats in vivo. Three types of activity are expressed by the maturating EC: (1) delta oscillations (~ 1.5 Hz), (2) theta bursts (~ 6 Hz) and (3) gamma oscillations (~ 35 Hz) superimposed on the theta oscillation. The transition from discontinuous to continuous oscillatory activity mirrors the maturation of connectivity and takes place, as in hippocampus, around P9. To assess the impact of entorhinal activity on the oscillatory patterns of PFC and hippocampus, cross-correlation and coherence analysis as well as Granger causality analysis were performed. They revealed a strong coupling PFC and HPC as well as between HPC and EC, but comparably weaker interaction between PFC and EC. These findings were backed up by stimulation of one brain region and recordings in the other two regions of the prefrontal-entorhinal-hippocampal network.

Thus, the early prefrontal activity seems to be controlled by both hippocampal and entorhinal rhythms whereby the hippocampal drive exerts a stronger influence on prefrontal oscillatory patterns.

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# Different cell properties of Retzius cells in ganglia controlling male and female reproductive organs of the medical leech

Till Sacher[1], Jutta Kretzberg[1]

[1]University Oldenburg, Computational neuroscience, Carl-von-Ossietzky-Straße 9-11, 26121 Oldenburg, Germany

The medicinal leech Hirudo medicinalis has a relatively simple nervous system consisting of 21 mid-body-ganglia. 19 of these share the same set of ~400 individually characterized neurons with very similar cell properties. The remaining ganglia 5 and 6, which locally control the male and female reproductive organs of the hermaphrodite organism contain some additional neurons. In this study we show that the morphology and physiology of the two Retzius neurons differ considerably between ganglia 5 and other mid-body ganglia, suggesting a role in reproduction.

The paired Retzius (RZ) cells are known to be involved in swimming and production of mucus. They are very well characterized in mid-body-ganglia [Lent 1973], but much less is known about RZ cells in ganglia 5 and 6. We show that both RZ cells in these ganglia have a morphological different structure and also respond differently to intracellular electrical stimulation.

To characterize the physiological differences, spike rates, response latencies, spike amplitudes and hyperpolarization of the RZ cell responses of ganglion 5 (controlling the male reproductive organs) and ganglion 10 (a typical mid-body ganglion) were compared.
While we did not find significant differences in response latencies and hyperpolarization responses, spike rates and amplitudes differ considerably between the RZ cells in both ganglia. RZ cells in ganglion 5 show higher spike rates than in ganglion 10 (e.g. 1.4 spikes compared to 0.4 spikes in response to 250ms injection of 1nA) and produce spikes with smaller amplitudes (26.2mV in ganglion 5 compared to 36.7mV in ganglion 10).

The electrical coupling of both Retzius cells in ganglion 5 and 10 were analyzed by electrophysiological double recordings. In ganglion 10, both RZ cells are coupled symmetrically, with each presynaptic spike eliciting a postsynaptic spike. In ganglion 5, postsynaptic responses are much weaker and seem to be asymmetric (e.g. stimulating the right cell with 1nA yields 13.5 spikes presynaptic and 5.0 spikes postsynaptic, stimulating the left cell with 1nA yields 11.8 spikes presynaptic and 1.8 spikes postsynaptic).

This difference in coupling can also be observed in staining experiments (Neurobiotin conjugated with Streptavidin Cy3). When injecting one of the RZ cells in ganglion 10, a strong staining can also be observed in the second RZ cell, while the same injection does not lead to an equally strong postsynaptic staining in ganglion 5.

To test if these results are specific for Retzius cells, we also recorded and stained mechanosenory touch cells, which respond to light touch of the skin in each body segment. As expected, their cell properties do not differ between the different ganglia.
Therefore, the morphological and electrophysiological differences between Retzius cells in reproductive ganglion 5 compared to other midbody ganglia are specific for this cell type. This finding supports the hypothesis that Rezius cells are involved in the control of reproductive organs of the leech. Since the properties of Retzius cells in ganglion 6 seem to be more similar to mid-body ganglia than to ganglion 5, Retzius cells are probably involved in the control of the male rather than the female reproductive organs.

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# Disentangling the retinal cable mess – FIB-SEM based 3D-reconstruction of the anchovy inner retina in high resolution

Martin Heß[1], Petra C Koch[1], Gerhard Wanner[1]

[1]BioZentrum LMU München, Großhaderner Straße 2-4, 82152 Planegg-Martinsried, Germany

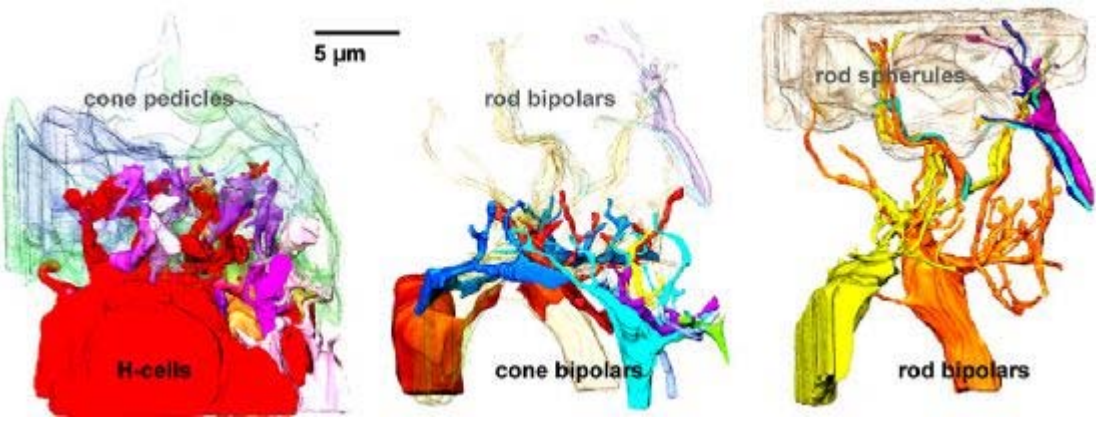
The anchovy retina is distinguished by an exceptional photoreceptor fine structure suggesting polarization contrast vision. Concomitantly two cone types (long cones, LC and short cones, SC) with orthogonally oriented e-vector analyzers are arranged in a tessellate pattern sustained down to the level of their synaptic pedicles. This implies that the impressive geometric regularity is continued through the synaptic layers of the retina, last but not least representing the structural basis of bioelectric image processing handling polarization information instead of spectral information.

A morphological investigation of a suchlike neuronal network has to cope with reams of very fine, three-dimensionally interwoven dendrites (diameters only few nm) on the one hand, and with cell dimensions of several dozen micrometers on the other: horizontal extension of dendritic fields, radial extension of bipolar cells between outer and inner plexiform layer (OPL + IPL). Therefore an electron-microscopic approach has to be followed that provides both, high resolution and contrast in 2D, and high z-resolution and perfect alignment of subsequent image planes for 3D-reconstruction. The latter can be hardly achieved with ultrathin-section series using TEM due to inevitable image distortion and comparatively "rough" slicing.

In order to visualize every single cell of a small piece of the inner retina and to clarify the wiring rules (cell-type-specific connectivity, 3D wiring geometry) in the plexiform layers of the European anchovy Engraulis encrasicolus we used a FIB-SEM crossbeam workstation (Zeiss Auriga®) as imaging device on Os/U-stained, resin embedded and carefully oriented retinal fragments in combination with the 3D-rendering software Amira®. To balance resolution and image acquisition speed we used voxel sizes between (9x9x10)nm3 and (14x14x50)nm3 depending on the retinal layer that was imaged (field-of-view 28µm x 21µm, total milling distance 150µm). Altogether the raw data comprise about 5000 image planes and a total data volume of about 30 GB. The "final-product" of manual segmentation followed by surface rendering is a digital 3D-model of the investigated retinal fragment that can be virtually explored by free choice of perspective and zoom, as well as composition, colour and transparency of single objects (cells and cell constituents).

The first results of the ongoing evaluation show 3 types of horizontal cells arranged in distinct layers disclosing constant wiring rules (H1 contact SC and LC, H2 only SC and H3 only LC, a rod-specific H-cell was not found yet), and at least 3 types of bipolar cells concerning their connectivity in the OPL (mixed rod-cone bipolars, specific LC- and SC-bipolars). The contact pattern of H-cell dendritic endings embracing all synaptic ribbons in their synaptic fields laterally can be determined. The same is true for cone-to-bipolar synapses that are found at central positions of the synaptic triades or at the pedicle bases. The dendritic fields of different bipolar types interdigitate, those of the same type do not.

Generally the findings indicate separation of (LC- and SC-dependent) information channels and parallel processing in the inner anchovy retina. The convergence sites/rules essential for contrast generation or encoding e-vector orientation are expected in the IPL – its reconstruction will give further insights while approaching an understanding of the mechanisms of polarization vision in vertebrates.


5 µm
cone pedicles
H-cells
rod bipolars
cone bipolars
rod spherules
rod bipolars

# Effects of anodal slow oscillation transcranial direct current stimulation (tDCS) in the rat

Sonja Binder[1], Paul Christian Baier[2], Matthias Mölle[1], Jan Born[1], Lisa Marshall[1]

[1]Universität zu Lübeck, Institut für Neuroendokrinologie, Ratzeburger Allee 160, 23538 Lübeck, Germany[2]Christian-Albrechts-Universität Kiel, Abteilung für Psychiatrie und Psychotherapie, Niemannsweg 147, 24105 Kiel, Germany

Background:
In humans, transcranial electric stimulation alternating at the frequency of the sleep slow oscillation (SO) improves declarative memory, leads to an increase in endogenous slow oscillatory activity and is directly followed by pronounced reverberations; i.e. EEG activity after stimulation is briefly entrained to the stimulation frequency (Marshall et al., 2006). In the rat, little is known about the after-effects of slow-oscillation stimulation. Therefore, this study aimed to develop a protocol which is comparable to studies in human subjects regarding stimulation parameters and procedure.

Methods:
Rats were implanted bilaterally with stainless steel screw electrodes for EEG recordings (two frontal, two occipital) and two EMG electrodes in the neck muscles. Two additional screw electrodes (diameter 1.6 mm) served as stimulation electrodes and were positioned laterally on the cranium at the height of bregma. Anodal stimulation oscillating at a frequency of 1.5 Hz (i.e., the dominant frequency of the sleep SO in the rat), began 1 min after the animals showed the first signs of continuous NonRem -sleep (NR) after lights on. Animals received 5-6 consecutive stimulations, each lasting 30 s, separated by a stimulation-free interval of 60 s. Current strengths ranged between 15 and 55 μA. Each animal underwent at least one stimulation session, preceded or followed by a stimulation-free 24h-period which served as the control condition in a balanced order. FFT-analyses were performed on EEG epochs, (i) during stimulation, (ii) on NR epochs between the subsequent stimulation epochs, and (iii) on matched EEG epochs during the stimulation-free recordings of the same animal. Furthermore, relevant sleep parameters (e.g. total sleep time and REM-latency) and sleep spindle count were semiautomatically calculated, and compared between stimulation and control recordings. EEG signals were also inspected visually for any signs of possible post-stimulation entrainment.

Results & Discussion:
Contrary to our hypothesis, preliminary results on EEG activity were not equivalent to those reported in humans. There was neither an enhancement in SO EEG power following stimulation, nor a substantial entrainment of EEG-rhythms after cessation of stimulation. Interestingly, we found an increase in REM latency on the days of SO-stimulation, as compared with control days (p ). Furthermore, on days of stimulation spindle count in the NR-intervals between stimulation periods was decreased. The discrepancy to the findings in human studies may be related to differences in sleep architecture between the species, as rats have very short sleep cycles, as well as to species specific neuroanatomical differences. Future studies should take this into account.

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# Enhancing Knee-Ankle-Foot-Orthoses with modular, adaptive neuro-control

Jan-Matthias Braun[1], Vishal Patel[2,3], Poramate Manoonpong[1,2,4], Florentin Wörgötter[1,2,4], Bernhard Graimann[3]

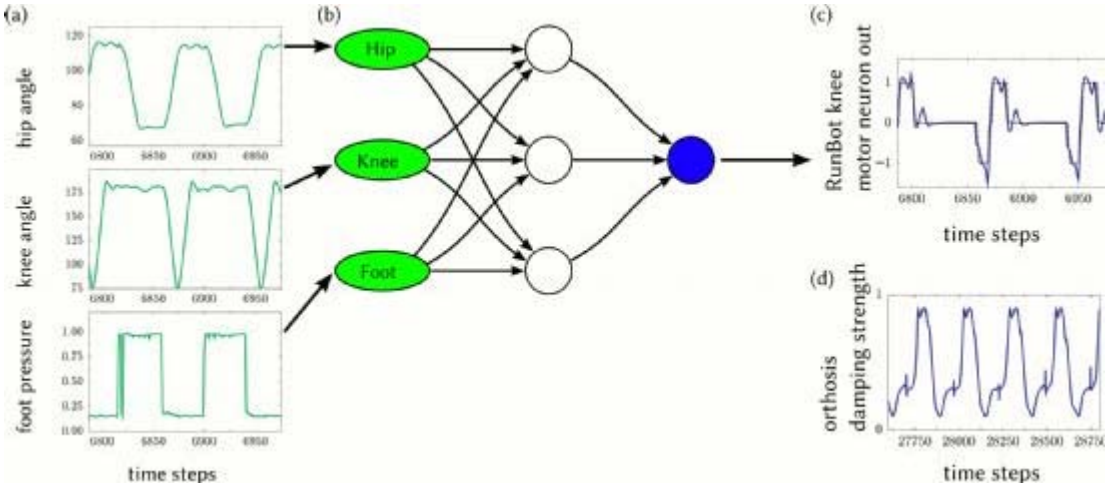
[1]Georg-August-Universität Göttingen, 3. Physikalisches Institut, Göttingen, Germany
[2]Bernstein Focus Neurotechnology Göttingen, Germany
[3]Otto Bock HealthCare GmbH, Duderstadt, Germany
[4]Bernstein Center for Computational Neuroscience, Göttingen, Germany

A Knee-Ankle-Foot-Orthosis (KAFO) is a modular lower-extremity orthosis prescribed to people with gait disability which might be e.g. caused by diseases or injury to brain or spinal-cord. The KAFO should support, correct and assist the movement of the corresponding affected joints. Traditional KAFOs are restricted by a gait depending switch of the joints based on (electro-) mechanic non-adaptive switches. So common disturbances (floor unevenness, obstacles, ramps) can not be mastered in a satisfactory way. Novel approaches include active elements into the orthosis, which do not directly act on the movement, but, instead, adjust the compliance generating the very difficult problem of efficient control of such actuators.

Thus new technologies have to be developed to improve control, to overcome the shortcomings of traditional non-adaptive approaches, solving the problem of efficient actuator control. As development of advanced orthotic devices is additionally held back by the vast number of possible indications as well as by the wide range of neuromuscular variability within a specific patient group (Yakimovich et al., 2009), the development of advanced devices is imposing the need for individual (online) adaptation of gait parameters to allow adaptation (1) to changing environments like slopes, stairs etc. and (2) to the individual patients physiological conditions using reflexive neuro-control as inspired by RunBot (Manoonpong et al., 2007) and central pattern generators to control gait parameters like frequency and stride length.

In this poster we present a novel type of KAFO based on a controllable hydraulic damper, derived from OttoBock's C-Leg©. The neuro-control was first developed on RunBot and then transferred to the orthosis (s. Fig. 1), with the addition of an adaptation to the patient by means of direct feedback which allows the patient to actively interact with the controller. Moreover we will show the integration of ground detection mechanisms (flat terrain, slopes and stairs) and suitable adaptation of gait parameters.
We acknowledge support by BFNT Göttingen.

Figure 1: (a) Showing sensory inputs of RunBot, which are transformed via a reflexive neuro-control (b) to the desired motor neuron output (c). (d) The result of transferring this setup onto the orthosis.


(a)
hip angle
knee angle
foot pressure
time steps
(b)
Hip
Knee
Foot
(c)
RunBot knee
motor neuron out
time steps
(d)
orthosis
damping strength
time steps

# Flight and walking in locusts - cholinergic co-activation, temporal coupling and its modulation by biogenic amines

Jan Rillich[1], Paul Anthony Stevenson[2], Sergej Hartfill[1], Hans-Joachim Pflüger[1]

[1]Freie Universität Berlin, Institut für Neurobiologie, Königin-Luise-Str. 28-30, D-14195 Berlin, Germany[2]Universität Leipzig, Institut für Biologie, Talstr. 33, D-04103 Leipzig, Germany

Walking and flying in locusts are rhythmical behaviors generated by central pattern generators (CPG) that are tuned in intact animals by phasic sensory inputs. Although these two motor patterns are mutually exclusive in freely behaving locusts, leg movements during flight may be coupled to the wing-beat cycle. To analyze the extent to which these two CPGs are interconnected we bath applied cholinergic and aminergic drugs to evoke walking and flight while recording wing- and leg - muscle motor units from the cut nerves of fully isolated thoracic ganglia. We show that the muscarinic, cholinergic agonist pilocarpine induces fictive walking at comparatively lower concentrations (10 -4 M), whereas higher concentrations are required to elicit fictive flight (10 -3 M). Interestingly, flight did not suppress walking motor activity so that the two patterns were produced simultaneously (18 out 21 preparations). Frequently, at least one or two leg motoneurons (e.g. the fast trochanteral depressor), but rarely more, were temporally coupled to the flight rhythm, so that each spike in a step cycle volley occurred synchronously with wing depressor units firing at flight rhythm frequency. Interestingly, this coupling was abolished after adding the octopamine antagonist epinastine (in 5 of 6 preparations). We therefore analyzed the influence of biogenic amines on the interactions between both networks. Tyramine, the precursor of octopamine, also readily induced both fictive walking (at 10- 3 M) and flight (at 10-2 to 10-1 M), whereby temporal coupling between the units was seldom. Contrasting this, octopamine readily evoked flight (10 -2 – 10- 1 M), but usually failed to elicit walking (80%). Despite this, when flight occurred numerous leg motor units exhibiting the walking motor pattern were temporarily coupled to the flight rhythm throughout the whole fictive flight sequence. Flight motor activity elicited by the natural releasing stimulus (wind) to head hairs served as a reference for motor activity initiated by pharmacological agents. In 9 out of a total of 14 preparations we observed temporal coupling of walking motor units to the wing beat cycle of varying degrees. In preparations without or only slight coupling to the flight rhythm, octopamine application (5x10-2 M) increased the coupling of leg motoneurons to the wing beat cycle. Our results indicate that the degree of coupling between the CPGs for walking and flight is subject to aminergic modulation. We speculate that octopamine biases the whole motor machinery of a locust to flight activity. We are currently investigating the role of the suboesophageal ganglion in the initiation and maintenance of walking. Preliminary data shows that pilocarpine initiates walking, and furthermore that the step-frequency of walking bouts increases with increasing pilocarpine concentration. (supported by the DFG)

# Hippocampal network patterns in Kv7/M-channel-deficient mice

Jasper Grendel[1], Quyen Le[1], György Buszáki[2], Dirk Isbrandt[1]

[1]Zentrum für Molekulare Neurobiologie Hamburg, Experimental Neuropediatrics (ENP), Falkenried 94, 20251 Hamburg, Germany [2]Center for Molecular and Behavioral Neuroscience, Rutgers University, Aidekman Building #301, 197 University Avenue, Newark, NJ 07102, USA

We addressed the physiological roles of M channels by suppressing KCNQ/M-channel activity in the brains of transgenic mice expressing a KCNQ2 subunit with a dominant-negative pore mutation that suppressed M-channel activity by co-assembling with other KCNQ subunits. To achieve temporal and spatial control over transgene expression in the nervous system, we used the Tet-Off system. This strategy allowed us to generate viable transgenic mice deficient in functional KCNQ/M-channels. The restriction of transgene expression to defined developmental periods revealed that M-channel activity plays a critical role in the development of normal hippocampal morphology during the first postnatal weeks. Suppression of the M-current after this critical period resulted in normal brain morphology but increased neuronal excitability and deficits in hippocampus-dependent spatial memory.

In order to investigate the effect of M-channel suppression on hippocampal network patterns and unit activities in the intact brain, we recorded multiple single neurons with silicon probes obtained from recordings in freely moving control and mutant mice. In addition, chronically implanted 16-site linear silicone probes were implanted in the CA1-dentate gyrus/CA3 axis to record the depth profiles of network activity patterns in the hippocampus. Stable recordings were obtained from each animal for a period of up to two months during slow wave sleep, rapid eye movement (REM) sleep, exploration in the home cage, and during voluntary wheel or linear track running. The experiments revealed hippocampal network patterns that were comparable to those previously reported (Buzsaki et al. 2003), including the presence of theta oscillations in CA1 during wheel and linear track running and REM sleep, with maximal amplitudes in the CA1 stratum lacunosum-moleculare and sharp-wave ripple fast oscillations during SWS.

Preliminary results demonstrate that mutants expressing the dominant-negative transgene show decreased theta and gamma power in both voluntary wheel running and REM sleep sessions. Unit phase locking in REM sleep to theta and gamma oscillations was decreased in these mutants. No difference was seen in the length, amplitude, or occurrence of high-frequency oscillations in CA1 (ripples) during slow wave sleep.

# Identification of lateral habenular neurons relaying hypothalamic input to monoaminergic hindbrain circuits

Wolfram C. Poller[1], René Bernard[1], Vince I. Madai[1], Thomas Kahl[2], Gregor Laube[1], Rüdiger W. Veh[1]

[1]Charité, Institut für Integrative Neuroanatomie, Philippstraße 12, 10115 Berlin, Germany[2]Charité, Institut für Vegetative Anatomie, Philippstraße 12, 10115 Berlin, Germany

The lateral habenular complex (LHb) is an evolutionary conserved epithalamic structure, involved in the regulation of ascending monoamine systems. The LHb is implicated in various biological functions, such as reward, arousal, feeding, pain processing, and reproductive behavior. Several studies indicate an important role of the LHb in the etiology of psychiatric diseases. The regulatory effects of the LHb on monoamine release are mediated by spontaneously active LHb neurons that inhibit dopaminergic neurons in the VTA. It is unknown how the activity of these LHb neurons is regulated and where modulating input to the LHb arises. Detailed tracing studies found a strong projection from the lateral hypothalamus (LH) to the LHb. As the vegetative center of the brain the LH is in a good position to convey important homeostatic information to the LHb. To get an idea of the potential effects of this LH projection, we set out to identify the neurons targeted within the LHb and the neurotransmitter used. Here, we hypothesized the LH projection to directly target VTA-projecting neurons in the LHb and to use glutamate as their neurotransmitter. To test our hypotheses we simultaneously injected the anterograde tracer Phaseolus vulgaris Leucoagglutinin (PhaL) into the LH and the retrograde tracer FluoroGold (FG) into the VTA of adult, male rats. Immunocytochemical visualization of both tracers in the LHb and subsequent scans in the confocal laser scanning microscope (CLSM) revealed the existence of various potential contacts of PhaL-positive terminals on FG-positive cells. The existence of synapses at these contact sites was confirmed by co -visualization of the protein synaptophysin and overlaying with the tracer signals. Combining the visualization of PhaL and vGluts we found many LH-terminals in the LHb to be vGlut2 positive. The existence of vGlut2-positive vesicles in traced LH terminals was confirmed in the electron microscope.

# Individual Potassium Channel Proteins Display Characteristic Patterns in Rat Cerebellum and Olfactory Bulb

Angelika Görtzen[1], Daniela Hüls[1], Eva Lichtendahl[1], Heike Heilmann[2], Rüdiger W. Veh[2]

[1]Klinik für Neurologie,St. Josef-Hospital, Mülheimer Straße 83, 46045 Oberhausen, Germany[2]Institut für Integrative Neuroanatomie, Charité, Philippstraße 12, 10115 Berlin, Germany

The biological functions of excitable cells are predominantly controlled by their decoration with potassium channels. In line with the diverse and complex tasks of such cells there is a bewildering diversity of potassium channel activities. This is due to about 70 different genes, using splice variants to produce even more proteins, which may form heterooligomeric complexes as biologically active channels. The biological properties are further modified by the interaction with smaller auxiliary subunits. Despite much data on the physiological properties of molecular characterized channel complexes often are poorly understood, especially in a natural environment.
The organization of channels in native organs may be helpful to better understand their function. This is especially true, when tissues show a systematical function-related organization, as is the case in the mammalian cerebellum and olfactory bulb. This principle has already been used to understand the different subcellular sorting of Kv1.1 and Kv1.2 potassium channels in the mitral cells of the olfactory bulb (Veh et al, 1995, Eur J Neurosci 7:2189-2205).
In the present work we used a battery of more than 90 different, newly prepared or commercial antibodies to study the regional, cellular, and subcellular distributions of potassium channel proteins in the cerebellum and the olfactory bulb of the rat. A selected panel of other antibodies showed the basic patterns of tissue organization such as the distribution of neuronal cell bodies (calbindin; calreticulin; parvalbumin), of dendrites (microtubule-associated protein 2, MAP2; regulator of G protein signa-ling 4, RGS4), of axons (unphosphorylated and phosphorylated neurofilament; myelin basic protein), of astrocytes (S100beta protein; glial fibrillary acidic protein, GFAP) and other glial cells (microglial calcium binding protein, Iba-I; chondrotin sulfate proteoglycan, NG2).
Many potassium channels displayed similar, but not identical distribution patterns as compared to the marker proteins. In contrast, a few detected highly specific and unexpected profiles, which may represent localizations on rare and potentially unknown subpopulations of neurons or glial cells. From our data one may expect that they provide more information of the detailed organization of the cerebellum and the olfactory bulb as well as to increase our understanding of the detailed biological functions of potassium channels in native tissues.

# Inhibitory and Excitatory Synaptic Conductances during Sharp-Wave Ripples in vitro

Jose R. Donoso[1], Nikolaus Maier[3], Dietmar Schmitz[1,3], Richard Kempter[1,2]

[1]Bernstein Center for Computational Neuroscience Berlin, Germany
[2] Institute for Theoretical Biology, Humboldt Universität zu Berlin, Germany
[3] Neuroscience Research Center, Charité-Universitätsmedizin Berlin, Germany

During slow-wave sleep or immobile resting periods, the rodent hippocampus displays a peculiar pattern of fast (approximately 200 Hz) short-lived network oscillations superimposed on slower sharp waves. Such sharp wave-ripple complexes (SPW-R) have been implicated in memory consolidation. Information about inhibitory and excitatory currents received by a CA1 cell during a SPW-R event would allow us to get insights about the mechanisms involved in the generation of this oscillatory pattern.

To quantify the time course of excitation and inhibition, patch clamp methods can be used to measure the synaptic input that a neuron experiences within the network during the field event of interest. In the analysis method proposed by Borg-Graham et al.(1996), current traces obtained at two different holding potentials are mathematically combined in order to disentangle the excitatory and inhibitory components.

By means of a single compartment cell model we illustrate the difficulties in ensuring the fundamental requirement for a consistent estimation. Namely, that the current traces combined correspond to the same trajectory in the conductance space. Here we show that the variability and voltage dependence of the current traces pose serious difficulties for the alignment process required by the single-cell-voltage-clamp method.

We present an alternative method of conductance estimation based on paired cell recordings that relies on the correlation between excitatory currents in both cells. Our analysis of in vitro data provides evidence that excitation and inhibition are both oscillatory during SPW-R, suggesting the presence of a phasic component in both of them.

Selected reference:

Borg-Graham LJ, Monier C., Fregnac Y. Voltage-clamp measurement of visually evoked conductances with whole-cell patch recordings in primary visual cortex. J Physiol (Paris) 1996; 90:185-8

# Integration of coordinating information into an oscillator

Carmen Ramona Wellmann[1], Brian Mulloney[2]

[1]Universtität zu Köln, Institut für Zoologie / Abt.Tierphysiologie, 50674 Koeln, Germany
[2]Dept. Neurobiology, Physiology and Behavior, Univ. of California Davis, Davis, CA, USA

Coordination of oscillating central pattern generators (CPG) is an important feature accomplished by the central nervous system. A lot of work was done to understand the organization of CPGs on the cellular level, but little to understand how CPGs interact to synchronize their activity with a stable phase lag. The crayfish swimmeret system is an excellent model to understanding coordination. The system's modular CPGs are anatomically separated and distributed in different segments, but they still show synchronization with a precise phase lag independent of the frequency of the rhythm.
The activity of each module is generated through reciprocal inhibition of two sets of nonspiking interneurons, Int1A and Int2A. Their alternating oscillations control the firing of the powerstroke (PS) and return stroke (RS) motor neurons, which can be recorded extracellularly to monitor the status of each module. Each module holds another nonspiking interneuron, ComInt1, that integrates the coordinating information from neighboring modules. ComInt 1 is responsible for their synchronization (Smarandache et al, 2009). Each module has two types of coordinating neurons, ASC and DSC. During one cycle ASC sends information about the timing, duration and strength of PS firing in its own module to other more anterior modules. DSC sends information about RS firing to more posterior modules. In summary, within each module: while the membrane potential of Int2A is depolarized, PS motor neurons and ASC are active, but while Int1A is depolarized, RS motor neurons and DSC are active.
In this work we want to show three important results obtained with paired intracellular recordings from neurons in the same module. First we demonstrate that Int1A directly inhibits ASC. We now understand how coordinating information is shaped and transmitted to the neighboring modules. Second, ComInt1 only affects one of the two interneurons in the modules kernel; it makes a direct excitatory synapse onto Int2A, but does not connect with Int1A neurons. This result is consistent with our previous knowledge of synaptic connections that Int1A and Int2A neurons make with swimmeret motor neurons. This pathway explains the effects of perturbations of ComInt1 neurons on the module's motor output. Third, ComInt1 neurons receive coordinating information as periodic bursts of large, brief excitatory post-synaptic potentials (EPSPs) elicited by bursts of spikes in coordinating axons from other modules. These EPSPs are integrated and conducted passively to ComInt 1's output synapse onto Int2A. To measure the attenuation and filtering of these EPSPs in ComInt1, we recorded simultaneously from two sites in ComInt1: near where these EPSPs arise and near its output synapses onto the pattern-generating circuit. The anatomical distance between these sites approaches 1 mm. The individual EPSPs within the burst remained discrete despite this filtering. This preservation of discrete EPSPs in the presynaptic voltage-transients that regulate transmission from ComInt1's graded synapses means that the details of activity in other modules that are transmitted from coordinating axons to ComInt1 might be relayed faithfully to the kernel of the CPG.
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# Investigation of neural circuits related to acoustic startle response and prepulse inhibition using behavioural positron-emission-tomography

Cathrin Rohleder[1], F Markus Leweke[1], Rudolf Graf[2], Heike Endepols[2]

[1]Central Institute of Mental Health, J5, 68159 Mannheim, Germany
[2]Max Planck Institute for Neurological Research, Gleueler Str. 50, 50931 Köln, Germany

Prepulse inhibition (PPI) is a measure of sensorimotor gating and refers to a reduction of startle reflex when a non-startling prestimulus (prepulse) is presented up to 500 ms before the startling stimulus. PPI is regarded as an endophenotype for neuropsychiatric disorders like schizophrenia and is therefore used to investigate antipsychotic potential of new drugs or explore new animal models. For interpreting the results of such studies appropriately, it is important to understand the underlying neural mechanisms of PPI.
So far, invasive methods like brain lesions, intracerebral infusions of transmitter - or receptor-specific pharmacologic agents and systematic administration of neurotoxins have been used to identify brain areas related to startle and PPI. In the present study metabolic brain activity during acoustic startle response (ASR) and PPI of ASR in healthy and untreated rats using 2 [18F]fluoro-2-deoxyglucose positron-emission-tomography (FDG-PET) was investigated.
After intraperitoneal injection of FDG, rats (n = 5) were placed in the startle box (San Diego Instruments) and were either exposed to continuous background noise (65 dB SPL LIN, control condition) only, or a PPI-paradigm was applied (test condition) for 45 min. One hour after FDG-injection the PET-Scan was conducted for 30 min (Focus 220, Siemens). To analyse alterations in glucose metabolism, percent change in the test condition was calculated relative to the control condition. Furthermore, PPI was calculated as percent decrease of startle amplitude following a startle stimulus preceded by a prepulse.
Statistical analysis (1way RM ANOVA) revealed that the higher the sound pressure level of the prepulse the higher the PPI ($p < 0.001$), indicating that PPI depends on prepulse salience against background noise.
FDG-PET based on the behavioural paradigm revealed metabolic changes, which reflect activation of the primary startle circuit (increased activity in parts of the caudal pontine reticular nucleus of up to 12.6 %) and mediation of PPI (increased activity of the right penduculopontine tegemental nucleus and substantia nigra pars reticulata of up to 20 %). Metabolic activity changes in other brain regions are presumably caused by modulation of startle processing: The mPFC, especially the left infralimbic cortex, plays an important role in PPI, while all other parts of the mPFC were not altered. Additionally, metabolic activity was increased in the right lateral orbitofrontal cortex, a structure that has not been in the focus of PPI-research so far. Not only activity in the basolateral amygdala was increased, but also other parts of this nucleus including an output pathway of the amygdala, the stria terminalis, were active during PPI processing. It is known from the literature that the septohippocampal system modulates PPI and owing to our PET results especially the left septofimbrial nucleus and both septohippocampal nuclei were identified to be important. Moreover, parts of the dorsal and ventral hippocampus showed increased glucose metabolism. No activity change was observed in the mediodorsal thalamus. The nucleus accumbens, ventral pallidum and ventral tegmental area required only partly more glucose compared to the control condition so that no general effect could be observed.
In summary, FDG-PET turned out to be a suitable tool to examine PPI processing in healthy, untreated rats and can be used to complement previous lesion and infusion studies.

# Local neurons in the auditory system of Ensifera

Andreas Stumpner[1], Tim Daniel Ostrowski[1,2]

[1]Georg-August Universität Göttingen, Johann-Friedrich-Blumenbach-Institut für Zoologie und Anthropologie, Abt. Zelluläre Neurobiologie, Berliner Str. 28, 37073 Göttingen, Germany
[2]Division of Biological Sciences, University of Missouri, 204 Tucker Hall, Columbia, MO, 65211, USA

Auditory processing in insects involves sensory neurons in the ears and local neurons and intersegmental neurons in the central nervous system. Much attention has been directed to sensory cells, intersegemtal neurons projecting into the brain and very few large local neurons. However, from experiments like cell killing it is obvious that larger numbers of local neurons must provide important contributions to auditory processing. Here we compile data on a bush cricket (with some comparisons to crickets) demonstrating the likely importance of local neurons for frequency processing, directional processing and also temporal processing. In the prothoracic ganglion, the first level of auditory processing, frequency dependent inhibition, directional inhibition and temporal inhibition are found. The only well characterized local neuron, the omega neuron (homologous to ON1 in crickets) has been demonstrated to produce strong directional inhibition – however there must be additional inhibitory elements. Such elements, which should show clear directional responses and have bilateral branches in the ganglion have not been found yet. Additionally, frequency dependent inhibition has been found, which may rely on groups of DUM-cells. Preliminary data show that there exist several DUM-cells, which differ in their frequency tuning. Whether DUM-cells with similar properties exist in crickets, is unknown. However, in crickets a second omega neuron (ON2) might be responsible for high frequency inhibition. In the brain of bush crickets, local neurons show sharpened frequency tuning as compared to ascending neurons, but again frequency dependent inhibition is found which takes place in the brain and is likely produced by additional local elements. Temporal processing in the prothorax appears to involve specific inhibition as well, since blocking $Cl^-$-channels with picrotoxin changes the response of temporally selective neurons like AN2. Among local neurons in the brain we find several elements with temporal selectivity for duration or repetition of single pulses in a way which is not apparent in the thorax. Finally, we see an influence of ascending elements upon each other in the brain (most likely AN3 evoking a graded potential in TN1-axons), which probably is mediated by local neurons in the brain.

# Locust flight control: performance of different network modelling approaches

Harald Wolf[1]

[1]University of Ulm, Institute for Neurobiology, Albert-Einstein-Allee 11, D-89069 Ulm, Germany

The locust flight oscillator is among the best understood motor control networks of intermediate complexity. This is true in particular with regard to the functioning of the central pattern generating circuit and the integration of sensory feedback. The flight control network is composed of more than 100 interneurons, supplies about 60 flight motoneurons, and receives feedback from several hundred sensory neurons associated with wing structures. Based on good knowledge of connectivity and function for both interneurons (Robertson & Pearson 1984, J Insect Physiol 30, 95-101) and sensory receptor organs (e.g. Pearson KG, Wolf H 1988, J Exp Biol 135, 381-409), modelling approaches with Hodgin- Huxley neuromimes reproduced essential features of the control network (Ausborn, Stein & Wolf 2007, J Neurosci 35, 9319-9328). These include reset properties and interneuronal activity patterns. The network model also predicted as yet unknown properties of sensory feedback integration and suggested novel control strategies during flight (Ausborn, Wolf & Stein 2009, J Comput Neurosci 27, 245-257). These predictions were tested and verified by physiological experiments in the locust, illustrating the power of these modelling approaches.

Yet the question arises how realistic a model has to be to reproduce particular features of the real oscillator (for instance, tailoring the model to the task at hand may allow economical use of computing power, or analysis of structure–function relationships). I therefore modelled the locust flight oscillator with a much simpler set of neuromimes, consisting of sigmoid input -output transfer functions (i.e. without spiking properties; Hülse, Wischmann & Pasemann 2004, Connection Science 16, 294–266; spiking frequency of neuronal cells may be represented in these neuromimes by output potential values, for example). No individual properties of identified interneurons were implemented, except their specific connectivity patterns in the network. In particular, synaptic characteristics such as facilitation or depression were absent, and synaptic weights were equalised and normalised for each neuron to a maximum input of 1.

This simplified network model exhibited reliable oscillations but did not reproduce several detailed characteristics, for example, the exact timing of individual interneuron activities. The model readily reproduced essential network properties, though, including reset characteristics of key interneurons. This is illustrated below for interneuron 301. Depolarising input to interneuron 301 reset the rhythm, as illustrated by the superposition of 7 stimulus presentations, taking stimulus onset as reference (ordinate, output voltage interneuron 301; abscissa, time in modelling steps). Regardless of the phase of stimulus presentation, the rhythm continues after the stimulus (and a certain latency) always with a depolarisation.

I currently examine responses of the model network to sensory input, and its performance in closed loop feedback conditions. This should allow further assessment of the relationship between modelling approach and reproduction of functional network features.

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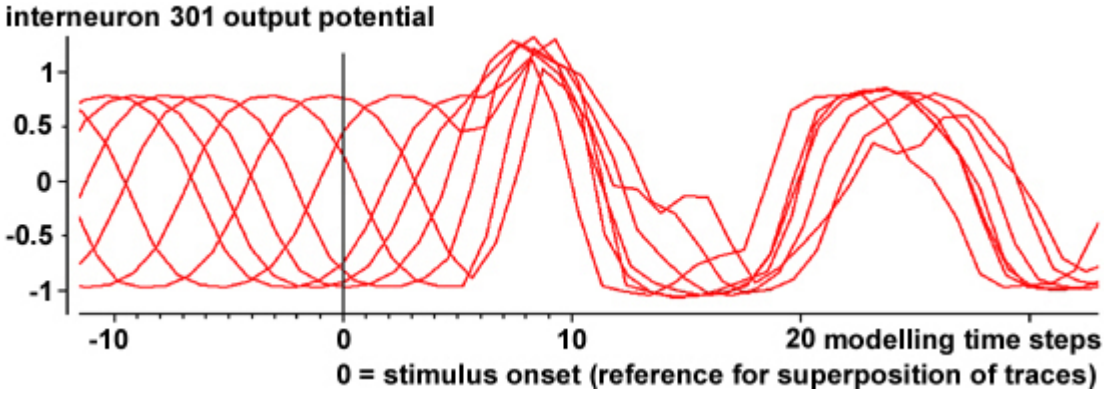
interneuron 301 output potential
1
0.5
0
-0.5
-1
-10
0
10
20 modelling time steps
0 = stimulus onset (reference for superposition of traces)

# Long-range correlation of the membrane potential in neocortical neurons during slow oscillation

Maxim Volgushev[1], Sylvain Chauvette[2], Igor Timofeev[2]

[1]University of Connecticut, Psychology, 406 Babbidge Road, 06269-2050 Storrs, CT, USA

[2] The Centre de recherche Université Laval Robert-Giffard (CRULRG), Laval University, Québec, G1J 2G3, Canada

Large amplitude slow waves are characteristic for the summary brain activity, recorded as electroencephalogram (EEG) or local field potentials (LFP), during deep stages of sleep and some types of anesthesia. Slow rhythm of the synchronized EEG reflects an alternation of active (depolarized, UP) and silent (hyperpolarized, DOWN) states of neocortical neurons. In neurons, involvement in the generalized slow oscillation results in a long-range synchronization of changes of their membrane potential as well as their firing. Here, we aimed at intracellular analysis of details of this synchronization. We asked which components of neuronal activity exhibit long-range correlations during the synchronized EEG ? To answer this question, we made simultaneous intracellular recordings from 2- 4 neocortical neurons in cat neocortex. We studied how correlated is the occurrence of active and silent states, and how correlated are fluctuations of the membrane potential in pairs of neurons located close one to the other or separated by up to 13 mm. We show, that strong long-range correlation of the membrane potential was observed (i) only during the slow oscillation, but not during periods without the oscillation, (ii) only during periods which included transitions between the states, but not during within-the-state periods, and (iii) only for the low frequency (<5 Hz) components of membrane potential fluctuations, but not for the higher frequency components (>10 Hz). In contrast to the neurons located several millimeters one from the other, membrane potential fluctuations in neighboring neurons remain strongly correlated during periods without slow oscillation. We conclude that membrane potential correlation in distant neurons is brought about by synchronous transitions between the states, while activity within the states is largely uncorrelated. The lack of the generalized fine-scale synchronization of membrane potential changes in neurons during the active states of slow oscillation may allow individual neurons to selectively engage in short living episodes of correlated activity – a process that may be similar to dynamical formation of neuronal ensembles during activated brain states.

# Microarray analysis of habenula: identification of cDNAs differentially expressed in the medial and lateral habenular complexes and in the thalamus of the rat

Franziska Wagner[1], Christian Derst[1], Rüdiger W. Veh[1]

[1]Charité, Institut für Integrative Neuroanatomie, Philippstraße 12, 10115 Berlin, Germany

The habenula including its medial (MHb) and lateral complexes (LHb) plays an important role in various behavioural systems, including central pain processing, sleep-wake cycles, reproductive behaviors, stress responses and learning. One common aspect of these behaviors is an emotional component, which may be positive or not. Recently, work of several groups (Ullsperger, Shepard, Hikosaka; 2003) strongly suggests that the predominant biological function of the LHb is the translation of unsuccessful or erroneous behavior into negative reward.
While understanding the function of the LHb has substantially increased during the last years, the knowledge on its internal organization is scarce. In an attempt to separate and identify individual neuron types within the area of the LHb we looked for genes, which are exclusively or differentially expressed in the area of the habenular complexes. We used the Rat Gene 1.0 ST expression catalog array (Affymetrix) for analyzing total mRNA samples of the rat MHb, LHb and the surrounding thalamus as control tissue.
The most prominent signal was related to a G-protein coupled Receptor (Gpr151), which is known to be strongly expressed in the habenula, confirming the reliability of the chip system. In addition, Gene ontology hierarchy analysis predominantly identified genes of ion transport and synaptic transmission as differentially expressed in habenula versus thalamus. Several candidate genes such as nicotinic acetylcholine receptor subunits alpha3 and beta4, muscarinic acetylcholine receptors M2 and M3, Preproenkephalin 1, Neuropeptide Y receptor 1, voltage-gated potassium channel Kv3.2 and various 5-HT receptors were identified with strong expression in habenula. These and several other genes were chosen for extended laboratory-based analysis combining in situ hybridization, immunocytochemistry, and quantitative PCR. We expect that these data will help to identify novel neuron groups in the habenula, thereby facilitating the analysis of the internal organizations of the habenular complexes in the rat.

# Multisensory processing within visual-somatosensory cortical networks of the juvenile Brown Norway rat

Kay Sieben[1], Ileana L. Hanganu-Opatz[1]

[1]Center for Molecular Neurobiology, University Medical Center Hamburg-Eppendorf, Developmental Neurophysiology, Falkenried 94, 20251 Hamburg, Germany

Fully perception of environment and adaptation of directed behaviour to various stimuli requires integration of information from different sensory systems. For example, for multisensory localization, events initially coded in modality specific coordinate systems, e. g. retinotopic and somatotopic coordinates, have to be integrated. There is evidence from both human and primate work that multisensory localization is based on an external-visual representation of space in primary sensory cortices and maturates later during development. However the mechanisms underlying the multisensory localization and its maturation remain unknown. Here we developed an experimental approach that mimics in rodents the misalignment of somatosensory and visual coordinates achieved in humans by crossing of limbs. Congruent and incongruent uni - and bimodal visual (light spot) and tactile stimulation of crossed- and uncrossed whiskers was performed simultaneously with extracellular multielectrode recordings in the primary visual (V1) and somatosensory (S1 barrel field) neocortices of postnatal day 15 to 30 Brown Norway rats in vivo. The stimulation-evoked potentials, oscillatory discharge in different frequency bands and firing rate were analyzed. Unimodal stimulation in an uncrossed configuration led after 20-50 ms to a large amplitude response in the contralateral hemisphere that is accompanied by prominent, layer-specific sinks and sources and strong spike discharge. Bimodal congruent stimulation leads to an enhancement of these effects and augments the amplitude of oscillatory activity in theta and gamma frequency bands. Crossing of whiskers modifies the patterns of evoked oscillatory rhythms and the firing of V1 and S1 neurons. These data support the role of primary sensory areas for the multisensory integration of signals and the remapping of a common external coordinate-system.

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# Myoinhibitory peptide immunoreactivity in the circadian system of the cockroach *Leucophaea maderae*

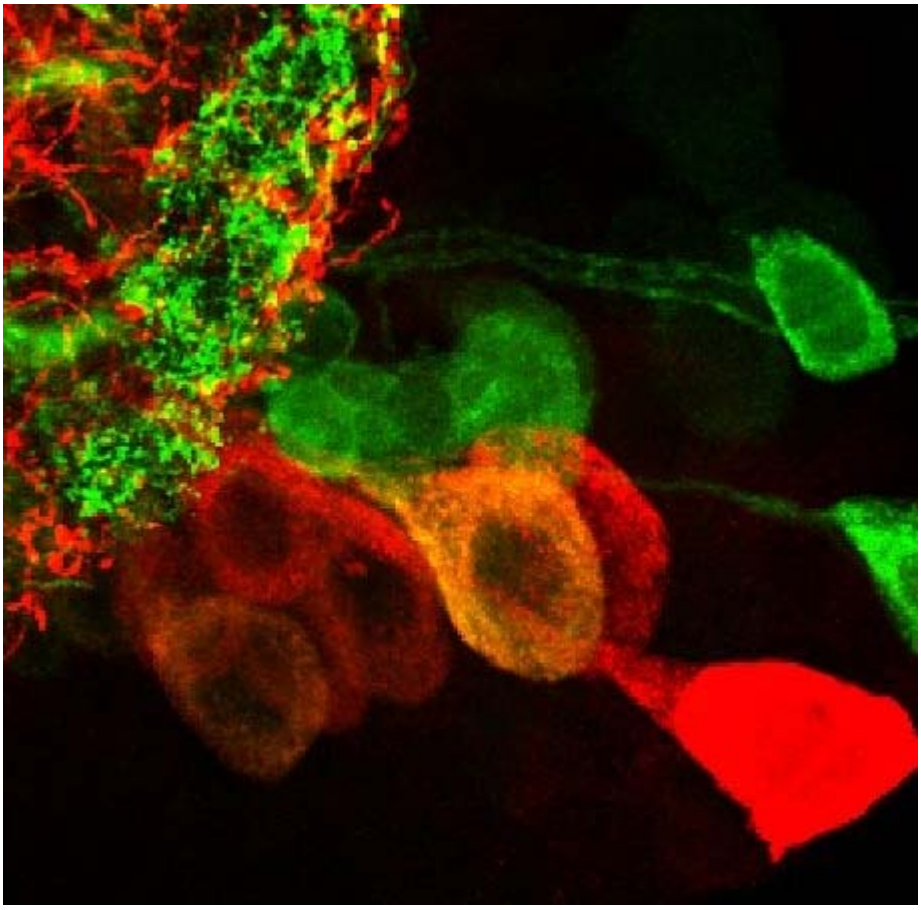
Julia Schulze[1], Susanne Neupert[2], Lilia Schmidt[3], Reinhard Predel[2], Uwe Homberg[3], Monika Stengl[1]

[1]University of Kassel, Biology / Animal Physiology, Heinrich-Plett-Straße 40, 34134 Kassel, Germany

[2]Friedrich Schiller University, Department of General Zoology and Animal Physiology, 07743 Jena, Germany

[3]Philipps-University of Marburg, Animal Physiology, 35032 Marburg, Germany

Myoinhibitory peptides (MIPs) are a family of insect W($X_6$)Wamide peptides with inhibitory effects on visceral muscles and juvenile hormone synthesis (Nässel 2002, Progr Neurobiol 68:1). Although MIPs are also widely distributed within the nervous system, a detailed analysis of their distribution and function in insect brains is still missing. In this study, we analyzed the distribution of MIP in the brain of the cockroach *Leucophaea maderae*. We focused on the accessory medulla (aMe), a small neuropil medioventrally of the medulla. The aMe is the circadian pacemaker center that controls locomotor activity rhythms in the cockroach. MALDI-TOF mass spectrometry identified Lem-MIP in the aMe and corpora cardiaca. Immunocytochemistry revealed wide distribution of MIP-related peptides in the cockroach brain. Dense immunostaining was observed in the superior median protocerebrum, in parts of the central complex and in the tritocerebrum. In contrast, only a few local interneurons of the antennal lobes were immunostained and some extrinsic neurons of the mushroom bodies, including a giant extrinsic neuron innervating the calyces. In the optic lobes, the noduli of the aMe showed dense immunostaining, and all neuronal cell groups of the aMe except the anterior neurons were labeled. Double-labeling experiments showed colocalization of pigment-dispersing factor (PDF) and MIP immunostaining in three neurons (PDFMe) of the aMe, namely in one strongly MIP-immunostained large, one more weakly immunoreactive medium-sized, and one faintly immunolabeled small PDFMe neuron. No colocalization of MIP- and PDF immunostaining was detected in the anterior optic commissure, but two thin PDF-immunoreactive fibres that ran parallel to the main fibre bundles of the posterior optic commissure showed colocalized MIP immunostaining. The results suggest that MIP participates in different functional circuits of the circadian system and is involved in multiple neuronal circuits of the midbrain of the Madeira cockroach. [Supported by DFG STE531/21-1 to M.S.]



# Neuronal intrinsic discharge properties and hippocampal network activity in ASA deficient mice

Christina Albus[1], Matthias Eckhardt [2], Volkmar Gieselmann [2], Heinz Beck[1], Thoralf Opitz[1]

[1]Laboratory of Experimental Epileptology and Cognition Research, Department of Epileptology, Life & Brain Center, Sigmund-Freud Str. 25, 53127 Bonn, Germany

[2]Institute of Biochemistry and Molecular Biology, Nussallee 11, 53115 Bonn, Germany

Arylsulfatase A (ASA) is a lysosomal enzyme catalyzing the degradation of sulfatides. Mice deficient in ASA show neuromotor deficits and mild behavioural disturbances. Invasive EEG recordings have revealed a marked cortical hyperexcitability, with episodes of recurrent spontaneous epileptiform activity. ASA-deficient mice accumulate sulfatides in glial cells, microglia, and neurons. The phenotypic abnormalities have so far been mainly ascribed to the progressive demyelination and axonal damage, but a direct examination of the consequences of sulfatide accumulation on neuronal excitability has been lacking.

We have therefore performed sharp microelectrode and field potential recordings to compare intrinsic, synaptic, and network properties of hippocampal CA1 pyramidal neurons from ASA-deficient mice and littermate controls. Two different age groups (8-10 weeks and 5-6 months) were used for recordings. We did not find changes of passive or active membrane properties in CA1 pyramidal cells between ASA-deficient mice and littermate controls in either age group.

Despite the lack of changes in intrinsic excitability, we found evidence of altered network excitability. We measured the incidence of sharp waves (SPW) in the CA1 and CA3 subfields under conditions of increased excitability (3 mM $K^+$, 2.5 mM $Ca^{2+}$, 1.3 mM $Mg^{2+}$) and found no differences between ASA-deficient mice and littermate controls in either subfield. However, we observed that the fraction of sharp waves with superimposed ripple oscillations (sharp wave ripple or SWR-events) was significantly increased in the CA3 region of ASA-deficient mice compared to control mice (8-10 weeks). An increased incidence of SWR-events in ASA-deficient mice was not found in the CA1 subfield. These results suggest that ASA deficiency causes a selectively increased propensity to generate high frequency network activity within the CA3 subfield. Candidate underlying mechanisms for increased ripple oscillations include CA3 -specific changes in GABAergic inhibition, intrinsic neuronal properties or gap junction coupling.

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# Octopaminergic/tyraminergic neurons in the CNS of *Drosophila* larvae

Mareike Selcho[1], Dennis Pauls[1, 2], Christian Wegener[2], Reinhard Stocker[1], Andreas Thum[1]

[1]University of Fribourg, Department of Biology, Zoology, Chemin du Musée 10, 1700 Fribourg, Switzerland; [2]Philipps-University Marburg, Department of Biology, Neurobiology/Ethology, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

The biogenic amine octopamine is involved in many different behaviors in adult and larval *Drosophila*. For instance it was shown to play a role in sugar reward learning, aggression and larval locomotion. The anatomical description of octopaminergic/tyraminergic neurons will provide a basis for understanding the cellular circuits underlying these behaviors.
Therefore we describe the innervation patterns of single octopaminergic/tyraminergic neurons in the larval CNS. We show by single-cell analysis that the approximately 40 octopaminergic/tyraminergic neurons in the central brain and suboesophageal ganglion (SOG) innervate nearly all parts of the brain including the antennal lobes, larval optic neuropiles, mushroom bodies and SOG. Contrary the ~43 cells located in the ventral nerve cord project mainly to the periphery and seem to play a role in larval locomotion (see Abstract by Dennis Pauls).

# A highly active subnetwork of neocortical neurons identified by in vivo IEG expression

Jean-Sébastien Jouhanneau[1], Lina Yassin[3], Brett L. Bennedetti[3], James F. A. Poulet[1,2], Alison L. Barth[3]

[1]Max Delbruck Center for Molecular Medicine
Robert Rossler Strasse 10
D-13092 Berlin-Buch, Germany
[2]NeuroCure
Neuroscience Research Center (NWFZ)
Charité - Universitätsmedizin Berlin
Campus Charité Mitte
Charitéplatz 1
10117 Berlin
[3]Department of Biological Sciences and Center for the Neural Basis of Cognition
Carnegie Mellon University
4400 Fifth Avenue
Pittsburgh, PA 15213

Whole-cell recordings in vivo indicate that firing rates of neocortical neurons, especially those in layer 2/3, are very low. Although a small subset of neurons sometimes display higher firing rates in vivo, it has not been clear whether these are excitatory neurons and whether these firing rates are an intrinsic property of the neuron or an emergent feature of the network.
Here we use expression of the activity-dependent immediate-early gene c-fos to target electrodes to specific neural subsets in layer 2/3 of primary somatosensory cortex in a fosGFP transgenic mouse. We hypothesized that expression of the fosGFP transgene activated under normal, unstimulated conditions might mark an active population of neurons and facilitate analysis into the cellular and molecular mechanisms that control firing rates and response selectivity in vivo.
Paired recordings in urethane-anaesthetized mice show that fosGFP expressing neurons exhibit elevated spontaneous firing activity compared to neighboring fosGFP- neurons. In acute brain slices, fosGFP+ pyramidal cells continue to show significantly elevated spontaneous firing activity compared to fosGFP- neurons. Elevated firing could not be attributed to increased intrinsic excitability of this cell population. However, in vitro paired recordings showed that fosGFP+ neurons received more excitatory and less inhibitory drive during spontaneous network activity. At least some of this excitatory drive is likely to come from other fosGFP+ neurons, since they showed a higher probability of being connected to each other compared to other combinations (fosGFP+ to fosGFP- or fosGFP- to fosGFP-). Thus, expression of the immediate-early gene c-fos is an indicator of a highly active, synaptically coupled subnetwork of excitatory neurons in primary somatosensory cortex.

# Optical imaging reveals autonomous seizure activity in the dentate gyrus of chronic epileptic animals

Florian Weissinger[1], Martin Holtkamp[1], Katharina Buchheim[1,2], Mark Elsner[1], Julia Matzen[1], Hartmut Meierkord[1]

[1]Charit¨¦ - Universitätsmedizin Berlin, Neurologie, Charit¨¦platz 1, 10117 Berlin, Germany
[2]deceased

Purpose

The dentate gyrus (DG) has been shown to be a strongly inhibitory structure protecting the highly vulnerable hippocampus proper. In previous imaging experiments in tissue of chronic epileptic animals, we observed epileptic activity in the DG that was independent from seizure-like events (SLEs) in other limbic structures. The aim of this study was to analyze the frequency and characteristics of this unexpected finding.

Methods

Male Wistar rats underwent electrical stimulation of the perforant path to induce status epilepticus (SE) of 3-5 h duration. Animals were sacrificed for *in vitro* experiments and histopathology after at least 56 d (mean 101.6 ± 33.7 d). Combined hippocampal entorhinal cortex slices were prepared and stored in artificial cerebrospinal fluid (ACSF). Epileptic activity was induced by 0-$Mg^{2+}$ ACSF. Extracellular field potentials were recorded using microelectrodes in the temporal or entorhinal cortex and in the DG, respectively. Synchronously, intrinsic optical imaging of the entire slice was performed. Changes in light transmission associated with SLEs were recorded using a CCD camera and in-house software. Thus, onset and spread patterns of SLEs could be analyzed. In some slices, paired pulse recordings in the DG granule cell layer were performed by stimulation of the perforant path; interpulse intervals (IPIs) were 5, 10, 15, 20, and 25 ms, respectively. To assess loss of inhibitory basket cells in the DG, immunocytochemical staining was done in a subset of animals. All glutamate decarboxylase (GAD)/cholecystokinin (CCK) and GAD/parvalbumin (PV) double labelled neurons were quantified.

Results

A total of 28 SE animals and 15 controls was used for this study. Upon omission of $Mg^{2+}$, SLEs were generated in all slices. In 18 SE animals (64.3%), the DG itself was capable of generating epileptic activity independently from entorhinal and temporal cortex. Onset of these SLEs was confined to the DG, while maximum spread only involved hilus and area CA3. We termed this phenomenon, which to our knowledge has not been described before, *autonomous DG activity*. This kind of activity was never seen in slices from control animals. SE animals with or without autonomous DG activity were not different with regard to SE duration, latency from SE to in vitro experiment, or occurrence of spontaneous seizures in EEG video monitoring post SE. When comparing SE slices with (n=32) and without (n=45) autonomous DG activity, there were no differences regarding hemisphere of slice origin, latency to first SLE in entorhinal or temporal cortex, and frequency of SLEs in these areas. DG inhibition assessed by paired pulse recordings was significantly diminished in SE slices compared to controls (IPI 10 ms and IPI 25 ms, $p < 0.05$; all other IPIs, $p = 0.01$); however, there was no difference when comparing SE slices with or without autonomous DG activity. Immunocytochemistry revealed significant loss of GAD/CCK ($p < 0.001$) and GAD/PV ($p < 0.05$) positive neurons in DG.

Conclusion

Intrinsic optical imaging revealed for the first time that in the majority of chronic epileptic animals the DG is hyperexcitable allowing initiation of epileptic activity by itself. Though DG inhibition in the chronic epileptic state was impaired, this finding was not specific for slices with autonomous activity. The current data indicate that loss of inhibitory basket cells may contribute to this phenomenon. Prospective studies should address further underlying mechanisms.

# Optical recording of action potential propagation in identified neurons of the crab stomatogastric nervous system

Carola Staedele[1], Peter Andras[2], Wolfgang Stein[1]

[1]Institute of Neurobiology, Ulm University, Albert-Einstein Allee 11, 89081 Ulm, Germany

[2]School of Computing Science & Institute of Neuroscience, Newcastle University, England

The processing of sensory information is essential for the adequate functioning of the nervous system. We are studying the influence of proprioceptive sensory feedback to central pattern generating circuits using the crustacean stomatogastric ganglion (STG), a well-characterized prototype central pattern generator (CPG) system. In particular, we focus on the muscle tendon organ AGR (anterior gastric receptor), a single neuron which innervates a muscle that protracts a tooth in the gastric mill chamber of the crab foregut (Smarandache & Stein, J Exp Biol 210, 2007; Combes, Meyrand & Simmers, J Neurosci 19, 1999). AGR is a bipolar neuron in the STG which protrudes a posterior axon to the muscle and an anterior axon that projects to its postsynaptic target cells. We have shown previously that AGR generates tonic activity which originates spatially distant from the innervated muscle in the anterior axon and that this activity is functionally relevant for the generated network activity (Daur, Nadim & Stein, Europ J Neurosci 30, 2009). The location of this spike initiation zone in the axon, however, is unknown. Since it can only be estimated with electrophysiological methods, we here demonstrate the use of optical imaging with voltage-sensitive dyes for tracking action potentials propagation and to determine the site of action potential initiation.

For this, we first tested the applicability of optical recordings to measure membrane potential changes in cell bodies of CPG neurons in the STG. Second, we tracked action potential conduction in the nerves containing the AGR axon. For both, the voltage-sensitive dye di-4-ANEPPS was bath-applied at a concentration of 5µM, which stained all neuronal membranes in the STG and its connecting nerves (Stein & Andras, J Neurosci Methods 188, 2010). Nerves and STG were desheathed to allow access for the dye. The optical signal was recorded using an Olympus BW51 microscope equipped with a high-speed CCD camera (MiCAM02) sampling at >600 Hz. We additionally recorded the activity generated by AGR and the other STG neurons with extracellular recordings from the motor nerves *lvn* (containing 16 motor neuron axons) or *dgn* (6 motor neuron axons) and with intracellular recordings in the STG.

Our optical recordings show that we can reliably detect both slow and fast changes in neuronal membrane potential in the CPG neurons in the STG even without averaging (i.e. during a single recording sweep). We also demonstrate the recording of AGR's action potentials in the nerve. For the latter, however, averaging over multiple action potentials was needed to detect the corresponding optical signal. This was probably due to the high background activity caused by the large number of axons from neurons other than AGR in the nerve. We are currently testing whether we can track action potential conductance along the nerve in order to determine the spike initiation zone of AGR.

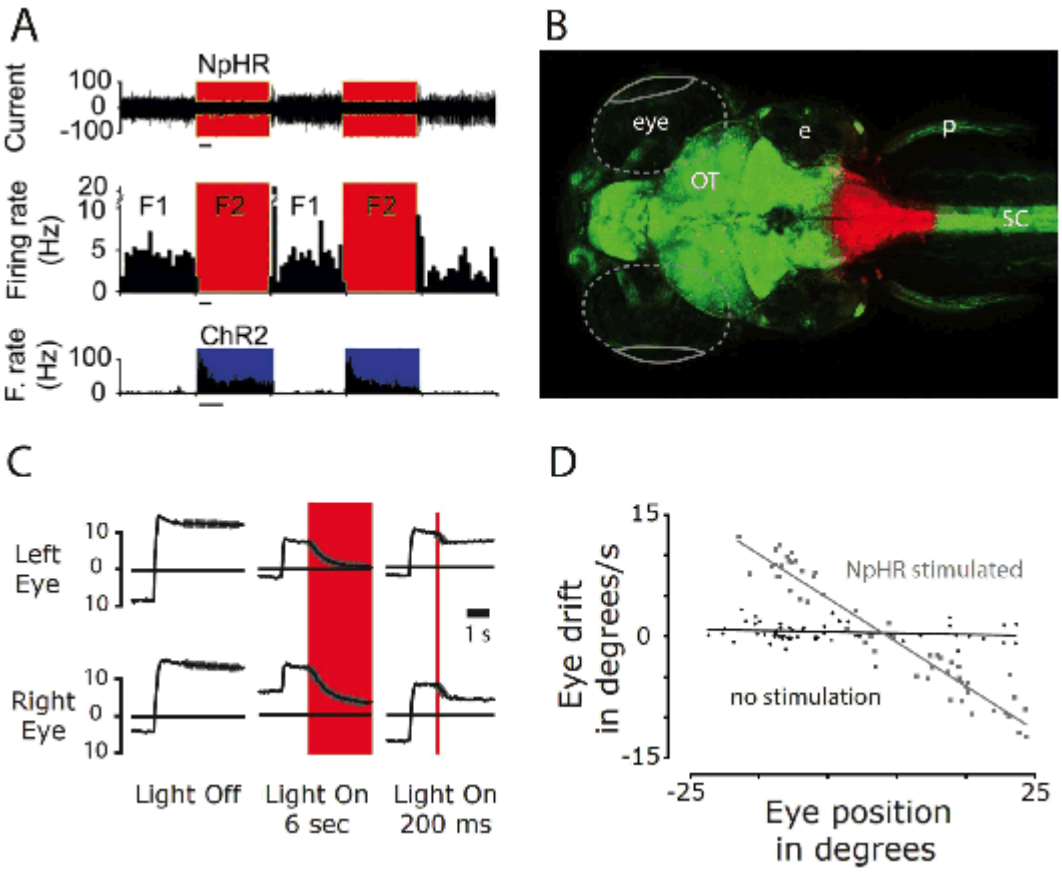
# Optogenetic dissection of the oculomotor system in zebrafish

Aristides Arrenberg[1,4], Pedro Gonçalves[2,3], Peter Schoonheim[1], Wolfgang Driever[4], Christian Machens[2,3], Herwig Baier[1]

[1]UCSF, Department of Physiology, Program in Neuroscience, 1550 4th Street , San Francisco, CA, 94158-2324
[2]Group for Neural Theory, Ecole normale supérieure, Paris, 75005, France
[3]INSERM Unite 960, Ecole normale supérieure, Paris, 75005, France
[4]Current address: University of Freiburg, Biologie I, Hauptstraße 1, 79104 Freiburg

Expression of halorhodopsin (NpHR), a light-driven chloride pump, allows for reversible silencing of neurons (Fig. 1A). The transparency of zebrafish (Fig. 1B) facilitates optogenetic approaches. We study the premotor circuits underlying horizontal eye movements during the optokinetic response (OKR) and free viewing. We use optic fibers to target small groups of cells and test the effect of their silencing on the generation of saccades (fast eye movements) during the OKR. Previously, we identified a cell population in rhombomere 5 that is required and sufficient for the generation of saccades in zebrafish larvae. Bilateral stimulation of NpHR suppressed saccades completely but left slow phase eye movements unaffected. Unilateral stimulation blocked saccades of both eyes in the direction of the stimulated side, but not in the other direction. Conversely, ChR2 mediated activation elicited saccades. These results are consistent with the hypothesis that the saccade generator of fishes is homologous to that in mammals. Currently, we are studying the neural integrator of horizontal eye movements in zebrafish. This circuit stabilizes the eye position following a saccade by firing at a constant rate. To understand how this persistent activity is maintained, we measure eye movements following spatially and temporally defined perturbations of the integrator neurons. NpHR-mediated silencing of the integrator produces transient drifts of the eyes towards the null position (Fig. 1B-D). Unilateral inactivations are sampled across eye positions to understand the coupling of the left and right half-integrators. To take full advantage of this new tool, we develop an analytical framework to model instantaneous perturbations of the system. Our approach can be used to add new constraints to the architecture of the integrator circuit and to test the predictions of competing network models.

*Figure 1. Halorhodopsin (NpHR) assisted silencing of the caudal hindbrain induces leaky eye movements. (A) Electrophysiological recordings from hindbrain neurons expressing NpHR or channelrhodopsin (ChR2). Middle row: Single unit firing rate histogram of a NpHR expressing cell that stops firing during illumination (red, F2) and of a ChR2 expressing cell (bottom) that increases its firing rate during illumination (blue). (B) Dorsal View of a 6 day old larva (anterior is left). The photoconvertible protein Kaede is expressed throughout the CNS in this Et(E1b:Gal4-VP16)s1101t, Tg(UAS:NpHR-mCherry)s1989t, Tg(UAS:Kaede)s1999t transgenic fish. The red region (caudal hindbrain) is photoconverted and labels the tissue volume silenced in the experiments shown in (C-D). Due to its cell surface localization, NpHR-mCherry is less visible than cytosolic, photoconverted Kaede. Abbreviations: SC, spinal cord, e, ear, p, pectoral fin, OT, optic tectum. (C) Angular eye positions of the NpHR expressing fish from (B) are plotted over time. After a saccade, the eye position is stable (left). Middle: NpHR is activated in the caudal hindbrain one second after a spontaneous saccade (red shade). The eyes drift back to the midline. Right: Short NpHR activation (200 ms, red shade) induces transient eye drift. (D) NpHR-induced eye drifts across different initial post-saccadic eye positions. Bilateral illumination (200 ms) of the hindbrain in the animal shown in (B) induces large eye drifts across eye positions (slope Light On: $k_{On}$=-0.54 $s^{-1}$, slope Light Off: $k_{Off}$=-0.02 $s^{-1}$).*


A
NpHR
Current
100
0
-100
Firing rate (Hz)
20
10
5
0
F1
F2
F1
F2
ChR2
F. rate (Hz)
100
0
B
eye
e
p
OT
SC
C
Left Eye
Right Eye
10
0
10
1 s
Light Off
Light On 6 sec
Light On 200 ms
D
Eye drift in degrees/s
15
0
-15
NpHR stimulated
no stimulation
-25
25
Eye position in degrees

# Oscillatory Synchronization in Large-Scale Cortical Networks Predicts Perception

Joerg F. Hipp[1], Andreas K. Engel[1], Markus Siegel[2]

[1]Department of Neurophysiology and Pathophysiology, University Medical Centre Hamburg-Eppendorf, 20246 Hamburg, Germany
[2]Centre for Integrative Neuroscience, University of Tübingen, 72076 Tübingen, Germany

Normal brain function requires the dynamic interaction of functionally specialized but widely distributed cortical regions. Long-range synchronization of oscillatory signals has been suggested to mediate these interactions within large-scale cortical networks, but direct evidence is sparse. Here we show that oscillatory synchronization is organized in such large-scale networks and provide behavioral evidence for their functional relevance. We implemented a new analysis approach that allows for imaging synchronized cortical networks and applied this technique to EEG recordings in humans. We identified two networks: beta-band synchronization (~20 Hz) in a fronto-parieto-occipital network and gamma-band synchronization (~80 Hz) in a centro-temporal network. Strong perceptual correlates support their functional relevance: the strength of synchronization within these networks predicted the subjects' percept of an ambiguous audio-visual stimulus as well as the integration of auditory and visual information. Our results suggest that oscillatory neuronal synchronization may regulate neuronal communication across frequency-specific, large-scale cortical networks.

# Participation of hilar mossy cells in sharp wave ripple oscillations *in vitro*

Alexei V. Egorov[1], Maura M. Zylla[1], Martin Both[1], Andreas Draguhn[1]

[1]Interdisciplinary Center for Neurosciences (IZN), Institute of Physiology and Pathophysiology, Department of Neurophysiology, University of Heidelberg, Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

The medial temporal lobe contains a system of anatomically related structures that are essential for memory formation, including the hippocampus, entorhinal and perirhinal cortices. Within the hippocampus, high frequency oscillations known as sharp wave ripple (SPW-R) complexes are associated with synchronous discharges of transient neuronal assemblies in multiple hippocampal sites, and presumably underlie spatial memory consolidation. The dentate gyrus is part of the hippocampal formation, and is an essential entorhinal–hippocampal relay station. The dentate gyrus consists of three layers: the molecular layer, granule cell layer and hilus. The hilar region of the dentate gyrus includes a variety of neuronal types such as hilar mossy cells (MCs), basket cells, spiny and aspiny interneurons. Because of their strategic position between the granule cell and pyramidal cell layers, neurons of the hilar region of the hippocampus are likely to play an important role in the information processing between the entorhinal cortex and the hippocampus proper. Although MCs are important components of the dentate circuitry, their physiological function and exact involvement in various network patterns (e.g., SPW-R oscillations) has remained unclear. Here we investigated participation of MCs in SPW-R oscillations using sharp microelectrodes, juxtacellular and field-potential recordings in horizontal mouse brain slices. MCs were identified based on their characteristic electrophysiological properties, including a rather depolarized resting membrane potential (RMP: -59 ± 4 mV), bursting in response to depolarizing current steps and the presence of large ongoing spontaneous synaptic activity. Location and morphological identification of MCs were revealed using single-cell juxtacellular labeling with neurobiotin. We observed a tight correlation between field SPW-R in area CA1 and neuronal spiking of MCs in the vast majority of neurons at RMP (8 out of 9 cells in intracellular recordings). On average, ~ 20 % of spontaneous spikes were coupled to SPW-R of CA1. These spikes were associated with membrane depolarization on top of high spontaneous background synaptic activity of MCs. SPW-R of CA1 were frequently correlated with a single spike of MCs, but occasionally with double spikes or bursts at ~ 100 Hz. We also found differences in spike waveform for action potentials outside and inside SPW-R. Thus, phase-coupled spikes were preceded by a more prolonged depolarization. Negative current injection increased background synaptic activity in amplitude, but strongly decreased the occurrence of spontaneous spikes (non-coupled and coupled), and membrane hyperpolarization beyond ~ -70 mV led to disappearance of spikes. Using juxtacellular recordings from MCs we detected a characteristic pattern of spontaneous unit activity that included single spike, double spikes and bursts. The cross-correlations of units to field SPW-R in CA1 were similar to those occurring in intracellular recordings. These results indicate recruitment of MCs in the dentate circuitry during SPW-R *in vitro*. Diversity of synaptic targets, including a recurrent excitatory projection to granule cells, suggest a possible integrative function of MCs in sustaining neuronal assemblies durig SPW-R. Supported by BMBF (BCCN)

# Pharmacologically induced antennal movements in the stick insect *Carausius morosus*

Andrea Winkelmann[1], Volker Dürr[1,2]

[1]Bielefeld University, Department of Biological Cybernetics, Universitätsstraße 25, 33615 Bielefeld Bielefeld, Germany
[2]Cognitive Interaction Technology- Centre of Excellence, University of Bielefeld, Germany

Stick insects use their antennae for active exploration of the environment. During walking, they rhythmically move their antennae in order to get information about obstacles and gaps in the legs' working ranges. In contrast to the antennal movement pattern, the generating mechanisms of antennal movements in stick insects are unknown. There are several studies on arthropods that hint at the existence of central pattern generators (CPG) for rhythmical movement generation in limbs such as legs, mouthparts and antennae. To analyse the influence of centrally generating networks on antennal movements, CPGs can be activated by pilocarpine, a muscarinic agonist of cholinergic receptors. As a first step of the present study, a reliable and robust animal preparation was established. It turned out to be best to apply pilocarpine directly onto the exposed brain in an *in situ* preparation. The invoked antennal movements did not differ significantly for pilocarpine concentrations in the range between 5 mM and 10 mM. 5 mM was the minimal effective concentration.

To analyse the antennal movement pattern, a retro-reflective marker was stuck onto one antenna. In order to measure the 3D-position of this marker, antennal movements were video-recorded from above and, via a mirror, from the side. Due to the fact that the two antennal joints were hinge joints, each position in space could be attained by a unique set of joint angles, only. These pairs of joint angles were calculated from the 3D-position of the marker.

In contrast to the cycle frequencies, which were counted observationally, working ranges and inter-joint coupling were analysed on the level of single joint angles. The latter characteristics were used to compare pharmacologically induced antennal movement patterns and spontaneous antennal movement during walking. Although the antennal cycle frequency in pharmacologically induced antennal movements was between 0.3 Hz and 0.6 Hz instead of 1.5 Hz to 2 Hz in spontaneous movement, the working range of a single joint did not differ significantly between spontaneous and pharmacologically induced antennal movement. Both groups showed strong inter-joint coupling, where the scape-pedicel joint led the head-scape joint. The time lag between both joints was around 200 ms in induced antennal movements and around 90 ms in spontaneous antennal movements.

Transection experiments were made to identify the parts of the nervous system, which were sufficient and/or necessary for generation of rhythmic antennal movement. For this, stick insects were de-cerebrated by means of cutting both circumoesophageal connectives. After transection, stick insects were still able to walk but they no longer moved their antennae during walking. However, antennal movement could be invoked by subsequent application of pilocarpine. These results suggest that the pattern-generating networks for antennal movement are located in the brain. However, the ventral ganglia (suboesophageal and/or thoracic ganglia) appear to be necessary for spontaneous antennal movement during walking.

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# Physiological properties of non-local, horizontal projections onto layer 5 pyramidal neurons

Philipp Schnepel[1,2], Martin Paul Nawrot[3], Ad Aertsen[1,2], Clemens Boucsein[1,2]

[1]Neurobiology and Biophysics, Faculty of Biology, University of Freiburg, Germany
[2]Bernstein Center Freiburg, University of Freiburg, Germany
[3]Neuroinformatics/Theoretical Neuroscience, Inst. of Biology, Freie Universität Berlin, Germany

Local cortical networks and their role in information processing in the brain have been studied on many different levels and scales, both experimentally and theoretically. Although the properties of local and inter-laminar synaptic connections have been investigated in great detail, the question remains if this is sufficient to describe more generic properties of cortical networks in terms of information processing and propagation. In recent years, the impact of long-range horizontal connections on neocortical networks has therefore drawn increasing attention, since several neuroanatomical studies (Hellwig 2000; Binzegger et al., 2004; Stepanyants et al., 2009; Voges et al. 2010) have consistently suggested that an estimated 50-75% of the connections a neuron receives originate outside the local volume (radius: ~250µm). Due to the strong drop in connection probability of laterally displaced pairs of neurons and the resulting methodological constraints for classical investigation with paired recordings, the properties of these connections have not been elucidated yet, although their impact on local information processing could be substantial.
Here, we used photostimulation to map long-range horizontal projections to layer 5B pyramidal neurons in acute cortical slices. For lateral distances of 200-1500µm, we found intact projections which were preserved in the slice and characterized their physiological properties as well as their layer of origin. The average amplitude of EPSCs slightly dropped with distance, while strong connections were still present over long distances. Short and long range connections showed an equally high synaptic reliability of 100% in most tested synapses, the same level of amplitude variability, and an equally high temporal precision of <1ms. In summary, our data provide an initial parameterization of long-range connections, which could be used to refine structured models of cortical networks. We conclude that long-distance horizontal connections could represent a substantial fraction of inputs to the local, cortical network. Secondly, although they showed a slight drop in amplitude with increasing distance, they contribute with reliable and precise inputs to the single neurons in layer 5, thus impacting the local computation considerably.

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# Propagation of Activity Fronts in Patterned Neural Cultures

Jordi Soriano[1], Shimshon Jacobi[2], Núria Amigó[1], Sara Teller[1], Jaume Casademunt[1], Elisha Moses[2]

[1]Departament d'ECM, Facultat de Física, Universitat de Barcelona, 08028 Barcelona, Spain
[2]Department of Physics of Complex Systems, Weizmann Institute of Science, 76100 Rehovot, Israel

Dissociated neurons cultured on a dish show a rich repertoire of spontaneous activity that is strongly influenced by the connectivity of the network. To study the role and importance of neuronal connectivity in network dynamics, we introduce a new experimental approach consisting in the confinement of neurons on predefined regions of a substrate. We investigate two configurations, one-dimensional (1D) and two-dimensional (2D) patterns. For the 1D, the substrate is prepared by chemically imprinting thin, long lines along a glass coverslip. For 2D patterns we use a PDMS topographical mould where neurons grow and connect only along the valleys or crevices of the mould. Our experiments show that the activity repertoire that emerges from such a 'patterned neuronal network' is much richer and complex than the all-or-none behaviour of a standard two-dimensional culture, with the observation of travelling pulses and nucleation centres. Together with numerical models that combine concepts of graph-theory with the integrate-and-fire nature of the neurons, we show that the patterned substrate dramatically reduces the degrees of freedom for the connectivity, constraining and guiding the circuitry of the network in such a way that strongly shapes its dynamics.

# Recruitment of interneurons into inhibitory feed-back microcircuits in the epileptic hippocampus

Leonie Pothmann[1], Christina Müller[1,2], Stefan Remy[2], Heinz Beck[1]

[1]University of Bonn, Laboratory of Experimental Epileptology and Cognition Research
[2]German Center for Neurodegenerative Diseases (DZNE)

CA1 microcircuits show profound reorganization in human and experimental temporal lobe epilepsy due to synaptic sprouting, loss of certain neuron types, and functional synaptic changes. Changes in the recruitment of inhibitory interneurons (INs) are thought to be important for the generation and maintenance of aberrant synchronous activity. In addition, the recruitment of INs during physiologically occurring activity patterns may be disrupted. We have therefore examined the recruitment of interneurons into feed-back inhibitory microcircuits in the CA1 region in the pilocarpine model of chronic epilepsy. We recruited feed-back inhibition by electrical stimulation of CA1 pyramidal cell axons in the alveus and recorded the resulting EPSPs from identified INs in the stratum oriens and pyramidale in the whole cell configuration. In control tissue, a first group of INs showed a strong initial response at the beginning of theta-patterned stimulus trains with a subsequent depression (theta-depressing, 10/15 cells), while a second group showed facilitating responses (theta-facilitating, 5/15 cells). Axonal reconstructions revealed that theta -facilitating INs projected to stratum lacunosum moleculare, whereas theta-depressing INs predominantly projected perisomatically. In epileptic animals, both somatically and dendritically targeting INs showed a theta facilitating response (14/16 cells), with only two INs classified as theta depressing. An analysis of the inhibitory drive onto INs during alveus stimulation revealed that dynamic changes in IN inhibition cannot account for this effect. An alternate mechanism that might account for these epilepsy-related changes is a change in the release properties at CA1-interneuron synapses.
These data suggest a marked change in the synaptic recruitment of perisomatically inhibiting INs into feedback microcircuits that may be relevant for the initiation of seizure activity in the CA1 ensemble.

# Slow oscillating population activity in developing cortical networks: Models and experimental results

Thomas Baltz[1], Andreas Herzog[2], Thomas Voigt[1]

[1]Institute of Physiology, Otto-von-Guericke-University Magdeburg, Developmental Physiology, Leipziger Str. 44, 39120 Magdeburg, Germany

[2]Biosystems Engineering, Fraunhofer-Institut für Fabrikbetrieb und -automatisierung IFF, 39004 Magdeburg, Germany

During early development neuronal networks express slow oscillating synchronized activity. The activity can be driven by several, not necessarily mutually exclusive, mechanisms. Each mechanism might have distinctive consequences for the phenomenology, formation or sustainment of the early activity pattern. Here we study the emergence of the oscillatory activity in three computational models and compare the results with multisite extracellular recordings that we obtained from developing cortical networks in vitro. The modeled networks consist of leaky integrate-and-fire neurons, which were driven by either neurons that are intrinsically bursting, intrinsically random spiking or driven by spontaneous synaptic activity. A plausible network behavior emerged in all model types over a large volume of parameters. In embryonic cultured cortical networks, we found evidence that support two of the three models, namely spontaneous synaptic activity and intrinsically bursting neurons. The activity of model networks driven by intrinsically bursting cells best matched the phenomenology of one-week-old cultures, in which early oscillatory activity has just begun. Intrinsically bursting neurons were present in cortical cultures, but we found them only in those cultures that were younger than three weeks in vitro. On the other hand, synaptically dependent random spiking was highest after three weeks in vitro. In conclusion, model networks driven by intrinsically bursting cells show a good approximation of the emergent recurrent population activity in young networks, whereas the activity of more mature networks seems to be better explained by spontaneous synaptic activity.

# Sound-driven modulation of sub- and suprathreshold activity in mouse primary visual cortex.

Paolo Medini[1]

[1]Fondazione Istituto Italiano di Tecnologia, Neuroscience and Brain Technologies, Via Morego, 30, 16163 Genova, Italy[1]

Integration of multimodal information is essential for the integrative response of the brain and is thought to be accomplished mostly in sensory association areas. However, available evidences in humans and monkeys indicate that this process begins already in primary sensory cortices. However, how cross-modal synaptic integration occurs in vivo along cortical microcircuitries remains to be investigated. Primary sensory cortices of rodents are well-suited to address this issue as they have a well-known anatomy and synaptic physiology.
Here we quantified how acoustic stimulation (white noise, 50 ms duration) affects spontaneous and sensory-driven activity of pyramidal neurons in different layers of primary visual cortex by intrinsic signal imaging-targeted in vivo whole-cell recordings in lightly anesthetized and awake, head-fixed mice. Acoustic stimuli reliably evoked hyperpolarizations - lasting about 200-300 ms - in layer 2-3 and 6 neurons, but not in the main thalamorecipient lamina, layer 4. We found depolarizing responses to sound only in layer 5 (about 1/4 of recorded neurons), whereas the remaining cells exhibited no response or hyperpolarizations.
To explore the synaptic nature of sound-driven hyperpolarizations in supragranular pyramids, we measured the inhibitory and excitatory conductances elicited by sound. Hyperpolarizations were due to the combined effect of activation of inhibitory conductances along with a withdrawal of excitatory ones. In agreement with this, sound-driven hyperpolarizations were significantly reduced by intracellular perfusion with a cesium-based solution containing 1 mM picrotoxin to block GABA-B and GABA-A receptors, respectively (-3,3 ± 0,4 mV vs -1,1 ± 0,3 mV, t-test, $p<0,001$).
We next quantified the impact of sound-driven inhibition on visual responsiveness by coupling flashed or moving light bars with white noise. Sub- and suprathreshold responses were significantly reduced in the case of bimodal stimulation compared to the pure visual modality (of about 30 and 50%, respectively, paired statistics, $p<0.05$).
Finally, transection experiments guided by intrinsic signal imaging indicated that auditory-driven synaptic inputs onto visual cortical neurons persisted despite inactivation of horizontal connections between primary visual and auditory cortices.
Taken together, our findings illustrate a simple scheme by which spontaneous and evoked activity in a retinotopic column of the mouse primary visual cortex can be shaped by the acoustic external environment in a layer-specific manner.

# Spontaneous and evoked population activity in a urethane sleep model

Tim Wanger[1], Kentaroh Takagaki[1], Michael T. Lippert[1], Frank W. Ohl[1,2]

[1]Leibniz Institute for Neurobiology, Auditory Learning and Speech, Brenneckestr. 6, 39118 Magdeburg, Germany

[2] Otto-von-Guericke University, 39106 Magdeburg, Germany

The integrated activity of neuron populations is a useful and informative measure of both behavioral state and of ongoing dynamics in brain activity (Freeman, Mass Action in the Nervous System, 1975). Population activity can be observed on various levels, and observations on the mesoscopic scale have recently received much revived interest. While unanesthetized preparations are ideal for investigating physiological brain states, many methods used to record or image neural mass-action are invasive and challenging under unanesthetized conditions. Furthermore, the unanesthetized brain changes rapidly between many non-discrete states, which further complicates analysis. For these reasons, anesthetized preparations have been and will continue to be invaluable to the investigation of neurophysiology.

Here, we characterize the population activity patterns of the rat cortex in two discrete, reproducible, and stable states, which can be induced by urethane anesthesia. The preparation is highly stable (>48h with proper physiological support) and exhibits a robust and spontaneous alternation between a low amplitude ECoG-desynchronized state and a high amplitude ECoG- synchronized state (Detari et al., Brain Res. 1997 Jun 6;759(1):112-21). This biphasic preparation has been used successfully to study state-dependent changes of unit activity patterns in responses to sensory stimuli (Murakami et al., Neuron. 2005 Apr 21;46(2):285-96.; Curto and Harris, J Neurosci. 2009 Aug 26;29(34):10600 -12). In this report, we characterize evoked and spontaneous patterns of population activity using several experimental modalities: electrophysiologically, optically and neurovascularly. Our data support the proposition that this preparation bears a striking resemblance to natural sleep and awake states (Clement et al., PLoS One. 2008 Apr 16;3(4):e2004.). Analyses of responses to graded visual stimuli and analyses of spatial velocity patterns from spontaneous activity suggest that different cortical networks may be engaged under these two states.

# Suppression of synchronous population activity in developing neuronal networks by targeted stimulation of functional hubs -- a modeling study

Birgit Kriener[1], Carsten Grabow[2], Marc Timme[2,3,4]

[1] Institute of Mathematical Sciences and Technology, Norwegian University of Life Sciences, As, Norway
[2] Network Dynamics Group, MPI for Dynamics and Self-Organization Goettingen, Germany
[3] BCCN Goettingen, Germany
[4] Fakultaet fuer Physik, Georg-August University Goettingen, Germany

A recent experimental study by Bonifazi et al. (2009) showed that the functional topology of developing hippocampal networks has scale-free features, implying that some few neurons, so-called hubs, are heavily connected while most neurons have only a small number of connections. Morphological analysis revealed that these hub neurons are a sub-population of GABA-ergic interneurons with widespread axonal arbors. In the early stages of development GABA acting via GABA_A receptors is known to cause excitatory responses in immature neurons due to a high level of intracellular chloride (Ben-Ari et al. 2007). In an ongoing spontaneous activity regime this excitatory effect of GABA is a key feature in the generation of synchronous population events, so-called Giant Depolarizing Potentials (GDP).
By targeted depolarizing current stimulation of a hub neuron these GDPs are suppressed, while no observable effect is generated by applying the same current step to a non-hub neuron.

Here, we present a biologically inspired network model that accounts for the basic neuron and connectivity properties and reproduces the experimental findings. This allows to analyze the respective roles of topology on the one hand, as well as the electrophysiological properties of neurons and synapses on the other hand in the observed suppression of synchrony.
Moreover, we discuss our findings within the framework of a reduced coupled oscillator model.

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# The effects of spike frequency adaptation on synchronization of coupled oscillating neurons in the presence of conduction delays

Josef Ladenbauer[1], LieJune Shiau[2], Klaus Obermayer[1]

[1]Technische UniversitÃ¤t Berlin, Neural Information Processing Group, Franklinstr. 28/29, 10587 Berlin, Germany
[2]University of Houston, Department of Mathematics, Clear Lake 2700 Bay Area Blvd., Houston, TX 77058

Synchronized oscillating activity in cortical circuits is considered to be fundamental to cognitive function, selective attention and consciousness. Spike frequency adaptation (SFA) has previously been shown to play an important role in the dynamics of cortical networks, promoting stable synchronization of neurons coupled through excitatory synapses when the influence of conduction delays is negligible [1].

In the present study we describe how SFA affects the synchronization properties of coupled pairs of neurons driven to repetitive firing at various frequencies, in dependence of axonal conduction delays. We use the two-dimensional adaptive exponential integrate-and-fire (aEIF) neuron model, considering recurrent excitation and feedback inhibition mediated through conductance based AMPA - and GABA -like synapses. The aEIF model exhibits rich dynamical behavior while being computationally relatively inexpensive, and its parameters can easily be related to electrophysiological quantities [2]. We apply phase response curves (PRCs) and phase reduction theory based on the assumption of weak coupling in order to determine stable in-phase (synchronous) and out-of-phase locked states [3]. The PRC is an experimentally assessable quantity that measures the response of an oscillating neuron to transient inputs received at different phases of its cycle. We calculate infinitesimal PRCs, which purely reflect the neural dynamics, and combine these with biologically plausible synapses, in order to obtain effective PRCs and interaction functions applicable to coupled phase models.

Our analysis predicts inevitable stable phase locking for identical pairs of excitatory or inhibitory cells, independent of the delay. Excitatory-inhibitory pairs on the other hand are more unlikely to phase lock. We further find that SFA favors synchronous spiking if the conduction delays are short compared to the oscillation periods. When delays are increased stable phase locked states shift towards anti-synchrony. We confirm these predictions by numerical simulations of the coupled neuron pairs. Supported by comprehensive parameter explorations, our results emphasize the role of SFA as a mechanism synchronizing neural oscillations and reveal its effectiveness in dependence of conduction delays.

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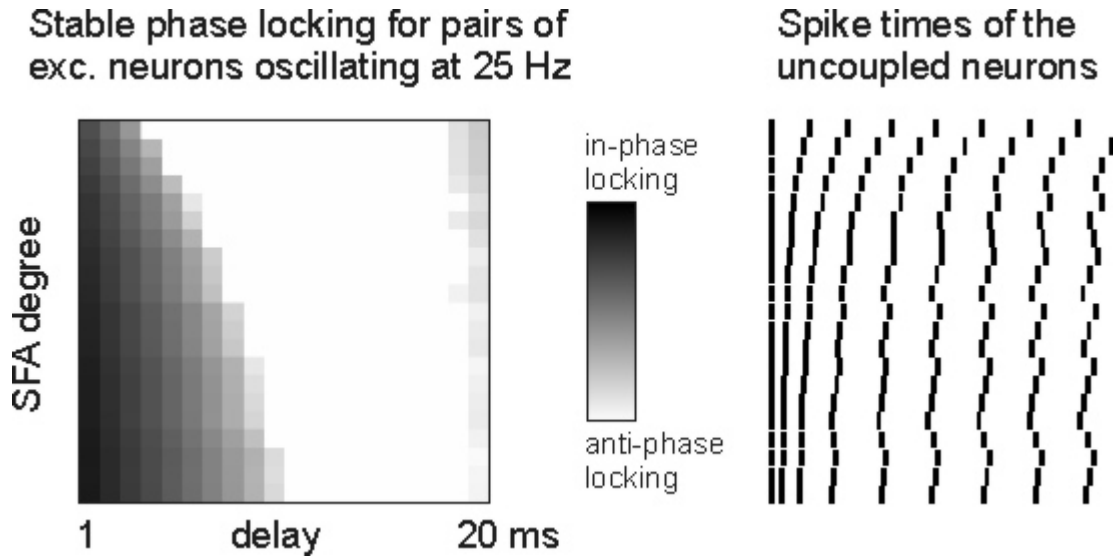
Stable phase locking for pairs of exc. neurons oscillating at 25 Hz
Spike times of the uncoupled neurons
SFA degree
in-phase locking
anti-phase locking
1
delay
20 ms

# The role of TGF-ß2 on the development of neuronal networks

Jan Manuel Speer[1], Eleni Roussa[1], Kertsin Krieglstein[1,2]

[1]Albert-Ludwigs-Universität Freiburg, Department of Molecular Embryology, Albertsstr. 17, 79104 Freiburg, Germany
[2]Albert-Ludwigs-Universität Freiburg, FRIAS, Albertsstr. 19, 79104 Freiburg, Germany

Transforming growth factors-beta2 (TGF-B2) has been indicated to play an important role during development and function of the respiratory neuronal network. By taking advantage of TGF-B2 deficient mice, previous works by this research group demonstrated impaired currents of excitatory glutamatergic and inhibitory GABAergic synaptic transmission in the preBötzinger complex, which is responsible for rhythmic respiratory activity (Heupel et al., 2008). However, a systematic analysis addressing the role of TGF-B2 in synaptogenesis and neuronal function in mammals is still missing.
The formation of functional synapses is a crucial event in neuronal network formation, and with regard to regulation of breathing it is essential for life. The functional or morphological changes in the respiratory center of the brain may cause of the perinatal death of the TGF-B2 knock-out mice.

The aim of the present work is to elucidate the direct modulation of vesicle release mediated via TGF-B2 induced synapsin phosphorylation.
In order to circumvent lethality and investigate development in TGF-B2 deficient mice, we developed primary hippocampal dissociation cultures from mice at embryonic day (E)18.5. Gain-of-function and loss-of-function experiments were performed after 12 days in culture.
We investigated the direct effect of TGF-B2 on synapsin phosphorylation by immunocytochemistry and western blotting. Synapses were identified by using synaptophysin and synapsin as general synaptic markers, vesicular glutamate transporter (vGlut2) as a marker for excitatory glutamatergic presynaptic terminals, and vesicular GABA transporter (vGat) as a marker for inhibitory GABAergic presynaptic terminals. Visualization of recycled synaptic vesicles in vital neurons has been shown by internalized fluorescent dye FM1-43.
Including subsequent rescue experiments using cultures obtained from TGF-B2 deficient mice.
A detailed investigation of impaired synaptic transmission mediated by TGF-B2 is essential. Furthermore this points to the possibility that TGF-B2 could modulate the motility of vesicles through actin-binding proteins resulting in an effect on the transmitter release machinery in all synapses.
Results of this work will contribute to clarify how TGF-B2 is involved in development and function of neuronal networks.

# The spatial phase relationship between oscillations and spikes varies during maturation of the rat prefrontal cortex

Nicole Cichon[1], Michael Denker[2], Marco D. Brockmann[1], Ileana L. Hanganu-Opatz[1], Sonja Gruen[2,3]

[1]Developmental Neurophysiology, Center for Molecular Neurobiology, Falkenried 94, 20251 Hamburg, Germany

[2]Laboratory of Statistical Neuroscience, RIKEN Brain Science Institute, Wako-Shi, 351-0198 Japan

[3]Theoretical Systems Neurobiology, Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM©\6), Research Center Juelich, Germany

The interactions between prefrontal cortex (PFC), hippocampus, and subcortical nuclei within complex neuronal networks are critical for attentional and mnemonic abilities, yet their maturation is poorly understood. We previously showed that discontinuous oscillatory patterns with characteristic spatial and temporal organization synchronize in theta-gamma frequency band the neonatal PFC. With ongoing maturation they are replaced by continuous theta-gamma rhythms. The intricate spatio-temporal relationship of slow theta and fast gamma oscillatory components during various stages of development may reveal changes in the flow of activity within and between the different brain structures, yet experimental proofs are missing.

Here, we analyze the relationship between spikes and local field potential (LFP) oscillations in the theta and gamma frequency band (i) across different layers and (ii) across recording sites in parallel to the cortical surface in the prelimbic region of the P (postnatal day) 6-15 rat PFC. At the end of the first postnatal week the theta bursts lasting on the order of several seconds are accompanied by short gamma episodes that are nested on the theta rhythm. The amplitude of gamma episodes is coupled to the phase of theta oscillations. The preferred phase of coupling between theta bursts and gamma episodes shifts about 45 degrees from upper to lower cortical layers. This phase shift of theta-gamma coupling is due to a phase shift of theta bursts, whereas the short episodes of gamma activity occur simultaneously at different recording sites. Besides highlighting a consistent layer-dependent phase-amplitude coupling between theta and gamma oscillations, we show that the coherency of theta oscillations drops faster across layers than within the layer. In contrast, gamma coherency shows an isotropic distance dependence. Spike occurrences are strongly locked close to the trough of the gamma rhythm. In addition, we find that the neuronal firing rates change as a function of cortical depth and strongly depend on the theta amplitude.

Towards the end of the second postnatal week the theta-gamma activity switches from a discontinuous to a continuous pattern. In contrast to the tight neonatal theta-gamma coupling the strength of the phase-amplitude coupling between theta and gamma oscillations is reduced in juvenile rats. While episodes of gamma activity still emerge simultaneously at different recording sites, their amplitude is hardly modulated by the phase of the theta rhythm. In addition, the phase shift of theta oscillations across prefrontal layers is reduced in juvenile when compared to neonatal rats.

The different phase coupling between theta and gamma oscillations as well as between LFP and spikes during various developmental phases may mirror the reorganization of prefrontal-hippocampal-subcortical networks and/or the maturation of underlying cellular mechanisms.

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# Unravelling the Central Pattern Generator for Cricket Singing

Stefan Schöneich[1], Berthold Hedwig[1]

[1]University of Cambridge, Department of Zoology, Downing Street, CB2 3EJ Cambridge, United Kingdom

Acoustic signalling in crickets represents a classical example of a genetically fixed action pattern driven by a central pattern generator (CPG). For mate attraction male crickets generate species -specific calling songs by rhythmically opening and closing their forewings. A short sound pulse is produced during each closing movement. The pulses are sequentially grouped in the species-specific pattern which matches the sharply tuned auditory recognition mechanism of the conspecific females. Over the last decades the neuronal mechanisms underlying cricket singing behaviour attracted much scientific interest but nevertheless it remained a largely unsolved neuroethological question.

Currently we identify the neuronal components of the singing CPG in the Mediterranean field cricket *Gryllus bimaculatus* by intracellular recording and staining of singing interneurons in the CNS of fictively singing males. Singing behaviour is elicited by microinjection of Eserine into the brain which activates the descending command neurons for calling song. The singing motoneurons are housed in the mesothoracic ganglion and extracellular recording of their activity from a wing nerve monitors the ongoing motor pattern.

We identified local and descending interneurons in the metathoracic and ascending interneurons in the abdominal ganglia as elements of the singing CPG. These neurons discharged in phase with the singing rhythm. Most importantly, perturbing their activity with intracellular current injection modified or reset the motor pattern.

Understanding the cricket singing CPG at the level of identified neurons will not only provide insight into the control mechanisms of a very conspicuous insect behaviour. Linked with a genetic approach this may also reveal which neuronal changes underlie the modification of song patterns during evolutionary segregation of cricket species.

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# Poster Topic

## T24: Attention, Motivation, Emotion and Cognition

T24-1A Aggression, anxiety and serotonin: phenotyping *Tph2*-deficient animals
*Daniel Marius Beis, Valentina Mosienko, Bettina Bert, Heidrun Fink, Michael Bader, Natalia Alenina*

T24-2A Attentional Modulation of Human Spiral Motion Discrimination
*Sepideh Fazeli, Anselm Rothe, Stefan Treue*

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T24-8A Conflict and error processing of the rat:
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T24-9A Decoding visual stimuli from ~40 Hz gamma -band oscillations at their neural representations by surface EEG recordings
*Rafael Polania, Walter Paulus, Michael A Nitsche*

T24-10A Distinct Stages of Emotional Face Processing under Different Task Conditions as Revealed by Independent Component Analysis
*Julian Rellecke, Annekathrin Schacht, Werner Sommer*

T24-11A Dopamine in the dorsomedial striatum supports contingency learning in rats
*Stephanie Braun, Wolfgang Hauber*

T24-12A Effects of physical and social environmental enrichment on 50-kHz ultrasonic vocalizations in rats
*Juan C. Brenes, Rainer K.W. Schwarting*

T24-13A Exploring the Neural Basis of Grapheme-Colour Synaesthesia- a fMRI Study
*Christopher Sinke, Janina Neufeld, Wolfgang Dillo, Hinderk Meiners Emrich, Markus Zedler*

T24-1B Feedback inhibitory control of excitatory signal integration in CA1 pyramidal neurons during theta patterned pyramidal neuron firing.
*Christina Mueller*

T24-2B Forward and backward fear conditioning in rats measured by fear-potentiated startle: Characterization and neural basis.
*Markus Fendt, Stefan Imobersteg*

T24-3B Gating of visual processing by selective attention as observed in LFP data of monkey area V4
*David Rotermund, Udo A. Ernst, Klaus R. Pawelzik, Simon D. Neitzel, Sunita Mandon, Andreas K. Kreiter*

T24-4B Implicit and Explicit Effects of Authenticity on the Perception of Emotional Prosody in Speech
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T24-5B Influence of authenticity on the emotional expression in speech
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T24-6B Local field potentials and spikes in monkey posterior parietal cortex convey independent information about movement plans in a reversing prism task
*Shenbing Kuang, Alexander Gail*

T24-7B Maternal Separation-Induced Histone Modifications in Frontal Cortex of Mice
*Lan Xie, Anna Katharina Braun, Jörg Bock*

T24-8B Motivational influences on effort-based decision making in rats:
The role of dopamine in the nucleus accumbens
*Bettina Mai, Wolfgang Hauber*

T24-9B Neurons in the laberal habenular complex project either to the dopaminergic VTA or to the serotonergic raphe nuclei in the midbrain of the rat
*René Bernard, Rüdiger W. Veh*

T24-10B Optimal time perception with multisensory cues: Dissecting the effects of perceived and performed motion
*Joachim Hass, Stefan Blaschke, J. Michael Herrmann*

T24-11B Pupil dilation reflects unexpected uncertainty: a role for noradrenalin in decision-making
*Wolfgang Einhäuser, Bernard Marius 't Hart, Kerstin Preuschoff*

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*Fingal Orlando Galashan, Hanna Christiane Rempel, Andreas K. Kreiter, Detlef Wegener*

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*Sonia Baloni, Daniel Kaping, Stefan Treue*

T24-1C Subchronic administration of ketamine produces long-lasting cognitive inflexibility in rats.
*Agnieszka Nikiforuk, Piotr Popik*

T24-2C Task-dependent attentional modulation of human direction discriminability.
*Elena Spanou, Stefan Treue*

T24-3C Task-evoked pupillary response in dual-task situations
*Yiquan Shi, Elisabeth Müller, Martin Buss, Erich Schneider, Torsten Schubert*

T24-4C Temporal dynamics of the early posterior negativity (EPN) in emotional verbs and nouns.
*Marina Palazova, Werner Sommer, Annekathrin Schacht*

T24-5C The gradual nature of rivalry
*Stefan Frässle, Marnix Naber, Wolfgang Einhäuser*

T24-6C The neural correlates of coloured music:
A functional MRI investigation of auditory-visual synaesthesia
*Janina Neufeld, Christopher Sinke, Wolfgang Dillo, Hinderk Meiners Emrich, Markus Zedler*

T24-7C The relation between morphological and electrophysiological properties, and topographical allocation of neurons within the rat lateral habenular complex
*Torsten Weiß, Rüdiger W. Veh*

T24-8C The role of dopamine in the dorsomedial striatum in general and outcome-selective Pavlovian-instrumental transfer
*Steffi Mareen Pielock, Björn Lex, Wolfgang Hauber*

T24-9C The saliency of attention-capturing events modulates the duration of exogenous attention
*Anja Lochte, Jonas Krebs, Stefan Treue*

T24-10C The utilization of acoustic landmarks for orientation in humans without vision.
*Daniel Schmidtke, Sarah Galinski, Karl-Heinz Esser*

T24-11C To threat or to punish – what makes the difference
*Sylvia Richter, Ulrike Kraemer, Catherine Libeau, Anna Deibele, Adriana Barman, Constanze Seidenbecher, Bjoern Schott, Thomas F. Muente*

T24-12C Ultrasonic vocalizations throughout the rat´s lifespan: effects of strain, sex, and testing conditions
*Claudia Natusch, Alexandra Lewik, Rainer K W Schwarting*

T24-13C Very Early Emotion Effects for Positive Words in Event-related Brain Potentials
*Mareike Bayer, Werner Sommer, Annekathrin Schacht*

T24-14C Spatial representation of dynamic objects within working memory in a collision avoidance task
*Gregor Hardiess, Thomas Müller, Stephan Storch, Hanspeter A. Mallot*

# Aggression, anxiety and serotonin: phenotyping *Tph2*-deficient animals

Daniel Marius Beis[1], Valentina Mosienko[1], Bettina Bert[2], Heidrun Fink[2], Michael Bader[1], Natalia Alenina[1]

[1]Max Delbrück Center for Molecular Medicine (MDC), Berlin-Buch, Germany
[2]Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Free University Berlin, Germany

Clinical and experimental investigations indicate a broad involvement of serotonin (5 -HT) in mental health. Tryptophan hydroxylase 2 (TPH2) is the rate-limiting enzyme in serotonin synthesis in the central nervous system (Walther et al., 2003). We recently created a *Tph2*-deficient mouse model ( *Tph2*$^{-/-}$) which lacks 99% of brain serotonin. *Tph2*$^{-/-}$ mice display alterations in autonomic functions, such as breathing, thermoregulation and cardiovascular control, but no disruption of brain anatomy or severe stereotypes are visible in these animals (Alenina et al., 2009). Using *Tph2*-deficient mice as a model with altered brain serotonin we investigated the influence of 5-HT on aggression and anxiety-like behavior.

The anxiety-like behaviour was assessed in three different tasks: elevated plus maze (EPM), marble burying test, and novelty suppressed feeding (NSF). In all three tasks *Tph2*$^{-/-}$ mice displayed behavior associated with reduced anxiety: they spent more time in the open arm of the EPM, buried less marbles, and exhibited reduced latency to eat in the NSF in comparison to control mice.

Furthermore, mice deficient in central serotonin have an elevated level of aggression. In the residence-intruder test they showed a decrease in latency to attack an intruder, increased number of attacks, as well as attack duration. Interestingly, this aggressive phenotype was also prominent in female *Tph2*$^{-/-}$ mice, which were often fighting if sitting together in one cage.

Moreover, monitoring of female behavior showed that *Tph2*$^{-/-}$ mothers despite being fertile and producing milk have impaired maternal care leading to less then 45% survival of the offspring. Further analysis showed that *Tph2*$^{-/-}$ mothers have an impaired ability to collect scattered pups in the pups retrieval test and used to cannibalize their offspring at much higher frequency as wild type animals. However, *Tph2*-deficient mothers exhibit normal olfaction, detecting and differentiating different odors in buried food and olfaction habituation/dishabituation tasks, indicating that other mechanisms are responsible for the alteration in maternal instincts in *Tph2*-deficient mice.

Our findings suggest an important role for serotonin signaling in maternal instinct, anxiety- and aggressive-like behaviors.

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# Attentional Modulation of Human Spiral Motion Discrimination

Sepideh Fazeli[1,2], Anselm Rothe[1], Stefan Treue[1,2]

[1]Cognitive Neuroscience Laboratory, German Primate Center, Goettingen, Germany
[2]Bernstein Center for Computational Neuroscience, Goettingen, Germany

The human visual system has to deal with large amounts of incoming information. For example, when moving through the environment, it has to interpret a complex input of optical flow patterns. Dealing with this barrage of information is impossible without a mechanism for attentional selection. Not much is known about the effects of attention on the perception of optic flow patterns. Spiral motion patterns are an important member of the general class of optic flow patterns. In monkeys, neurons in the medial superior temporal area are tuned to spiral motion stimuli and in humans selectivity for these patterns has been observed in fMRI studies in a region adjoining the middle temporal area.

Here we investigated the role of attention in the discrimination of spiral motion patterns by human subjects. Subjects had to report whether a brief spiral motion pattern contained clockwise or counterclockwise motion. We systematically varied the strength of the rotational component to determine the direction discrimination threshold. To examine the effects of attention, this threshold was measured in three different task: a single task, a dual task, and a Posner paradigm.

In all three conditions, subjects first fixated a central fixation point on a computer screen. In the single task and the Posner paradigm, a small square (the cue) then appeared for 470 ms at one of two possible locations chosen randomly on each trial. One location lay to the left, the other to the right of the fixation point. After 470 ms, two stimuli appeared on the screen, one at the cued location and the other at the uncued location. One stimulus was a task-relevant probe stimulus that consisted of an expanding or contracting spiral motion for 106 ms which was was followed by a mask (randomly moving dots for 235 ms), while the other stimulus was a distracter patch containing randomly moving dots throughout the 341 ms.

In the single task condition the cue always correctly signaled the location of the probe stimulus. In the Posner paradigm, in 80% of trials, the probe stimulus appeared at the cued location. In the remaining 20% of trials, the stimulus locations were reversed. Finally, in the dual task condition, two spatial cues were presented simultaneously for 470 ms, one at each of the two locations. The cues were followed by two probe stimuli, one at each location. There were no distracter stimuli in this condition. Subjects were asked to report the direction of spiral motion for both probe stimuli.

Our results indicate a clear pattern for the effect of task condition on discrimination performance. Average discrimination thresholds were lowest for the single task (6.5 and 8.8 degree for expanding and contracting spirals respectively), higher for the validly cued location in the Posner task (7.6 and 10.1) and highest for the dual task (12 and 15.5) and the invalidly cued location in the Posner task (13.7 and 14.6). Our results are qualitatively consistent with an attentional strategy where the subjects allocated the greatest amount of attention to the cued location in the single task (where the cue correctly signaled the probe location on all trials), less attention to the cued location in the Posner task (where the cue was only valid on 80% of trials) and the least attention in the dual task (where the cue was not relevant).

Our results suggest that attention substantially affects the ability of humans to accurately perceive spiral motion stimuli.

The project was supported by a grant (01GQ0433) of the German Federal Ministry of Education and Research to the Bernstein Center for Computational Neuroscience, Goettingen.

# Attentional signals in macaque area MT show directional tuning during a working memory period

Vladislav Kozyrev[1], Anja Lochte[1,2], Stefan Treue[1,2]

[1]German Primate Center, Cognitive Neuroscience Laboratory, Goettingen, Germany
[2]Bernstein Center for Computational Neuroscience, Goettingen, Germany
[3]Institute for Neuroinformatics, Ruhr-University Bochum, Bochum, Germany

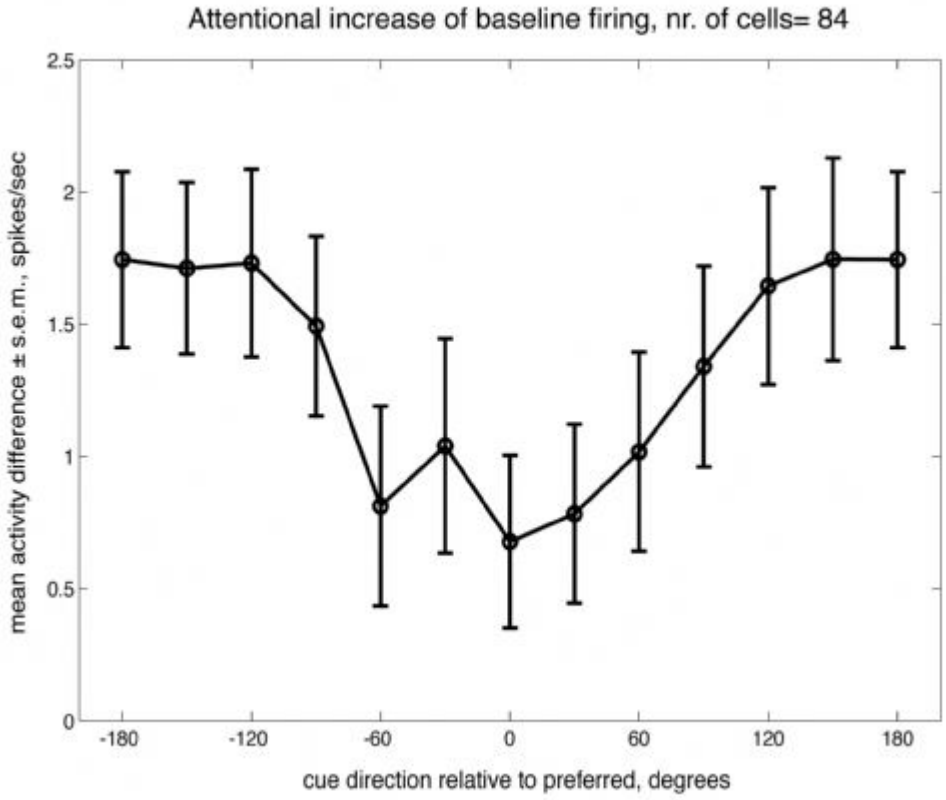
Attention is the ability to focus the brain's processing resources onto relevant sensory information. This can be based on sensory cues but they might only be present briefly. Therefore a short-term storage mechanism, often referred to as working memory, is required to allocate attention based on previous sensory information. Some current models of attention (such as the biased competition model of Desimone et al.) assume that attention directly modulates stimulus representations. In such models an attentional modulation without a stimulus is difficult to account for. Other models (such as the feature similarity gain model) argue that attention modulates neuronal gain; in this system an attentional signal could modulate neuronal responses even in the absence of a stimulus provided.

Here, using an attentionally demanding task, we investigated the responses of single neurons from the middle temporal area (MT) of two rhesus monkeys during working memory periods. The monkeys were trained to either passively fixate on a spot in the center of a screen or detect a change of motion (direction or speed) in one of four random dot patterns either inside the receptive field (RF) or far outside the RF. In every trial the currently relevant stimulus (the target of attention) was specified by a brief (600 ms) cue that appeared at the same location and moved in the same direction as one of the random dot patters appearing later. The direction of motion of the cue was systematically varied in 30 deg. steps. The cue and the following attention period were separated by an 800 ms delay, when no stimulus (except for the fixation spot) was visible. The animal had to maintain the cued information in memory to guide its attentional allocation when the stimuli appeared.

The recorded neurons showed robust direction-selective responses to the motion cue corresponding to the sensory tuning of those neurons. After the cue offset, the firing rates dropped within the first 150-200 ms of delay to a level which was usually higher than the spontaneous activity present in the passive fixation trials, i.e. when no cue had been presented. For each direction of the cue, we averaged the neuronal firing rate within 200-800 ms after the cue offset and subtracted the spontaneous activity from it. The resulting firing rates averaged across 84 cells (attention inside the RF), aligned to the preferred direction of each cell are presented in the Figure. The data show a clear directionality, with the smallest response around the preferred direction (0° along the x-axis), i.e. neuronal responses during the working memory period were significantly higher for directions close to the anti-preferred. Directional signals showed a similar trend when attention was directed outside the RF, providing further support for a global feature-based mechanism of attention.

We cannot completely rule out adaptation mechanisms, which might explain the observed effects. But since our cue was presented for a very brief period of time compared to the values used in typical adaptation studies (Van Wezel & Britten, 2002; Kohn & Movshon, 2004) and because of the similarity between the results obtained when directing attention inside and outside the RF, adaptation is not likely to be a major contributor. Rather, the MT activity during the memory period may be indicative for the involvement of sensory areas in short-term information storage (Zaksas & Pasternak, 2006).

The project was supported by a grant (01GQ0433) of the German Federal Ministry of Education and Research to the Bernstein Center for Computational Neuroscience, Goettingen.


Attentional increase of baseline firing, nr. of cells= 84
mean activity difference ± s.e.m., spikes/sec
2.5
2
1.5
1
0.5
0
-180
-120
-60
0
60
120
180
cue direction relative to preferred, degrees

# Attention-dependent dynamic changes in coherence between monkey area V1 and V4

Iris Grothe[1], Simon D. Neitzel[1,2], Sunita Mandon[1,2], Andreas K. Kreiter[1,2]

[1]University of Bremen, Bernstein Group for Computational Neuroscience, Hochschulring 16a, 28359 Bremen, Germany
[2]Brain Research Institute, Center for Cognitive Sciences, University of Bremen, 28334 Bremen, Germany

Attention is the mechanism which enhances the processing of a certain object or feature at the cost of reduced processing of others. It thereby allows for selective analysis of behaviorally relevant objects out of a complex (visual) scene. The need for such a mechanism becomes evident already in early stages of cortical information processing: Visual areas provide converging afferent projections towards downstream processing stages. This anatomical wiring results in visual areas like V4 having neurons with larger receptive fields (RFs) than upstream areas. In such large RFs multiple different objects can be present. Next to signals representing the attended object, a potentially much larger number of signals representing independent and behaviorally irrelevant objects provide input to a V4 neuron. Neurons in V4 therefore need a fast and reliable mechanism for changing their effective connectivity with both their input and target areas in order to dynamically route the ever-changing behaviorally relevant information.
Previous experimental work (Taylor et al., 2005) has shown a tight relation between selective attention and enhanced synchronous gamma-band activity in local populations of V4 neurons, providing evidence for a functional role of gamma-band synchronization as a mechanism of attentional modulation. Here we hypothesize that attention not only modulates synchronization within a local neuronal population, but also modulates the coherence between a target population and its input populations of neurons.
To investigate the dynamic coupling of neurons in V4 with its afferent neurons, we acquired single unit activity (SUA), multi unit activity (MUA) and local field potentials (LFP) from simultaneous recordings with multiple electrodes from macaque monkeys' areas V1 and V4. The monkeys were performing a shape-tracking task, where they had to attend to one of two closely spaced morphing shapes. Size and position of the shapes were adjusted such, that both fitted into a single V4 RF while covering two separate, non-overlapping V1 RFs.
Spike triggered averages (STAs) revealed that spikes in V4 can be precisely timed with respect to the phase of the gamma oscillation in the V1 LFP. This demonstrates convincingly that precise synchronization between a V4 neuron and the group of V1 neurons carrying its afferent signals is possible. Moreover, the STA was strongly attention dependent. With attention directed to the stimulus covering the RF of a V1 recording site a clear increase in the V4-V1 coherence was present. When the attention of the monkey was switched to the other stimulus, while visual stimulation remained the same, the pattern of coherence switched accordingly: the same V1 site representing the now unattended stimulus shows strongly reduced or disappearing coherence with the V4 neuron.
These results are compatible with the hypothesis that attention can induce dynamic changes in the effective connectivity between a V4 population of neurons and its anatomically hard-wired input populations by means of selectively modulating coherence.

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# Auditory Stream Segregation of SAM Tones: Psychoacoustics and fMRI

Lena-Vanessa Dollezal[1], Susann Deike[2], André Brechmann[2], Georg M. Klump[1]

[1]Animal Physiology and Behaviour Group, IBU, Carl-von-Ossietzky University Oldenburg, D-26111 Oldenburg, Germany

[2]Leibniz Institute for Neurobiology, Brenneckestr.6, D-39118 Magdeburg, Germany

The process of auditory stream segregation refers to the ability of the auditory system to separate signals into separate auditory streams or integrate them into one auditory stream according to their acoustic characteristics (Moore & Gockel 2002). Many studies so far have evaluated the influence of spectral cues on sequential streaming. During the last years also the question of whether signals with the same spectral excitation but different temporal structure would elicit stream segregation has come into focus (e.g., Roberts et al. 2002, Grimault et al. 2002).

Here, we used sinusoidally amplitude- modulated (SAM) tones. The SAM tones were presented in the ABA- stimulus paradigm (125 ms tone duration; the - indicates a silent interval of the same duration as the tone duration). The carrier frequency was 1000 Hz, the modulation depth was 100 % and up to three different reference modulation frequencies of the A tone (30 Hz, 100 Hz or 300Hz) have been presented. The modulation frequency of the B tone was adjusted relative to that of the A tone to evoke a one stream, a two streams or an ambiguous percept, respectively. In the ABA- stimulus paradigm subjects perceive a galloping rhythm if they integrate A and B signals into one stream and two streams with an isochronous rhythm if the segregate A and B signals.

Presenting these SAM tones we are able to analyse both the influence of temporal structure and the influence of spectral excitation on the auditory streaming effect. The pattern of spectral excitation elicited by the SAM tone is affected by both the carrier frequency and the modulation frequency. The temporal pattern of excitation is mainly affected by the modulation frequency. The bandwidth of the auditory filters tuned to the spectral components of the stimuli play a role in the encoding of spectral and temporal cues.

In psychoacoustics stream segregation can be determined either by asking the subject to report their subjective perceptual state (i.e., gallop or isochronous rhythm percept), or by using the "drunken horse" paradigm. The latter measures the detection of an irregularity in the rhythm due to a temporal shift of the B signal which is easier if perceiving a galloping rhythm (e.g., Cusack & Roberts, 2000). For evaluating the physiological correlate of auditory streaming, the SAM-tones in the ABA- sequence were presented to subjects in an fMRI experiment. The percept evoked in the subjects was related to their cortical activity measured in the BOLD-response.

In the subjective task perceptual stream segregation increases significantly with increasing modulation frequency difference between the A and the B SAM tones. Furthermore, in the "drunken horse" paradigm a significant improvement in the shift-detection threshold of the B tone is observed if the presented ABA- sequence evokes a one stream percept rather than a two stream percept. The results of the fMRI experiment showed a graded BOLD response with lowest for the one stream, intermediate for the ambiguous and highest amplitude for the two streams percept. Similar to the results of the psychoacoustical measurements, this gradient may reflect the increasing differences in modulation frequencies of the A and B SAM tones. Significant differences in BOLD responses were observed in the left and right planum temporale, left Heschl's gyrus and right middle frontal gyrus.

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# Cognitive deficits and their compensation following occlusion of the anterior cerebral artery in rats (Rattus norvegicus): A behavioural PET study

Elena Höfener[1], Rudolf Graf[1], Christiane Kleineberg[1], Christine Marx[1], Heike Endepols[1]

[1]Max-Planck Institut für Neurologische Forschung, Mulimodal Imaging Group, Gleulerstraße 50, 50931 Köln, Germany

The anterior cerebral artery (ACA) supplies the frontal and cingulate cortex as well as other parts of the limbic system in both humans and rats. In humans, focal cerebral ischemia in the ACA territory causes severe cognitive, emotional and mnemonic dysfunctions. However, little is known about brain mechanisms leading to cognitive deficits and subsequent recovery after ACA occlusion (ACAo). We therefore assessed ACAo-induced cognitive impairments in a rat model. To directly monitor metabolic activity in brain regions during a cognitive task, and to show behavioral deficits as well as metabolic losses in the ACA territory after ACAo, we combined behavior with positron emission tomography (PET) using the tracer [18F]fluorodeoxyglucose (FDG).

Cognitive functions were studied before and after stroke with the attentional set-shifting task (ASST) in an operant chamber. The ASST included different discrimination sub-tasks in which the animal had to focus on the relevant stimulus feature while ignoring another perceptual dimension to obtain food reward (e.g. visual, but not auditory stimulus dimension was rewarded). The sub-tasks (single discrimination, compound discrimination, and reversals) took place before the actual set-shifting and were necessary for the animals to establish the first attentional set. This was followed by an intradimensional shift (ID) where rats had to discriminate a novel stimulus pair of the previously relevant perceptual dimension, and an extradimensional shift (ED) where the relevant stimulus dimension changed.

Before and after ACAo, the ASST was combined with FDG-PET. Metabolic activity during set-shifting was measured in different brain areas and compared to activity during a control task, where no attentional shift was required.

Before ACAo (N=8), FDG-PET revealed brain areas associated with set-shifting reflected by increased metabolic activity in the infralimbic cortex,and dorsomedial striatum, which is in line with previous lesion studies highlighting the importance of the prefrontal cortex for set-shifting.

After ACAo (N=5), by tendency, behavioural data indicate impaired discrimination ability in different ASST sub-tasks which might be due to problems in maintaining an attentional set. However, present analysis steps of behavioral data did not reveal set-shifting impairment. Furthermore, increased reactions times suggest a motivational deficit following ACAo.

Structural MRI confirmed ischemic lesion in the ACA territory, which showed decreased metabolic activity during behavioral PET. Increased metabolic activity in the left and right hippocampus after, but not before ACAo, was associated with set-shifting. This indicates that hippocampal activity (outside ACAo territory) might be related to compensatory function. To conclude, despite lesions within the prefrontal cortex, ACAo did not induce set-shifting impairment which might be due to deficits in maintaining an attentional set or compensatory activity of areas outside ACA-territory. Furthermore, the combination with FDG-PET has been established as suitable tool to measure metabolic activity during cognitive tasks in rats.

# COMPETITION AND ATTENTIONAL SELECTION OF THREATENING FACES IN SOCIAL ANXIETY - EVIDENCE FROM STEADY-STATE VEPs

Matthias J Wieser[1], Lisa M McTeague[2], Andreas Keil[2]

[1]University of Würzburg, Department of Psychology
[2]University of Florida, Center for the Study of Emotion and Attention

Numerous studies have shown that emotional stimuli attract attention and enhance perception. This is especially true if the stimuli hold a special relevance for the observer like phobic stimuli. In ERP studies, where single faces were shown without any tasks, results point to a selectively enhanced processing of threatening faces. Complementing ERP research, steady-state visual evoked potentials (ssVEPs), which are defined by a resonant oscillatory response of visual cortex to repetitive stimulation, offer an avenue to observing electro-cortical facilitation as an index of enhanced attentional engagement. Using frequency tagging (i.e. simultaneously flickering multiple stimuli at multiple frequencies), it is possible to separate visual cortical activity to spatially overlapping stimuli. Thus, attentional selection can be tested even for the critical conditions in which multiple stimuli compete for attention resources. In the present study, a change detection task array was spatially overlaid over a facial expression (angry, happy, neutral), each flickering at a different frequency (15 vs. 20 Hz), while high-density EEG was recorded from 256 sensors. 17 low and 17 high socially anxious participants were investigated. Socially anxious participants showed only slightly enhanced ssVEP amplitudes to angry faces. However, the signal strength elicited by the change detection task array was reduced during the concurrent presentation of angry faces, which points at competition effects of threatening faces in social anxiety. No impairments were observed in accuracy of the change detection task. It is suggested, that phobia-relevant stimuli draw attentional resources when competing for attention resources, but competition is critically dependent on the stimulation of the same population of neurons (most likely of the V1, as beamformer source localization suggests).

# Conflict and error processing of the rat: a microPET and ERP study

Christine Marx[1], Markus Ullsperger[1,2], Rudolf Graf[1], Heike Endepols[1]

[1]Max-Planck-Institute for Neurological Research, MMI, Gleueler Straße 50, 50931 Cologne, Germany
[2]Donders Institute for Brain, Cognition and Behaviour Radboud University, Montessorilaan 3
6525 HR Nijmegen, The Netherlands

The Simon test is a neuropsychological interference task, which is commonly used to study conflict processing in humans. Response conflict occurs when the stimulus implies a certain response while the spatial characteristics of stimulus presentation indicate a different one. This conflict leads to longer reaction times and higher error rates (Simon effect). Functional imaging, neuropsychological, and electrophysiological studies suggest the involvement of the anterior cingulate cortex (ACC) in response conflict monitoring and error monitoring (Ridderinkhof et al., 2004). The ongoing monitoring of correct and incorrect behaviour is reflected in neural activity which can be recorded in form of event-related potentials (ERPs). Especially the error-related negativity (ERN) component of the ERP, assumed to be generated in the ACC, is suggested to reflect conflict and error monitoring (Falkenstein et al., 1991).
The aim of this study is to shed light on conflict and error monitoring in rodents with the help of two different techniques: temporal resolution through ERPs and spatial resolution through microPET imaging.
Imaging:
Four Lister hooded rats were used to monitor metabolic brain activity with positron emission tomography (PET) during four auditory Simon tasks: $T_I$ (only conflict trials), $T_C$ (only non-conflict trials), $T_R$ (conflict and non-conflict trials 50% each), and $T_N$ (without spatial dimension) as control. As a tracer we used [18F]fluorodeoxyglucose (FDG), which accumulates in active cells and therefore allows PET imaging after the rat has completed the cognitive tasks. We observed a robust Simon effect including sequential modulation. Both a subtractive and a correlative analysis of the PET data revealed specific activation patterns for different task settings. Enhanced metabolic activity during response conflicts was found in the prelimbic and anterior cingulate cortex, suggesting that in the rat, as in humans, conflict monitoring and resolution takes place in the prefrontal cortex.
Electrophysiology:
Our imaging results are concordant with the generally assumed prominent role of the ACC in the literature. Therefore we focused our further examinations on this brain region. While rats conducted an auditory Simon task, local field potentials (LFPs) were recorded using six stainless steel depth electrodes, three of which were placed in the left and three in the right ACC. Nine rats performed four different tasks with different levels of conflict: $T_{50I}$ with 50% conflict trials, $T_{20I}$ with 20% conflict trials, $T_{80I}$ with 80% conflict trials and $T_{20/20}$ with 20% conflict and 20% non-conflict trials and 60% trials without spatial dimension. The recorded LFPs were time locked for tree different time points: 1) stimulus locked, 2) reaction locked and 3) reward locked. We found robust and sustained differences in the modulation of the ERP depending on correct or incorrect responses starting at the time of response and prior to reward/no reward. Differences and similarities between human and rat ERPs will be discussed with focus on error processing and the possibility of the existence of an ERN in rats.

Ridderinkhof KR, Ullsperger M, Crone EA, Nieuwenhuis S (2004) The role of the medial frontal cortex in cognitive control. Science 306:443-447.
Falkenstein, et al. (1991). Effects of crossmodal divided attention on late ERP components. II. Error processing in choice reaction tasks. Electroencephalogr Clin Neurophysiol, 78(6), 447-455.

# Decoding visual stimuli from ~40 Hz gamma-band oscillations at their neural representations by surface EEG recordings

Rafael Polania[1], Walter Paulus[1], Michael A Nitsche[1]

[1]University of Göttingen, Clinical neurophysiology, Robert Koch Str 40, 37075 Göttingen, Germany

Sensing and perceiving involves patterns of activity being synchronized across large numbers of neurons in the human cerebral cortex, before, during and after stimulus presentation but with differing spatiotemporal patterns. Particularly, EEG activity in the gamma frequency (~40 Hz) band has been identified to play a key role in various perceptual and cognitive processes. In the current study we tested the hypothesis that gamma-band activity in humans reveals neural patterns that encode the representation of individual stimuli using non- invasive EEG recordings.

To this end, we have developed a novel method in order to decode patterns of relatively high frequency neural activity (>20 Hz) by means of non- invasive EEG recordings. Four healthy volunteers with normal vision or corrected to normal by glasses (age 22- 30 years) were included in the study. Subjects maintained fixation on a small white cross (subtending 1.5° of visual angle) displayed on a black background at the center of the screen. After 1.5- 2.0 s, a target stimulus (1.8°) was presented – letter T, letter L, letter T rotated 180° or letter L rotated 180° – at the center of the screen for 80 ms. Immediately after the target stimulus, a mask stimulus formed by all possible line segments forming the letter stimulus L or T (and their 180° rotated versions) was presented to interrupt visual processing of the target shape. The subject's task was to maintain fixation throughout the trial, pay attention to the presented stimuli, and discriminate as fast as possible the shape of the target by pressing the button under the right index finger when they saw the letter T (upright or rotated), and the button under the right middle finger when they saw letter L (upright or rotated). Visual stimuli time series from dipoles placed on the cerebral cortex, filtered in the gamma -band, were transformed to high dimensional binary arrays by use of nonlinear dynamics. Applying Bayes decision theory adapted to bit-level features, we were able to discriminate a set of visual stimuli largely above chance level about 100-450 ms after stimulus onset. These gamma-band patterns were decoded from dipoles located in visual areas V1-V2, which suggested that we were decoding bottom-up information processing. We carried out further analyses, where we demonstrate that beta-band activity (13-30 Hz) in the medial parietal cortex might encode top-down information processing, whose oscillations can be decoded as well. Thus, our results are in principle accordance with the hypotheses postulated by Engel, Fries and Singer (Engel & Fries 2010 Curr Opin Neurobiology; Engel, Fries, Singer 2001 Nat. Reviews) which states that gamma- and beta-band oscillations mediate bottom-up and top-down information processing respectively in the human brain. Hence, our results support the concept that the temporal coincidence of specific brain activities generates the functional states that characterize human information processing (Llinas 1998, R Soc Lond), which we show are possible to be decoded. Additionally, we believe that the novel method presented here is a promising approach in applications such as brain computer interfaces (BCIs), where intuitive and rapid communication might be restored by using neural correlates of specific human cognitive representations.

# Distinct Stages of Emotional Face Processing under Different Task Conditions as Revealed by Independent Component Analysis

Julian Rellecke[1], Annekathrin Schacht[1 2], Werner Sommer[1]

[1]Humboldt-Universität zu Berlin, Department of Psychology - Biological Psychology, Rudower Chaussee 18, 12489 Berlin, Germany
[2]University of Goettingen, CRC Text Structures - JRG Experimental Psycholinguistics, Nikolausberger Weg 23, 37073 Goettingen, Germany

Studies on the time course of emotional stimuli processing have consistently revealed two components: The Early Posterior Negativity (EPN) and the Late Positive Complex (LPC) – believed to reflect distinct successive processing stages. While the EPN has been referred to increased early perceptual encoding, the LPC has been reasoned to reflect higher cognitive operations following processes associated with the EPN. However, challenging the conceptualization of serial processing stages, the EPN and the LPC have also been reported in partly overlapping time windows. Thus, by employing Independent Component Analysis (ICA) we attempted to separately assess the development of both the EPN and LPC over time. ICA results revealed the EPN and LPC to be temporally overlapping, indicating parallel rather than serial processing stages. Such pattern indicates that processing of emotional stimuli occurs in parallel at perceptual and cognitive levels.
A further aim of our study was to investigate task-independency of both components. As faces are thought of as stimuli that are processed largely automatically, different facial expressions (angry, happy, neutral) were used as critical stimuli and presented under different task conditions. Overall, angry faces showed largest emotion effects; effects of task where only present for the LPC but not for the EPN. This suggests that increased perceptual encoding of angry expressions is a largely automatic process, whereas more cognitive stages of emotional face processing are subject to the observer's intentional state.

# Dopamine in the dorsomedial striatum supports contingency learning in rats

Stephanie Braun[1], Wolfgang Hauber[1]

[1]Universität Stuttgart, Biologisches Institut, Abteilung Tierphysiologie, Pfaffenwaldring 57, 70569 Stuttgart, Germany

It is well known that animals not only can encode the causal relationship between actions and their consequences but also detect changes in the causal efficacy of their actions. For instance, in instrumental conditioning, post-training degradation of one of two action-outcome contingencies selectively reduced performance of the action that is no longer causal to the delivery of a particular reward. Such a flexible control of actions in the pursuit of goals is crucial for adapting goal-directed behaviour to changing environments.
Recent studies suggest that the capacity to detect changes in the causal efficacy of actions is mediated by a number of brain areas including the dorsomedial striatum. For example, functional magnetic resonance imaging in humans revealed that neural responses in the dorsomedial striatum were modulated as a function of the contingency between the rate of button pressing and the amount of money earned [1]. Furthermore, a particular subregion of the dorsomedial striatum seems to be critical in mediating the sensitivity to changes in the instrumental contingency [2]. Studies in rats revealed that cell body lesions of the posterior, but not of the anterior part, of the dorsomedial striatum produced insensitivity to contingency degradation. Likewise, a dopamine (DA) depletion of the posterior part of the dorsomedial striatum (pDMS) produced an insensitivity to contingency degradation [3]. However, it is yet unclear whether pDMS DA depletion renders animals partially or completely unable to detect changes in the causal efficacy of their actions. Here, sham controls and rats with pDMS DA depletion were examined using contingency degradation protocols that differed in terms of complexity and duration. Results revealed that sham controls and animals with pDMS DA depletion both were sensitive to pronounced changes of action-outcome contingencies. However, unlike sham controls, animals with pDMS DA depletion failed to detect changes of action-outcome contingencies, if presented only in a few trials.
Taken together, our present findings suggest that DA signalling in the dorsomedial striatum is important for rapid detection of changes in the causal efficacy of actions and their consequences. Thus, striatal DA is a critical neurochemical substrate for adapting goal-directed behaviour to changing environments.

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# Effects of physical and social environmental enrichment on 50-kHz ultrasonic vocalizations in rats

Juan C. Brenes[1], Rainer K.W. Schwarting[1]

[1]Philipps-University of Marburg, Experimental and Physiological Psychology, Gutenbergstr. 18, 35032 Marburg, Germany

Enriched environments, i.e. housing conditions providing a combination of enhanced social relations, physical exercise and non- social stimuli, are used to study relationships between behavior and experience -dependent changes in the brain. These specific manipulations of the postnatal social environment in rats can result in chronic developmental changes, such that, when studied in terms of behavior and physiology under laboratory test conditions they provide important psychobiological models. At the behavioral level, it has been shown that rearing laboratory animals in a complex environment reduces anxiety, accelerates habituation, enhances several cognitive functions, and improves stress-coping abilities. However, behavioral studies have focused on cognitive rather than on emotional effects of enrichment. On the other hand, it is well-known that rats emit ultrasonic vocalizations (USVs) with different affective and social communicative functions depending on age and context. In adolescent and adult rats two types of USVs have been differentiated: the 22-kHz calls emitted as an alarm sign and in aversive situations like social defeat or fear conditioning; and the 50-kHz calls elicited by positive appetitive stimuli and in situations aimed to promote, maintain, or reestablish positive social encounters. In our knowledge, there is no formal study aimed to investigate the effect of environmental enrichment on USVs in rats. Although 50-kHz calls could possibly gauge a positive emotional subjective state in the rat, the question of whether improving the welfare of the laboratory animals can affect the utterance of 50- kHz calls has not yet been addressed. Therefore, in the present study male Wistar rats were reared postnatally from day 32 on: 1) physical enrichment, 2) social enrichment, 3) physical-social enrichment, or 4) standard housing for approximately 40 days. The effect of housing conditions was evaluated through three different paradigms: on social spontaneous calling due to a transient separation from congeners, on behavioral response to playback of 50-kHz calls, and on amphetamine-induced appetitive USVs. In addition, spontaneous open-field habituation and object recognition memory were measured as behavioral markers of the enrichment effect throughout the housing period. Evidence about the likely differential effect of social and physical enrichment on several USVs features and call subtype (Flat vs. frequency-modulated calls), and on habituation and memory parameters will be provided.

# Exploring the Neural Basis of Grapheme-Colour Synaesthesia- a fMRI Study

Christopher Sinke[1], Janina Neufeld[1], Wolfgang Dillo[1], Hinderk Meiners Emrich[1], Markus Zedler[1]

[1]Hannover Medical School, Clinic for Psychiatry, Social Psychiatry and Psychotherapy, Carl-Neuberg-Str 1, 30625 Hannover, Germany

Synaesthesia is a condition in which stimulation in one sensory modality leads to an involuntary perception in another unrelated (and unstimulated) modality. For example music can elicit taste, colour or shape. But not only couplings between modalities, but also within a modality are observed. In the most investigated form of synaesthesia, grapheme -colour synaesthesia, achromatic letters and numbers are perceived in certain colours. Psychopysiological and neuroimaging experiments have shown that this condition is real and not an illusion of the affected people. The letter-colour pairings are stable over time and the colour is automatically triggered. However, when looking at fMRI studies on grapheme-colour synaesthetes one finds quite heterogeneous results. For example have some studies found activation in the colour center V4 while others didn't find more activation there. This may be due to the fact that most studies had only a few subjects (<=8).

In the present fMRI study 19 grapheme -colour synaesthetes and 13 non-synaesthetic controls are presented with letters and pseudo-letters. In the within-group comparison letters compared to pseudo-letters activate a larger network in synaesthetes than in non- synaesthetic controls, involving frontal and parietal regions. In the between-group comparison, synaesthetes show higher activation in the right and left inferior frontal gyrus and in the left inferior parietal lobule. Neither the within - nor the between group comparisons show additional activation in the color center V4 or other visual areas. Thus grapheme -colour synaesthetes show greater activation in fontal and parietal cortex, but not in occipital cortex. This may be a hint that grapheme-colour synaesthesia is not so much a sensory but more a cognitive phenomenon.

# Feedback inhibitory control of excitatory signal integration in CA1 pyramidal neurons during theta patterned pyramidal neuron firing.

Christina Mueller[1]

[1]University of Bonn Medical Center, Department of Epileptology, Sigmund-Freud Strasse 25, 53127 Bonn, Germany

[2]Research group Dendritic Integration in the CNS, Department of Epileptology, University of Bonn Medical Center

[3]Deutsches Zentrum fuer Neurodegenerative Erkrankungen e.V (DZNE), Bonn

Recurrent inhibitory loops control excitability in the CA1 subfield of the hippocampus. Yet, relatively little is known about how feedback inhibition regulates dendritic excitatory signal integration. It is still unclear, how local dendritic EPSPs and dendritic spikes are suppressed by feedback inhibitory circuits targeting different compartments of the neuron. Since inhibitory interneurons play a key role in rhythmic oscillations such as theta rhythm, we have tested how feedback inhibition controls the integration of dendritic excitatory inputs during theta patterned pyramidal neuron firing. We exclusively recruited feedback inhibition by stimulation of pyramidal neuron axons in the alveus. At the same time we used fast microiontophoresis of glutamate in combination with two photon calcium imaging to elicit EPSPs and dendritic spikes at defined locations on the apical and basal dendritic tree. We found that EPSPs and associated local dendritic calcium transients were significantly reduced by feedback inhibition when excitatory input was elicited on basal and proximal radial oblique dendrites in strata oriens and radiatum. More distal apical dendritic inhibition was not powerful enough to suppress local Ca2+ transients. However, the somatic voltage deflection caused by distal EPSPs was more strongly suppressed than proximal input due to a stronger on path-inhibition by proximal inhibitory synapses on the way to the soma/axon.
During ongoing theta rhythm the proximal inhibitory suppression of excitatory input was strongly attenuated, therefore action potential output of the pyramidal neuron evoked by both EPSPs and dendritic spikes was facilitated. Local distal inhibition in str. lacunosum -moleculare was unchanged. Interestingly, we found that this target region specific dichotomy was due to a differential recruitment of interneurons into theta activity. Using interneuronal cell-attached and whole-cell recordings with subsequent morphological reconstructions to identify the axonal target compartments, we found that in respect to their theta recruitment hippocampal interneurons can be functionally divided into two groups: Interneurons that are innervating the distal apical tuft and receive constant or slightly facilitating input from CA1 PCs (late theta cells, putative O-LM interneurons)) and cells that are innervating the perisomatic region that receive depressing input from the PCs (early theta cells, basket cells and putative bistratified cells). In summary, we describe an activity- and input-zone dependent regulation of excitatory synaptic input by hippocampal feedback interneurons during theta rhythmic activity. This regulatory mechanism controls the probability of neuronal action potential output evoked by both EPSPs and dendritic spikes during theta activity in the CA1 area of the hippocampus.

# Forward and backward fear conditioning in rats measured by fear-potentiated startle: Characterization and neural basis.

Markus Fendt[1], Stefan Imobersteg[1]

[1]Novartis Institutes for BioMedical Research, Neuroscience Research, WSJ-386.3.28, 4056 Basel, Switzerland

In conditioned fear studies, the association between the unconditioned stimulus (US: e.g., an electric stimulus) and the conditioned stimulus (CS: e.g., a light or tone stimulus) is usually learned by a delay or forward fear conditioning procedure, i.e. the US is presented at the end of the CS. After forward fear conditioning, the CS induces conditioned fear which can, for example, be measured by a potentiation of the startle response (humans, rodents) or by avoidance behavior (flies). Recent studies in flies and humans showed that backward fear conditioning, i.e. the presentation of the CS after the US, results in another phenomenon: Now, the CS induced an inhibition of the startle response or approach behavior. This phenomenon was discussed as "pain relief learning".
In the present study, we investigated this phenomenon in rats using the fear-potentiated startle paradigm. We observed that a CS after backward fear conditioning induced a robust decrease in the startle magnitude. However, the exact timing between US and CS was not critical in rats (in contrast to the experiments in flies) indicating that the CS after backward fear conditioning served as a safety signal. In a second experiment, we temporally inactivated the amygdala and the nucleus accumbens, two brain structures which are crucial for fear or safety learning, respectively, and tested the effects of these inactivations on the expression on learned fear and learned safety. Preliminary data indicates that amygdala inactivation blocks learned fear but not learned safety, whereas nucleus accumbens inactivation blocks learned safety but not learned fear.

# Gating of visual processing by selective attention as observed in LFP data of monkey area V4

David Rotermund[1], Udo A. Ernst[1], Klaus R. Pawelzik[1], Simon D. Neitzel[1], Sunita Mandon[1], Andreas K. Kreiter[1]

[1]University of Bremen, Center for Cognitive Science, Hochschulring 18, 28359 Bremen, Germany

A major function of visual attention is the selective processing of behaviourally relevant visual information. Because receptive field (RF) sizes increase with increasing processing level, neurons in higher visual areas like V4 may receive afferent input associated with more than one object. For selective processing of the relevant object, afferent activity carrying information about this object needs to be more effective than activity related to other objects. In order to investigate attention- dependent routing of information flow in visual cortex, we developed a method which allows a direct and simultaneous estimate of the contribution of target and distracter objects to the neuronal activity pattern.

A macaque monkey was trained to perform a shape-tracking task (Taylor et al. 2005), in which the animal had to direct attention to one of two sequences of morphing shapes on a 21" CRT screen (100 Hz frame rate). Both shapes were within a typical RF in V4. They had a diameter of about 1° of visual angle and were separated by a gap of less than 1°. The luminance of the filled shapes was modulated pseudo-randomly for each frame. The animal's task was to signal the reoccurrence of the initial shape of the attended sequence. Recordings of V1's local field potentials (LFPs) were performed with an array of implanted microelectrodes. Simultaneously we recorded with up to three microelectrodes LFPs in a retinotopically matching part of V4.

The LFPs were split into their frequency components by a wavelet transform. As a measure for the effective contribution of each stimulus we computed the spectral coherence ($c$, $0 = c = 1$) between the time course of its luminance and the measured LFPs.

Despite of the close proximity of the two stimuli, they differed strongly with respect to the coherence of their flickering pattern with the LFP activity in V1. For the stimulus covering the V1 RFs, we found coherence values in a range from 0.1 to 0.25 (significance threshold is 0.015 for $p<0.01$) with the maximum below 10 Hz and a second peak around 20Hz. For the other stimulus, coherence was strongly reduced in the frequency range below 10Hz and vanished entirely for all higher frequencies. Preliminary analysis revealed no clear effect of attention.

In contrast, for recording sites in V4 with both stimuli inside their RFs, we found a strong effect of attention. For the attended stimulus the coherence was several times higher than for the non- attended stimulus. Switching attention was accompanied by a corresponding reversal of the coherence pattern. In comparison to V1, significant coherence was typically limited to a frequency range up to 15Hz with a maximum around 5 Hz and values between 0.07 and 0.14.

In summary our new method reveals that spatial selective attention gates temporal information in V4 in a highly effective manner and with high spatial resolution. Furthermore it characterizes the spectral filter properties of attentional gating, thereby providing constraints for possible underlying mechanisms.

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# Implicit and Explicit Effects of Authenticity on the Perception of Emotional Prosody in Speech

Matthis Drolet[1], Ricarda I. Schubotz[2], Julia Fischer[1]

[1]German Primate Center, Cognitive Ethology, Kellnerweg 4, 37077 Goettingen, Germany

[2]Max-Planck Institute for Neurological Research, Gleueler Str. 50, 50931 Koeln, Germany

[3]Goettingen Graduate School for Neurosciences and Molecular Biosciences, Justus-von-Liebig-Weg 11, 37077 Goettingen, Germany

Previous work has shown that the ability to recognize emotions is influenced by authenticity of the stimulus, with significant interaction effects found between stimulus authenticity and emotion.

The aim of the present fMRI study was to determine what neuronal substrates are modulated by judgments on emotional speech when applied to authentic and play-acted stimuli. In order to corroborate the presence of effects due to stimulus authenticity we employed an authenticity judgment task (to determine explicit effects) and an emotion judgment task (to determine implicit effects). The Theory of Mind network (including mesial prefrontal cortex , superior temporal sulcus and temporo-parietal junction), was a candidate for showing effects of emotion authenticity.

The study included 24 right-handed participants. Authentic recordings with emotional content were selected from the database of a radio station. Play-acted stimuli were reenactments made by professional actors. Recordings were up to 4.5 seconds in length and did not contain keywords from which the expressed emotion could be inferred (therefore recognition based only on prosody). Experimental runs consisted of 72 emotion judgments (EJ: fear, anger, joy or sadness), 72 authenticity judgments (AJ: real or play-acted), 16 word-detection tasks (WD) and 18 null-event trials for contrast and control. Imaging was performed with a 3T Siemens MAGNETOM TrioTim with 22 axial slices (3x3mm in-plane resolution) and a single-shot gradient EPI sequence (2s TR). The functional data were analyzed using the software package LIPSIA v1.5.0 for both preprocessing (highpass cut-off 1/95Hz; Gaussian filter 5.65mm FWHM) and statistical evaluation (1st level: least-squares estimation GLM; 2nd level: one-sample t-test group analyses). To correct for false-positive results, a voxel-wise z-threshold was set to $Z = 2.33$ ($p = 0.01$) with a minimum activation area of 270 mm3 (i.e. 10 contingent voxels).

The behavioral results indicated that anger was detected better when play-acted than when authentic and sadness was detected better when authentic than when play-acted. Results from authenticity judgments indicated that the authenticity of a stimulus was relatively difficult for subjects to detect explicitly ($d' = 0.88$; $c = -0.08$).

Regarding brain activity, active judgment of authenticity, more than judging emotion, was found to enhance activation in the Theory of Mind (ToM) network (medial prefrontal cortex, temporoparietal cortex, retrosplenium) as well as areas involved in the cognitive control of working memory and retrieval (BA 47). Direct contrasts further point to enhanced modulation of social cue perception and ToM by authentic as compared to play-acted emotional speech. In particular, mesial prefrontal cortex also showed significantly more activation during emotion judgments of authentic stimuli as compared to play-acted ones.

The behavioral results confirm the interaction effects of emotion detection and stimulus authenticity, while the brain activation results demonstrate both explicit and implicit modulation by authenticity. The behavioral and functional results clarify that emotional authenticity is an important property in prosody and influences human responses to such stimuli.

# Influence of authenticity on the emotional expression in speech

Rebecca Jürgens[1], Kurt Hammerschmidt[1], Julia Fischer[1]

[1]German Primate Center, Cognitive Ethology Lab, Kellnerweg 4, 37077 Göttingen, Germany

The ability to detect false emotions is crucial in social life, and also of great interest from a forensic point of view. The aim of the present study was to compare the acoustic structure of authentic speech tokens to play-acted ones. We hypothesized that actors would tend to perform emotional expressions in a more exaggerated fashion, which should affect variables that are related to speaker arousal such as fundamental frequency, vocal perturbation, distribution of energy in the higher frequency regions and speech rate. Our stimulus material was composed of 78 radio recordings in which the four emotions 'anger', 'fear', 'sadness' and 'joy' were expressed in natural situations, and of the corresponding re-enactments by 42 professional actors. We conducted a detailed acoustic analysis by measuring the speed of speech on the basis of short speech fragments and by analysing cut-out vowels. Our results showed that acoustic parameters that correlate with arousal were not affected by the encoding condition. Independent of the expressed emotions the authentic expressions differ from play-acted ones in terms of voice quality. Although our working hypothesis had to be rejected, these results nevertheless demonstrated that a difference between authentic and play-acted vocal expressions exists. The result is in line with previous studies on the same stimulus set which showed that even though listeners were not able to explicitly distinguish the encoding conditions, authenticity had an effect on emotion recognition and brain activation. Our acoustic analysis showed that the different perception of play-acted and authentic emotional stimuli is not just explained by higher arousal but by a general difference in the encoding. This illustrates that caution is advised when using play-acted emotional stimuli to draw conclusions about naturally occurring expressions, at least in the voice.

# Local field potentials and spikes in monkey posterior parietal cortex convey independent information about movement plans in a reversing prism task

Shenbing Kuang[1], Alexander Gail[1]

[1]German Primate Center, Sensorimotor Group, Kellnerweg 4, 37077 Goettingen, Germany

The primate posterior parietal cortex, in particular the parietal reach region (PRR), is strongly involved in the planning of goal-directed arm reaching movements. However, it is yet unclear if the planning activity reflects the visual representation of the motor goal, or the planned hand movement direction (proprioceptive motor goal). Previous studies led to conflicting conclusions: Neuron spiking activity in monkey PRR encoded the proprioceptive motor goal in a reversed joystick-cursor paradigm[1]. fMRI signals from the human PRR primarily encoded visual motor goals in a reversing prism paradigm[2].

Most likely reasons for the discrepancy could have been the use of different behavioral paradigms, with either overlapping (prism) or separated (joystick) manual and visual workspaces, and/or the difference in the signal types (spikes vs. fMRI BOLD) arises from distinct recording techniques.

To clarify the controversy and test for these possibilities, we recorded single unit activity and local field potentials in PRR of monkeys while they performed a visually-guided delayed center-out reach task under reversed vision. The left-right reversal of the spatial visual input with dove prisms allowed us to dissociate the visual motor goals from the physical hand movement directions required to achieve the goals (Fig. 1A).

Preliminary results show that PRR neuron spiking responses during the late planning phase were mostly linked to the direction of upcoming hand movements, therefore reflect mainly proprioceptive motor goals (Fig. 1B). In contrast, LFP signals during the early planning phase transiently encode visual motor goals (Fig. 1C). The results suggest that the controversy in previous studies might be due to signal type differences rather than the difference in the behavioral paradigms. Assuming that LFP signals mainly reflect the input and local intra-cortical processing while spiking activities in general measure the cortical output[3], our results indicate a dynamic transition in the parietal cortex during movement planning phase from the early, sensory-input driven transient visual motor goal representation in LFP signals, to the late, more motor-output related proprioceptive motor goal representation in the spiking signals.

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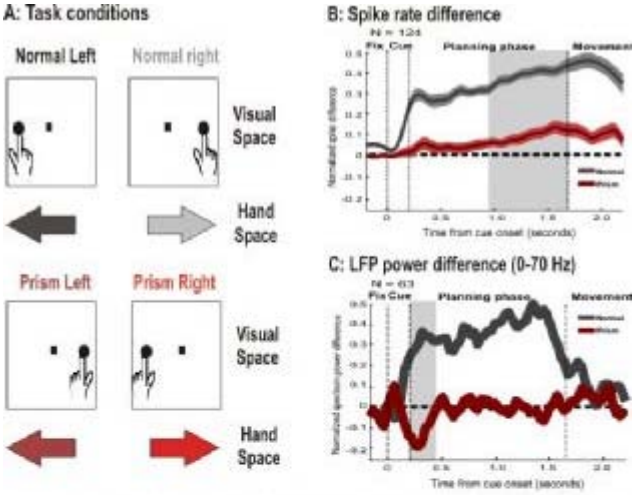


***Figure 1:*** ***(A)*** Delayed reach task conditions with 2 possible target locations (left or right) and two viewing context (normal or reversed) allow the dissociation of the physical direction of hand movement and visual representation of the motor goal. ***(B)*** Normalized population spiking rate differences between planned reaching to the preferred and non-preferred directions, separately for the normal and prism viewing conditions. Shaded area marks the time period in the planning phase when both curves are significant different from zero. The same signs between these two curves reflect unchanged direction selectivity across viewing conditions which indicates proprioceptive motor goal encoding. ***(C)*** Normalized population LFP power differences with the same plotting conventions as in (B). Note that significant direction selectivity across viewing conditions occurs only within a transient early phase during movement planning. The opposite signs reflect reversed direction selectivity which indicates visual motor goals encoding.

# Maternal Separation-Induced Histone Modifications in Frontal Cortex of Mice

Lan Xie[1], Anna Katharina Braun[1], Jörg Bock[1]

[1]Institute of Biology, Department of Zoology and Developmental Neurobiology, Leipziger Str. 44, 39120 Magdeburg, Germany

Histones are subject to a wide variety of posttranslational modifications including acetylation, methylation and phosphorylation, which are regulated by specific enzymes. Overall, posttranslational modifications of histones create an epigenetic mechanism for the regulation of a variety of normal and disease-related processes. The aim of the present study was to clarify if neuronal network adaptations to early stress experience which have been described previously are correlated to specific histone modifications. We established the technique of Chromatin immunoprecipitation (ChIP) to look into the details of histone-DNA interactions and the consequences of experience-induced histone modifications for gene expression in the prefrontal cortex. The present study concentrated on differences in the acetylation of histone H4 (Ac-H4) and histone H3 (Ac-H3) between maternally separated and control mice. After ChIP with Ac -H3 and Ac -H4 precipitated DNA was used for qPCR. We focussed on the promoter regions of Arg3.1/Arc or Egr1. It was observed that both Arg3.1/Arc and Egr1 in maternally separated animals were significantly elevated after ChIP with Ac-H3 than these in control, whereas only a trend towards increase was observed after ChIP with Ac-H4. The results indicate that the previous described neuronal alterations after stress are presumably correlated to the activation of Arc and Egr1 transcription, which is regulated by histone H3 acetylation.

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# Motivational influences on effort-based decision making in rats: The role of dopamine in the nucleus accumbens

Bettina Mai[1], Wolfgang Hauber[1]

[1]Universität Stuttgart, Biologisches Institut, Abteilung Tierphysiologie, Pfaffenwaldring 57, 70569 Stuttgart, Germany

Motivational states exert a profound effect on decision-making and the execution of behavioural strategies. Of particular interest are motivational states resulting from a physiological deficit, such as reduced reserves of energy, as these represent a universal problem for animals and are, therefore, key factors in decision-making. Recent studies suggest that the nucleus accumbens (NAc) and its dopamine (DA) input are critical components of a neural system that subserves a particular form of decision making, that is effort-based decision making. For instance, in rats tested in a T-maze cost-benefit task, excitotoxic lesions of the ACC caused a bias away from the response option requiring more effort to obtain greater reward [1]. However, little is known about the impact of motivational states such as hunger or satiety on effort-based decision making. Moreover, although there is considerable evidence suggesting that the mesoaccumbens DA system processes information about deprivation conditions [2], it is still unclear whether DA in the NAc conveys information related to such motivational states thereby influencing effort-related decision making.

The current study was designed to assess (i) the effects of hunger versus satiety on effort-based decision making in rats, and, (ii) the effects of a DA depletion of the NAc on effort-based decision making in hungry versus satiated rats. Therefore, we investigated the effects of a restrictive versus an ad libitum feeding regimen on effort-based decision making in a cost-benefit T-maze task. In this task, subjects could either choose to climb a barrier to obtain a high reward in one arm (high effort - high reward) or a low reward in the other arm without a barrier (low effort - low reward). Not surprisingly, results showed that food-deprived rats, unlike satiated rats, had a high preference for the high effort - high reward response option. Results further revealed that rats with a NAc DA depletion induced by local infusion of 6-hydroxydopamine had a reduced preference for the high effort - high reward response option. Furthermore, effort-based decision making both in sham controls as in rats with a NAc DA depletion was sensitive to a motivational shift induced by a change from a restrictive to an ad libitum feeding regimen, i.e. the preference for the high effort - high reward response option became lower in the latter condition in both groups. An immunohistochemical analysis further revealed a complete loss of tyrosine hydroxylase positive fibers in the core subregion of the NAc.

The present findings add further support to the notion that mesoaccumbens DA signals play a critical role in effort-based decision making. In addition, our preliminary findings point to the view that mesoaccumbens DA signals do not convey information related to motivational states thereby influencing effort-related decision making.

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# Neurons in the laberal habenular complex project either to the dopaminergic VTA or to the serotonergic raphe nuclei in the midbrain of the rat

René Bernard[1], Rüdiger W. Veh[1]

[1]Charité University Medicine, Institute for Integrative Neuroanatomy, Philippstr. 12, 10117 Berlin, Germany

The two habenulae including the lateral habenular complexes (LHb) are bilateral epithalamic brain structures involved in the modulation of ascending monoamine systems in response to afferent input from several limbic structures and basal ganglia. The LHb is implicated in various biological functions, such as reward, sleep/wake cycle, feeding, pain processing and memory formation. Habenular abnormalities have been demonstrated in mood disorders, such as major depression. The modulary role of the LHb is partly assumed by putative spontaneously active LHb neurons projecting to the dopaminergic ventral tegmental area (VTA), and serotonergic median (MnR) and dorsal raphe nuclei (DR). The habenular efferents to these three monoaminergic nuclei are glutamatergic and target local GABAergic interneurons in their projection areas. Like the LHb, also VTA, DR, and MnR are directly involved in processing of reward-encoded information. All four nuclei form a complex and coordinated network to form appropriate responses to reward-related stimuli.

At present it is not known, however, whether there are neurons or groups of neurons in the LHb, which together project to all of the three monoaminergic nuclei. Alternately, individual neurons or neuronal groups in the LHb may selectively project to one of the monoaminergic nuclei. To differentiate between these two hypotheses we made dual injections of two different retrograde tracers in the rat VTA and DR or MnR. Tracers were visualized by immunocytochemical detection and coronal sections were quantified for the different retrogradly labeled neurons within the boundaries of the LHb.

So far our results show that (1) the distribution of neurons in the LHb projecting to the three monoamine nuclei is similar and exhibits a great overlap, (2) the large majority of LHb projection neurons (>98,4%) target only one monoamine nucleus, and (3) the few LHb neurons that project to both, dopaminergic and serotonergic nuclei, display a heterogeneous distribution within the LHb.

In conclusion, these results imply that the habenula forms individual circuits with each monoamine nucleus, permitting the habenula to selectively fine-tune the output to different monoamine systems.

# Optimal time perception with multisensory cues: Dissecting the effects of perceived and performed motion

Joachim Hass[1], Stefan Blaschke[2], J. Michael Herrmann[3]

[1]Central Institute of Mental Health, AG Computational Neuroscience, J5, 68159 Mannheim, Germany
[2]Georg August Universitaet Goettingen, Abteilung fuer Medizinische Psychologie und Medizinische Soziologie, Germany
[3]University of Edinburgh, School of informatics, Institute for Adaptive and Neural Computation, UK

Time is a perceptual quantity that abstracts from sensory modality. Humans can judge durations of visual, auditory and multidimensional stimuli. Yet, it is unclear how temporal information from different cues is combined to form an integrated percept of time. Early proposals of a centralized, amodal clock have been challenged by recent findings of modality specific timing processes, e.g. in early vision. We therefore suggest that perceived time emerges from the convergence of various sensory streams into a "temporal hub". We support this hypothesis by a series of psychophysical experiments involving concurrent temporal and motor tasks, a paradigm which also allowed to assess potential links between time perception and motor timing, and the effect of attentional resources.

Participants were required to track the motion of a target drawn on a screen, using the end effector of a robot arm. At specific segments of this guided arm motion, namely the apices of the ellipse, two short tones (standard duration 100 ms) were presented which the participants had to discriminate according to their duration. Both the discrimination performance and the perceived duration of the tones were measured.

We find that the observation of the motion of the visual target distorted subjective duration, such that duration was perceived as longer when the observed motion of the target was faster. While such a distortion is well described for visual intervals, our study is the first to show that this effect carries over to the auditory domain. On the other hand, control experiments showed that the actual performance of the motion does not contribute to this effect, suggesting a separation of the mechanisms for time perception and continuous motor timing.

Furthermore, we show that the distortion effect does not depend on a global change of attention. To see that, we compared time perception of the dual-task condition with a single-task condition without the motor task. The motor task decreased the performance to discriminate the two intervals, consistent with a shortage of attentional resources in dual-task conditions which is frequently reported for interval durations in the seconds range. To see that this effect is independent from the distortion of subjective duration by motion speed, we included a training phase where participants practiced the motor task until they reached a defined level of performance. The training diminished the dual-task effect on discrimination performance, but did not affect the distortion of subjective duration. So unlike former studies on multisensory time perception, we provide evidence that the duration distortion is due to direct multisensory interaction rather than modulations of global attention.

Overall, the results are best explained by Bayesian integration of temporal information from the different sensory modalities into a centralized "temporal hub", which is independent from the mechanisms that govern the timing of continuous motion. This form of integration is the optimal solution of the problem to extract temporal information from independent sources with different variability.

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# Pupil dilation reflects unexpected uncertainty: a role for noradrenalin in decision-making

Wolfgang Einhäuser[1], Bernard Marius 't Hart[1], Kerstin Preuschoff[2]

[1]Philipps-University Marburg, Neurophysics, Karl-von-Frisch-Str. 8a, 35032 Marburg, Germany

[2]University of Zurich, Social and Neural Systems Lab and Institute for Empirical Research in Economics, Blümlisalpstrasse 10, CH-8006 Zurich, Switzerland

The fact that pupil dilation accompanies human decision processes has been well known for half a century. Which decision variables are reflected in this outwardly accessible physiological response, however, has remained largely unclear. We hypothesize that pupil dilation is related to uncertainty, in particular to errors in judging uncertainty. To test this hypothesis, we employ a straightforward auditory gambling task that manipulates the perceived uncertainty of a monetary outcome. We measure how various decision-making variables relate to pupil dilation during this task. We find that changes in pupil diameter reflect errors in judging uncertainty but neither the probability of the outcome nor the expected reward. As pupil diameter under constant illumination is likely to be mediated by noradrenalin (NA) released by the locus coeruleus (LC), we conclude that LC may be a key structure in uncertainty processing. Our data support a recent computational model, which links NA to the perception of decision-making variables, in particular to unexpected uncertainty. These findings suggest that NA plays a similar role in the processing of uncertainty as dopamine does for reward, namely the encoding of error signals.

# Single cell attention-dependent modulations in monkey area MT that correlate with reaction time differences in response to behaviorally relevant speed changes

Fingal Orlando Galashan[1], Hanna Christiane Rempel[1], Andreas K. Kreiter[1], Detlef Wegener[1]

[1]University of Bremen, Brain Research Institute, Am Hochschulring 16a, 28359 Bremen, Germany

In primates, selective visual attention modulates neuronal responses to behaviorally relevant objects throughout visual cortex. Covertly attending a relevant object has been convincingly shown to increase firing rates in response to attended objects and/or to decrease those in response to non-attended objects. This sustained attentional modulation is suggested to reflect the selection of the relevant location and to support detection of relevant events at the target location/object. We here address the question how the ongoing attentional modulation prior to the behaviorally relevant event relates to the attention-dependent representation of the event itself.

To this end, we trained two macaque monkeys on a speed change detection task. Stimulation consisted of two Gabor gratings, one being located at the receptive field (RF) of the currently recorded unit, and the other being mirrored across the vertical meridian. Both Gabors intrinsically moved in the preferred direction of the recorded neuron, with a constant speed of 2.1 deg/s. The monkeyâ€™s task was to start each trial by pressing a lever and to indicate an instantaneous increase in the speed of the target by release of the lever, while ignoring any speed increase at the distractor. A spatial cue at the beginning of each trial indicated the upcoming target position, which was either the RF stimulus or the stimulus outside the RF. Thus, recordings were obtained for stimuli currently attended as well as for those currently non- attended. Throughout the trial, monkeys had to gaze at a central fixation point and to keep fixation for minimally 300 ms following release of the lever.

Depending on their speed tuning profile, the majority of MT neurons responded to instantaneous speed changes with an increased transient and a somewhat lower sustained firing rate. On average, throughout the trial firing rates were significantly higher for attended Gabors, including transient and sustained post -change epochs, and the latency of the transient rate increase was slightly, but significantly reduced. However, no attention-dependent changes were observed regarding the relative increase of the firing rate in response to the speed-up of the Garbor, as measured by an Acceleration Index. To test whether these attention- dependent rate changes correlate with behavior, we sorted all successful trials according to the reaction time of the trial and then pooled over the fastest and slowest 20% of all trials. We found that reaction times correlate with the amplitude of the transient increase in firing rate, which also shows a significantly steeper slope, but not with latency or peak time. Interestingly, pre-change firing rates do neither correlate with the amplitude of the transient response nor with reaction times. Thus, the attention- dependent modulation of the firing rate prior to the speed change does not indicate the overall attentional state as expressed in the reaction time of a trial, whereas the firing rate in response to the relevant event reliably correlates with reaction times. Further analysis is attempted to identify neuronal pre-change correlates that are capable to predict the reaction time of a trial and that correlate with the rate increase in the transient post-change response.

# Spatial and feature-based attentional modulations in area MT and MST of macaque visual cortex

Sonia Baloni[1], Daniel Kaping[3], Stefan Treue[1,2]

[1]German Primate Center, Cognitive Neuroscience Laboratory, Kellnerweg 4, 37077 Goettingen, Germany
[2]Bernstein Center for Computational Neuroscience, Goettingen
[3]Laboratory for Neural Circuits and Cognitive Control, University of Western Ontario, Canada

Visual information processing in extrastriate visual cortex in primates is accomplished along two major pathways, namely a dorsal pathway involved in motion processing and a ventral pathway involved in object identification. Area MT and the dorsal part of area MST (MSTd) are two major areas in the dorsal pathway. Area MSTd receives the majority of its inputs from area MT. Cells in these areas respond strongly to moving stimuli, with MT specialized for linear motion stimuli (LMS) and MST for spiral motion stimuli (SMS). Here we aimed to determine how responses to LMS & SMS in MT & MSTd are modulated by spatial and feature-based attention.
We extracellulary recorded the activity of single MSTd and MT cells from two awake behaving macaque monkeys engaged in a spatial or a feature-based attention task. Monkeys had to maintain their gaze at a central fixation spot while stimuli were presented inside and outside (opposite hemi- field) of the receptive field. For determining spatial attentional modulation, the responses of a cell when attending the preferred direction (PD) inside the receptive field (inRF) were compared to attending the PD outside the receptive field (outRF). For determining feature-based attentional modulation, the response of a cell when attending the PD outRF was compared to attending the anti-preferred direction outRF.
55 and 33 cells were recorded from area MT with LMS & SMS respectively, while from MSTd 48 cells were recorded with LMS and 105 cells with SMS. We observed spatial attentional modulations for both SMS & LMS in both area MT and MSTd. The average spatial attentional modulations in both areas and for both stimulus types was around 30%.
In area MT we observed feature-based attentional modulation only for LMS and in area MSTd only for SMS. Both modulations were on average about 10%.
Our results demonstrate that the magnitude of spatial attentional modulations of motion processing in areas MT & MSTd is comparable and not depended on the motion pattern type. In contrast, feature-based attentional modulations in MT and MSTd seem restricted to the stimulus types the respective area is specialized for, i.e. linear motion in area MT an spiral motion in area MSTd.

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# Subchronic administration of ketamine produces long-lasting cognitive inflexibility in rats.

Agnieszka Nikiforuk[1], Piotr Popik[1]

[1]Institute of Pharmacology, Polish Academy of Sciences, Department of Behavioral Neuroscience and Drug Development, Smetna 12, 31-343 Kraków, Poland

Schizophrenic patients exhibit frontal-like deficits including reduced flexibility in modifying behavioral responses to changing stimuli. Similar cognitive inflexibility can be induced in healthy humans by administering a single dose of ketamine, a non-competitive glutamate NMDA (N-methyl-D-aspartate) receptor antagonist. Single dose of ketamine induces similar schizophrenia-like cognitive inflexibility in rats, as assessed in the attentional set-shifting task (ASST). Studies using short-term administration of another dissociative anesthetic phencyclidine (PCP) show long-lasting cognitive impairment associated with neurochemical and ultrastructural changes in the frontal cortex.
We thus investigated the effects of subchronic ketamine administration on rats' performance in the attentional set-shifting task. In this paradigm, rats have to select a bowl containing food reward, based on the ability to discriminate the odors and the media covering the bait. The ASST requires rats to initially learn a rule and form an "attentional set" within the same stimulus dimensions. During the extradimensional (ED) shift, the crucial part of the test, rats have to switch their attention to a new, previously irrelevant stimulus dimension (and discriminate between the odors but no longer the media covering the bait). The animals' ED set-shifting performance serves as a measure of cognitive flexibility.
Ketamine (0 and 30 mg/kg) was administered intraperitoneally to male Sprague-Dawley rats once daily for 5 or 10 consecutive days. The ASST was performed 14 days following the final drug administration.
The results of the present study demonstrate that ketamine treatment for 10 days, but not 5 days, significantly and specifically impaired rats' performance at the ED stage of ASST. There was no significant drug effect during any other discrimination stage. Present data suggest that ketamine-induced cognitive inflexibility reflects clinically relevant aspects of cognitive dysfunction encountered in schizophrenic patients. Therefore, this model may be useful in the evaluation of potential treatments for schizophrenia-related cognitive deficits.
Acknowledgements
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# Task-dependent attentional modulation of human direction discriminability.

Elena Spanou[1], Stefan Treue[1,2]

[1]Cognitive Neuroscience Laboratory, German Primate Center, Göttingen, Germany

[2]Bernstein Center for Computational Neuroscience, Göttingen, Germany

Directing attention to a particular location in the visual field is known to improve detection and discrimination and shorten reaction times in that location relative to others. When behavioral performance is at threshold, attention can reliably make the difference between success and failure (Cook, E.P. & Maunsell, J.H.R., 2002). Early psychophysical experiments with human subjects have used a variety of techniques to manipulate components of attention. Among the most popular are paradigms where a stimulus is proceeded by the appearance of an either informative or non-informative cue affecting the pre-allocation of spatial attention, and the 'dual-task' where attention needs to be distributed because subjects carry out two peripheral discrimination tasks simultaneously. We compare the performance of 8 human subjects in two random dot pattern (RDP) direction discrimination tasks designed to account for these two paradigms. All subjects were trained to discriminate RDP directions at a peripheral cued location to the left or right of central fixation ('single-task' condition) in order to achieve sufficient performance (discrimination threshold < 4 degs) at a very short stimulus duration (71 ms). Subjects were also trained in the two paradigms where attention needed to be distributed: in the condition containing cues that were not fully valid a target (coherently moving) and a distractor (randomly moving) RDP were presented to the left and right of fixation. Target location was accurately indicated by a 500 ms preceding cue (red square) in 80% of the trials (informative cue trials), while in the remaining 20% the cue appeared at the distractor location (non-informative cue trials). In the 'dual-task' condition two coherently moving RDPs were presented simultaneously to the left and right of fixation and subjects were asked to report the RDP direction of both patches. For the main experiment we measured the performance of subjects in all 3 conditions ('single-task', 'valid/invalid-cue', 'dual-task'), using a stimulus duration of 71ms followed by a mask. Our results show that performance is significantly affected by the attentional load in both paradigms. In the valid/invalid-cue condition discrimination performance in the non-informative cue trials deteriorates by 71% compared to the single-task condition (average thresholds 4.8 and 2.8 degs, respectively) and in the dual-task condition performance is reduced by 57% in comparison with the single-task condition (average thresholds 4.4 and 2.8 degs, respectively). Performance difference between the single-task condition and the informative-cue trials was not statistically significant (average thresholds 2.8 and 2.6, respectively). These results show that RDP direction discrimination is best in tasks where attention can be fully allocated to the target location ('single-task'), worse in tasks where attention has to be allocated in two spatial locations simultaneously ('dual-task') and even worse when only a small amount of attention is allocated to the target ('non-informative cue').

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# Task-evoked pupillary response in dual-task situations

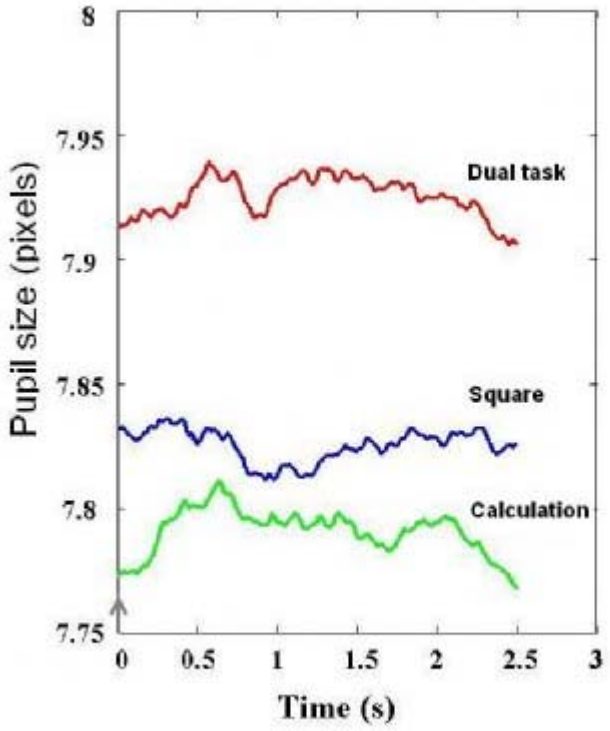
Yiquan Shi[1], Elisabeth Müller[1], Martin Buss[2], Erich Schneider[3], Torsten Schubert[1]

[1] Department for General & Experimental Psychology, LMU Munich
[2] Institute of Automatic Control Engineering, TU Munich
[3] Department for Clinical Neurosciences, University of Munich Hospital, Munich

Many studies have used task-evoked pupillary response as a measure of mental workload in humans. However, pupil size has been rarely measured in dual-task situations (but see Karatekin, 2004). Mental workload should be higher when someone has to do two tasks simultaneously than when a person does only one task. Additionally, most pupil studies took place in highly controlled laboratory settings which might restrict the validity of the data. The presented study wanted to test the usability of task-evoked pupillary response as a measure of mental workload in less controlled dual-task situations. Therefore a new head-mounted eye-tracker was used, the EyeSeeCam (Erich Scheider et al., 2008) to give the participants freedom of head movements while performing a sensori-motor task. We asked in detail if an additional task can produce higher mental workload which is reflected by larger pupil size even in a less controlled situation.

Three different conditions (single task "squares? single task "calculation? and dual task) were presented block-wise. In all conditions three green squares were presented with one of them changing its color to red. In addition to the color change , a tone of high or low frequency could occur in half of the trials. In the single task "squares?the subjects had to respond to the location of a square changing its colour by pressing a corresponding button. In the single task "calculation?the subject had to remember the number presented at the start of the block and add three when a high tone was presented and subtract three when a low tone was presented. In the dual task condition the subjects had to do both tasks. The procedure of the blocks for the three conditions only differed in one aspect: the cue at the beginning of the block, which told the subjects which task they had to do in this block.

First it was found that overall mean pupil size was significantly higher in the dual task blocks than in the single task blocks. Second, in the dual task blocks the pupil size was higher in trials with tone ("real?dual task trials) than in the trials without a tone, in which the subjects only had to remember the current calculation result.
These findings show that task-evoked pupillary response can be an indicator for mental workload which is produced by an additional task even in less controlled situations.


8
7.95
7.9
7.85
7.8
7.75
Pupil size (pixels)
Dual task
Square
Calculation
0
0.5
1
1.5
2
2.5
3
Time (s)

# Temporal dynamics of the early posterior negativity (EPN) in emotional verbs and nouns.

Marina Palazova[1], Werner Sommer[1], Annekathrin Schacht[1,2]

[1]Humboldt-Universitaet zu Berlin, Biological Psychology, Department of Psychology, Rudower Chaussee, 12489 Berlin, Germany

[2]University of Goettingen, CRC Text Structures, JRG Experimental Psycholinguistics, Nikolausberger Weg 23, 7073 Goettingen, Germany

Emotional content impacts visual word processing. However, it is unclear, whether and how this depends on word class and at which functional locus this influence occurs. Results by Schacht & Sommer, 2009 indicate that the EPN in verbs arises at a later point in time compared with the EPN in other word classes. In two experiments ERPs were recorded while subjects decided on the lexicality of positive, negative or neutral words. To examine the boundaries of the emotional ERP activation word class (Exp 1 & Exp 2), word frequency (Exp 1) and word concreteness (Exp 2) were manipulated. Results show that the EPN timing is not dependent on word frequency, but highly dependant on word class and word concreteness. In both experiments an EPN only occurred after or with the lexicality effect (the point where ERPs for words and pseudowords differentiate) indicating that EPN conceivably represents emotion activation at a post-lexical processing stage.

# The gradual nature of rivalry

Stefan Frässle[1], Marnix Naber[1], Wolfgang Einhäuser[1]

[1]Philipps-University Marburg, Neurophysics, Karl-von-Frisch-Str. 8a, 35032 Marburg, Germany

In rivalry, a constant physical stimulus evokes multiple distinct perceptual interpretations (percepts) that alternate over time. Distinct percepts are typically characterized as mutually exclusive for any given region of visual space. However, different regions of the visual space may belong to different percepts (piecemeal rivalry). Here we follow two strategies to address whether rivalry is an all-or-none process. First, we use two reflexes, pupil dilation and the gain of the optokinetic nystagmus (OKN), as objective gradual measures of rivalry; second, we use a continuous input device (analog joystick) for a gradual subjective report. Pupil size and speed of OKN's slow phase consistently show that transitions between percepts are smooth and gradual. Similarly, observers' joystick deflections, which are highly correlated with the reflex measures, indicate gradual transitions. In addition, the reflexes allow assessing rivalry without active report and reveal a significant effect of response mode (none, button press, joystick) on rivalry dynamics. This suggests that the previously described global all-or-none nature of rivalry may in part be a consequence of a discrete set of possible responses, shadowing gradual transitions between percepts. By simulating wave-like transitions between percepts, we find that the gradual nature of transitions is largely explained by piecemeal rivalry. We conclude that rivalry is a gradual phenomenon on a global scale, but likely discrete for any given point in visual space.

# The neural correlates of coloured music: A functional MRI investigation of auditory-visual synaesthesia

Janina Neufeld[1], Christopher Sinke[1], Wolfgang Dillo[1], Hinderk Meiners Emrich[1], Markus Zedler[1]

[1]Hannover Medical School, Clinik for Psychiatry, Social Psychiatry and Psychotherapy, Carl-Neuberg-Str.1, 30625 Hannover, Germany

In synaesthesia a certain stimulus automatically results in an additional internally generated sensation – most often colour. Most investigations of synaesthesia focused on grapheme-colour synaesthesia (coloured numbers or letters), which is one of the most common types, concerning approximately one percent of the population. In auditory-visual synaesthesia, which is believed to be also a common form, all kinds of sound (e.g. tones, music or noise) can induce additional visual experiences: colours, forms and textures.

It is still unknown, which mechanisms lead to these sensations: In the recent years there have been functional neuro-imaging investigations of grapheme-colour-synaesthesia, revealing quite inconsistent results. Some studies found increased brain activation in the visual 'colour-area' (V4/V8) in grapheme-colour-synaesthetes compared to controls during the perception of words and letters presented visually or acoustically. These results point to a direct cross activation of grapheme-area and colour area. But other studies failed to replicate these findings – a fact which puts the model into question. Additionally, increased activation has been observed by different studies especially in multimodal areas as for example in the inferior and superior parietal cortex, posterior inferior temporal cortex or prefrontal cortex regions – rather supporting alternative models as a 'hyperbinding' mediated by multimodal convergence areas. However, the current investigation is the first functional imaging study dealing with auditory-visual synaesthesia triggered by non-linguistic sounds.

To identify the brain regions mainly involved in this form of synaesthesia, the present study investigates brain activation during sound perception (chords and pure tones) in synaesthetes and non-synaesthetes, comparatively. First functional imaging data show a more diffuse and expanded activation pattern in synaesthetes compared to controls during all experimental conditions, involving more brain areas: especially in the left inferior parietal cortex and in the right middle frontal cortex – areas known to be involved in multimodal integration and cognitive control and attention-related mechanisms, respectively. These results point to an involvement of a more complex network of brain areas in synaesthesia and are in line with the 'hyperbinding' model.

Further, it will be determined if there are systematic differences between different photisms induced by different kinds of sound (different timbre and tonality) by assessing not only colour, but also other qualities (form, surface, material) of the perceived photisms. First results demonstrate that - though the photisms are highly individual (as it is also the case for colour patterns in grapheme-colour synaesthesia) - there are also some similarities: some geometrical shapes appear repeatedly and preferentially induced by certain sounds.

# The relation between morphological and electrophysiological properties, and topographical allocation of neurons within the rat lateral habenular complex

Torsten Weiß[1], Rüdiger W. Veh[1]

[1]Charite - Universitätsmedizin Berlin, Centrum für Anatomie, Institut für Integrative Neuroanatomie, Philippstr. 12, 10115 Berlin, Germany

Based on prominent relations with the monoaminergic systems, the lateral habenula complex (LHb) is indicated to be involved in the regulation of pivotal motor and cognitive behaviors. Accordingly, a highly complex and heterogeneous subnuclear organization of the LHb has been reported. Despite functional significance and neuroanatomical insights, however, there is less systematic information concerning the cellular constituents of the LHb, which may provide an essential prerequisite for understanding LHb functionality. The present investigation aimed to correlate structural and functional attributes of neurons within the different subnuclei of the rat LHb. Two main questions were addressed: First, are there differences in the somatodendritic and axonal morphologies between neurons of individual subnuclei? And second, do neurons located in separate subnuclei display particular electrophysiological characteristics? To that, we examined membrane properties and morphological characteristics of topographically identified LHb neurons in rat brain slice preparations using whole -cell recording and neurobiotin labeling. The subnuclear localization of recovered neurons was determined applying cytochemical and immunohistochemical criteria on adjacent sections.

The morphological analysis revealed a heterogeneous population of projection neurons randomly distributed throughout the LHb. According to somatodendritic characteristics four main neuron categories were classified including spherical, fusiform, polymorphic and vertical cells. The electrophysiological characterization of neurons within the different morphological categories demonstrated homologous profiles and no significant differences between groups. Typically, LHb neurons possessed high input resistances and long membrane time constants, displayed time-dependent inward rectification and distinct afterhyperpolarization. A salient electrophysiological feature of LHb neurons was their ability to generate rebound bursts of action potentials in response to membrane hyperpolarization. Based on the pattern of spontaneous activity, LHb neurons were classified as silent, tonic and bursting cells. The occurrence of the different physiological categories was not related to defined topographical allocation. Independent of the mode of activity at rest, the patterns of spontaneous firing and evoked discharge observed in LHb neurons were highly sensitive to alterations in membrane potential and merged upon de- and hyperpolarizing current injection and synaptic stimulation.

In summary, our data indicate that neurons within the different subnuclei of the rat LHb behave electrophysiologically more similar than expected, given their morphological heterogeneity. Hence, cytoarchitecture and intrinsic membrane properties alone do not allow the assignment of individual neurons to distinct subnuclei. We suppose that the formation of functional neuronal entities within the LHb may be achieved through defined synaptic inputs to particular neurons generating distinct temporal properties of responsiveness, rather than by individual neuronal morphologies and intrinsic membrane properties.

# The role of dopamine in the dorsomedial striatum in general and outcome-selective Pavlovian-instrumental transfer

Steffi Mareen Pielock[1], Björn Lex[1], Wolfgang Hauber[1]

[1]Universität Stuttgart, Biologisches Institut, Abteilung Tierphysiologie, Pfaffenwaldring 57, 70550 Stuttgart, Germany

Pavlovian stimuli predictive of appetitive outcomes can exert a powerful influence on the selection and initiation of instrumental behaviour [1, 2, 3]. For instance, Pavlovian stimuli can act to enhance those actions with which they share an outcome, e.g. sucrose-predictive stimuli increase responses associated with sucrose reward more than responses that earn different rewards [4, 5]. This phenomenon termed outcome-selective Pavlovian instrumental transfer (PIT) demonstrates that Pavlovian stimuli activate the memory of the sensory-specific properties of the associated outcome and selectively elevate instrumental behavior which leads to the same outcome. Furthermore, Pavlovian stimuli can invigorate an action by inducing a general appetitive arousal that elevates instrumental responding [4], a phenomenon termed general PIT.
The dorsomedial striatum has been implicated in outcome-selective, but not general PIT. However, the role of dopamine (DA) signals in this subregion in mediating PIT is unknown. Here we examined in rats effects of a 6-hydroxydopamine-induced DA depletion of the anterior (aDMS) or posterior (pDMS) subregion of the dorsomedial striatum on outcome-selective and general PIT as well as on instrumental performance on a FR-5 schedule (five lever presses earned one pellet). Results demonstrate that aDMS and pDMS DA depletions compromised the rate of responding on a FR-5 schedule suggesting that DA signals in the dorsomedial striatum are necessary to maintain high rates of instrumental responding. By contrast, aDMS and pDMS DA depletions did not affect general PIT suggesting that DA signals in the dorsomedial striatum do not mediate general activating effects of reward predictive stimuli to invigorate instrumental responding. Furthermore, aDMS DA depletions did not impair outcome-selective PIT, while pDMS DA depletions had no or only moderate effects. An immunohistochemical analysis revealed that 6-hydroxydopmaine infusions into the produced a circumscribed and near total loss of tyrosine hydroxylase positive fibers within either the aDMS or pDMS.
Collectively, these findings point to the view that DA signals in the aDMS and pDMS are necessary to maintain high rates of instrumental responding but may not mediate general activating effects or specific cueing effects of reward predictive stimuli.

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# The saliency of attention-capturing events modulates the duration of exogenous attention

Anja Lochte[1,2], Jonas Krebs[1], Stefan Treue[1,2]

[1] Cognitive Neuroscience Laboratory, German Primate Center, Göttingen
[2] Bernstein Center for Computational Neuroscience, Göttingen

Attention is a powerful mechanism to select potentially important information from the visual field. It can be controlled voluntarily (endogenous attention) or be triggered reflexively by sudden, salient external events (exogenous attention). The allocation of endogenous attention is known to be highly flexible in duration and magnitude and thus highly adaptable to the behavioral condition. Here we investigated whether exogenous attention - despite its automatic and transient nature - can be allocated in a flexible and graded fashion according to the saliency of the attention capturing event (ACE). By varying the magnitude of ACEs we quantified the influence of the ACE's salience on the duration of exogenous attention in a psychophysical experiment in humans. In our experimental design subjects viewed two spatially superimposed ('transparent') random dot patterns moving within a stationary aperture presented peripherally from a central fixation point. After an interval of 600ms a short acceleration of one of the direction components served as an ACE. A subsequent direction change of one of the two motion vectors occurred after a varying time interval either on the congruent or on the incongruent direction. Subjects were asked to respond to this event as fast as possible. A significant shortening of reaction times was observed for congruent trials relative to incongruent trials. This processing benefit was found to be of longer duration for higher salient ACE conditions than for less salient ones. A low- saliency ACE lead to a processing advantage for congruent events as compared to incongruent events only within an interval of 25 ms between the ACE and the response event. However, highly salient ACEs provided a processing benefit for up to 150 ms. These findings demonstrate that although exogenous attention is allocated in an automatic manner, its duration appears to be dependent on the saliency of the event that triggered it. This is the first report of a graded and flexible mechanism of motion-induced attention capture on the time-course of exogenous attention. Our findings show that exogenous attention is not an all-or-none phenomenon. Rather its allocation is more flexible and better adapted to the situation at hand than previously imagined, recruiting neither more nor less resources than necessary.

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# The utilization of acoustic landmarks for orientation in humans without vision.

Daniel Schmidtke[1], Sarah Galinski[1], Karl-Heinz Esser[1,2]

[1]University of Veterinary Medicine Hannover, Institute of Zoology, Auditory Neuroethology and Neurobiology Lab, Bünteweg 17, 30559 Hannover, Germany

[2]Center for Systems Neuroscience Hannover, Germany

It is known from several mammalian species, including humans, that female and male individuals favour different strategies when it comes to visually guided orientation. It was found that, in the investigated species, female individuals preferentially use visual landmarks as orientational aids, whereas males preferentially use Euclidean cues, e.g. angles, distances, and the like. Our lab could recently show, that the same is true for the utilization of acoustic landmarks in the laryngeal echolocating bat *Phyllostomus discolor* (Schmidtke & Esser 2010, J Comp Physiol A, DOI: 10.1007/s00359-010-0573-x). From this finding we hypothesized that the phenomenon of gender specific orientation strategies is independent of the modality that provides the sensory input for orientation. Therefore, gender differences in the utilization of acoustic landmarks should also be existent in other actively and/or passively acoustically orientating mammalian species.

To further test this hypothesis, we designed a study investigating the role of acoustic landmarks for the orientation of visually impaired/blind and sighted but blindfolded human subjects. In this study, the subjects ($n_{(visually\ impaired)}$ = 22; $n_{(sighted)}$ = 25, genders equally distributed) had to find their way through a small maze consisting of three adjacent rooms. Along the way from the starting position to the goal (shortest distance = 28.5 m), three individual (and qualitatively different) acoustic landmarks were installed that indicated important waypoints. After an initial briefing and a subsequent training phase, each subject had to walk through the maze for 27 times. In three arbitrarily interspersed trials per subject *and* landmark one of the acoustic landmarks was modified in order to provoke a reaction in those subjects that relied on the landmarks during wayfinding.

A first statistical screening of the data showed that there was a significant difference ($a = 0.05$) in the spatial performance between male and female subjects as well as between visually impaired and sighted subjects during the training phase, with the females and the sighted subjects performing worse than the respective reference group. We did not find any significant differences in the reaction to the modification of the individual acoustic landmarks between male and female subjects that support the working hypothesis given above. The number of the sighted subjects that reacted to the modification of the acoustic landmarks, however, was significantly ($a = 0.05$) higher than the number of visually impaired subjects. We are currently running some more sophisticated statistical tests on our data, the results of which will also be presented and discussed on this poster.

# To threat or to punish – what makes the difference

Sylvia Richter[1], Ulrike Kraemer[4], Catherine Libeau[5], Anna Deibele[5], Adriana Barman[5], Constanze Seidenbecher[5], Bjoern Schott[2,3,6], Thomas F. Muente[7]

[1]Helmholtz Centre for Neurodegenerative Diseases, Magdeburg, Neuropsychology, Leipziger Strasse 44, Haus 15, 39120 Magdeburg, Germany

[2]Department of Behavioral Neurology, Leibniz Institute for Neurobiology, Magdeburg

[3]Department of Neurology, Medical Faculty, Otto-von-Guericke University, Magdeburg

[4]Helen Wills Neuroscience Institute, University of California, Berkeley

[5]Department of Neurochemistry and Molecular Biology, Leibniz Institute for Neurobiology, Magdeburg

[6]Department of Psychiatry, Campus Mitte, Charite University Hospital Berlin

[7]Department of Neurology, University Hospital of Schleswig-Holstein, Luebeck, Germany

Aggressive behavior belongs to the normal range of human social behavior. Whether it turns into dissocial and destructive behavior, depends on a variety of internal and external influencing variables. While exploring the neurobiological basis of aggressive behavior, the preponderant part of neuroimaging studies focused on the consequences of brain lesions, psychiatric illness or investigated violent criminals. Yet, still little is known about the neuronal substrates of aggressive behavior in healthy, neurologically and psychiatrically normal people.

We used the Taylor Aggression Paradigm (TAP; Taylor, 1967) to investigate the neural correlates of reactive aggression and threat using functional magnetic resonance imaging (fmri). This task comprises a reaction-time competition between the subject and a same-gender opponent and authorizes the winner to punish the opponent with an aversive tone. A second condition only allows threatening the opponent. In addition, the subject could either be punished by the opponent during a loosing trial or be threatened, according to the particular experimental condition.

40 healthy, right-handed volunteers took part in this study (21 women). All subjects declared written informed consent.

We used a 3-T Siemens Magnetom Scanner to collect structural and functional images and performed random-effects analyses, based on a full-factorial anova model, on the functional data for the decision (choosing the strength of threat or punishment) and the outcome phase (punishing or threatening respectively getting punished).

Analyzing the behavioral data, we found significant differences between
threat and the punishment condition. Volunteers chose a significantly higher strength of punishment in comparison to the strength of chosen threat. In addition, men showed a significantly higher strength of punishment than women.

According to the functional data, we were able to identify different neuronal activation patterns concerning threat and punishment. Punishing the opponent is attended by higher activation in medial frontal gyrus, anterior cingulate cortex and caudate nucleus. Being punished is attended by a high bilateral activation of the amygdala. Those activation patterns also differed between men and women. Striatal activation during punishment condition was found to be significantly higher in men.

The results of our study show that threat and punishment seem to be perceived and evaluated differently by our volunteers. We were able to show behavioral and neuronal differences between threatening and punishing respectively being threatened and being punished. In addition we found significant behavioral and neuronal gender differences concerning punishment of the opponent.

# Ultrasonic vocalizations throughout the rat´s lifespan: effects of strain, sex, and testing conditions

Claudia Natusch[1], Alexandra Lewik[1], Rainer K W Schwarting[1]

[1]Philipps-University of Marburg, Department of Psychology, Gutenbergstr. 18, 35032 Marburg, Germany

Rats emit three types of ultrasonic vocalizations (USV's), which can be assigned to different motivationally relevant situations. The 40-kHz-distress calls of rat pups occur in isolation from the mother and the nest. They help the mother to localize the pups in order to carry them back to the nest. The 50-kHz calls emitted by adult animals often appear in appetitive situations, but also when rats are separated from their cage mates and are placed in a fresh cage (housing cage test, for details see Schwarting et al. Behav Brain Res 2007). In an aversive environment adult rats emit 22-kHz calls. Panksepp and coworkers suggested that these three types of vocalizations might serve as an index of the animal's subjective state (Knutson et al. Psychol Bull 2002). To study emotion, these calls can be a useful tool additional to recording overt behavior or measuring neurotransmitter levels of relevant brain structures.

We will show that it is important to consider the strain (here Long Evans, Sprague Dawley and Wistar Unilever), the sex and the testing conditions (here bedding) when conducting an experiment on ultrasonic vocalizations and emotion.

To elicit 40-kHz calls, we isolated the pups from their mother, nest and siblings. We used a tickling paradigm, the housing cage test, and an open-field amphetamine challenge for the recording of 50-kHz calls. To cause 22-kHz calls, we tested rats in a fear conditioning paradigm (Wöhr et al. Neurobiol Learn Memory 2005). Finally, HPLC was used to measure basal neurotransmitter levels in brain regions associated with positive and negative affect and ultrasonic calling.

# Very Early Emotion Effects for Positive Words in Event-related Brain Potentials

Mareike Bayer[1], Werner Sommer[2], Annekathrin Schacht[2,3]

[1]Humboldt-Universität zu Berlin, Berlin School of Mind and Brain
[2]Humboldt-Universität zu Berlin, Department of Psychology
[3]University of Goettingen, CRC Text Structures

A vast body of evidence suggests that emotional stimuli obtain preferential processing throughout the processing stream. Emotional content has been shown to impact already perceptual processes in the visual cortex, raising an ongoing debate about the degree of automaticity of emotional processing. Although emotion effects have been shown even when the emotional stimulus was not in the focus of attention, their occurrence might nevertheless be influenced by task demands. This holds true not only for biologically determined emotional stimuli such as faces and pictures but also for emotional words. However, the interplay of attention resources, task demands, and the level of processing is not yet fully understood.
The current study compared two task conditions: silent, uninstructed reading, and lexical decisions. Whereas in the first task, attentional resources are not diverted by any additional demands, lexical decisions require resources for the emotion -irrelevant discrimination of words from pseudowords, and also enforce a deeper processing by requiring overt responses. Stimulus material consisted of emotional and neutral single nouns well controlled for emotional, semantic, and orthographic features. ERPs were recorded from 62 EEG-channels.
The results show preferential processing of positive words, starting as early as in the time range of the P1, which are not influenced by task demands. At a later processing stage, starting around 280 ms, arousal effects have been shown in the form of Early Posterior Negativity and Late Positive Complex. While these arousal effects are consistent with previous findings that localized emotion effects at a post-lexical processing stage, the valence effects present evidence that emotion does also impact early processing stages of symbolic stimuli.

# Spatial representation of dynamic objects within working memory in a collision avoidance task

Gregor Hardiess[1], Thomas Müller[1], Stephan Storch[1], Hanspeter A. Mallot[1]

[1]University of Tübingen, Cognitive Neuroscience, Auf der Morgenstelle 28, 72074 Tübingen Tübingen, Germany

*Purpose*: The purpose of the study was the quantitative analysis of the working memory representation of dynamic objects related to gaze movement behavior.
*Methods*: Eighteen subjects participated in a virtual street-crossing paradigm. The primary task was collisions avoidance. To investigate the representation format, during a sub-task subjects were asked to reconstruct the traffic scene from memory and their performance in change detection was assessed.
*Results*: The distribution of cars positioned or detected correct during the sub-task reveals a task-dependent (i.e., collision-relevant) representation of about four cars. In contrast, analysis of gaze behavior did not show a preference for collision-prone cars.
*Conclusion*: Subjects avoided collisions efficiently by applying a gaze strategy adequate to create a representation that fulfills the demands of the task. Collision-prone cars are more likely to be represented in memory, but not more likely to be fixated.

# Poster Topic

# T25: Learning and Memory

T25-1A A behavioural odour-similarity 'space' in larval Drosophila
*Yi-chun Chen, Dushyant Mishra, Linda Schmitt, Michael Schmuker, Bertram Gerber*

T25-2A A critical role for PKA in the acquisition of gregarious behaviour in the Desert Locust
*Swidbert Roger Ott, Heleen Verlinden, Stephen Mark Rogers*

T25-3A A MODEL FOR INHERITANCE OF HIPPOCAMPAL PHASE PRECESSION: FROM CA3 TO CA1
*Jorge Hernan Jaramillo, Robert Schmidt, Richard Kempter*

T25-4A A virtual environment for insects: extracellular brain recordings in walking honeybees
*Nora Vanessa de Camp, Ruth Bartels, Sören Hantke, Randolf Menzel*

T25-5A Acetylation-dependent modulation of memory in the honeybees:
towards the identification of the regulated genes
*Katja Merschbächer, Uli Müller*

T25-6A Analysis of a Working Memory for Visual Orientation in Walking *Drosophila*.
*Sara Kuntz, Burkhard Poeck, Marla Sokolowski, Roland Strauss*

T25-7A Associative Olfactory Learning in *Schistocerca gregaria*
*Patricio Manuel Simoes, Swidbert R. Ott, Jeremy E. Niven*

T25-8A AVERSIVE VISUAL LEARNING IN *DROSOPHILA MELANOGASTER*
*Katrin Vogt, Christopher Schnaitmann, Simon Triphan, Hiromu Tanimoto*

T25-9A Avoidance training in infancy blocks adult avoidance learning in mice:
The impact of age and underlying epigenetic mechanisms
*Almuth Maas, Katharina Braun*

T25-10A BDNF modulates the postnatal extension of dendrites in the CNS in an area-specific way
*Anita Remus, Marta Zagrebelsky, Sabrina Schild, Martin Polack, Martin Korte*

T25-11A Behavioral and synaptic plasticity are impaired upon lack of the synaptic protein SAP47
*Timo Saumweber, Annika Weyhersmueller, Stefan Hallermann, Birgit Michels, Daniel Bucher, Natalja Funk, Dietmar Reisch, Georg Krohne, Stephanie Wegener, Erich Buchner, Bertram Gerber*

T25-12A Behavioural Phenotyping of Shank1 Null Mutant Mice: Social and Non-Social Cognition
*Stephan Röskam, Isabelle Fülber, Richard Dodel, Rainer Schwarting, Albert Hung, Morgan Sheng, Markus Wöhr*

T25-13A Brain proteome changes after memory-enhancing dopamine agonist treatment
*Nicole Reichenbach, Thilo Kähne, Horst Schicknick, Daniela C. Dieterich, Eckart D. Gundelfinger , Karl-Heinz Smalla, Wolfgang Tischmeyer*

T25-14A Cellular Site and Molecular Mode of Synapsin Action in Associative Learning
*Birgit Michels, Yi-chun Chen, Timo Saumweber, Dushyant Mishra, Hiromu Tanimoto, Benjamin Schmid, Olivia Engmann, Bertram Gerber*

T25-15A Common Requirement of Synapsin in Punishment- and Pain Relief-learning
*Thomas Niewalda, Birgit Michels, Ayse Yarali, Jennifer Bretzger, Sören Diegelmann, Bertram Gerber*

T25-16A Cortical neurodynamics during audiovisual category transfer in rodents
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T25-17A Discrimination learning depends on the arrangement of training stimuli
*Rodrigo J. De Marco, Shi-Bin Li, Tatiana Oviedo, Georg Köhr, Mario Trevino*

T25-18A Effects of chronic and acute BDNF deficiency on fear learning
*Thomas Endres, Ayse Özge Sungur, Anne Petzold, Volkmar Lessmann*

T25-19A Establishment of food vectors by desert ants, *Cataglyphis fortis*
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T25-20A Evidence for one-shot learning in the honeybee
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T25-21A Fear extinction learning in heterozygous BDNF knockout mice
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T25-22A Fear Learning and Extinction in an Automated Home Cage (DualCage) Environment
*Torben Hager, René Jansen, Anton Pieneman, Dawid Eckert, Stephan Schwarz, Christian Gutzen, Oliver Stiedl*

T25-23A Functional analysis of the octopaminergic/tyraminergic system in *Drosophila* larval classical olfactory conditioning
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T25-24A Generalization and transfer in honeybee navigation
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T25-25A Differential dopaminergic circuits for the formation of olfactory memory in Drosophila
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T25-1B Hebbian plasticity combined with Homeostasis shows STDP-like behavior
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T25-2B Homing pigeons with navigational experience show a more lateralised brain than pigeons without navigational experience
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T25-3B Honeybees integrate learned and communicated flight vectors in navigation
*Andreas Kirbach, Jacqueline Fuchs, Konstantin Lehmann, Uwe Greggers, Randolf Menzel*

T25-4B How outcome expectations organize learned behaviour in larval *Drosophila*
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T25-5B Increase of fearlessness and latency behavior of Prnp0/0 mice
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T25-6B Increased pupil size distinguishes familiar from novel images
*Marnix Naber, Ueli Rutishauser, Wolfgang Einhäuser*

T25-7B Interaction of genetics and environmental influences during fear extinction in 5-HTT knock-out mice
*Venu Narayanan, Jörg Lesting, Rebeca Heiming, Frederica Jansen, Nobert Sachser, Klaus-Peter Lesch , Hans-Christian Pape, Thomas Seidenbecher*

T25-8B Learning from positive and negative rewards
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T25-9B Learning to navigate: orientation flights of young honeybees
*Jacqueline Fuchs, Andreas Kirbach, Konstantin Lehmann, Uwe Greggers, Randolf Menzel*

T25-10B Mapping of regional brain activity during two- way active avoidance behavior using 2-FDG autoradiography and in-vivo SPECT imaging
*Anja Mannewitz, Jürgen Goldschmidt, Anett Riedel, Michael Gruss, Jörg Bock, Katharina Braun*

T25-11B Mapping the Individual Body-Size Representation Underlying Climbing Control in the Brain of *Drosophila melanogaster*
*Tammo Krause, Roland Strauss*

T25-12B Memotaxis: An Advanced Orientation Strategy in Fruit Flies and its Consequences in Visual Targeting and Temperature Orientation
*Christian Berg, José A. Villacorta, Valeri Makarov, Manuel G. Velarde, Paolo Arena, Luca Patane, Pietro S. Termini, Roland Strauss*

T25-13B Neural correlates of combined olfactory and visual learning in mushroom body extrinsic neurons of the honeybee (Apis mellifera)
*Ina Klinke, Randolf Menzel*

T25-14B Neurobehavioral changes in mice offspring induced by prenatal exposure to lipopolysaccharides
*Hossam Ebaid, Jamaan Ajarem , Gasem Abu-Taweel*

T25-15B Neuromodulation of avoidance learning by the ventral tegmental area and lateral habenula: Differential effects on acquisition, retrieval, long-term retention and extinction.
*Anton Ilango Micheal, Jason Shumake, Wolfram Wetzel, Henning Scheich, Frank Ohl*

T25-16B Newborn cells after cortical spreading depression – proliferation, survival and functional contribution to hippocampus dependent learning and memory
*Eileen Baum, Otto W. Witte, Anja Urbach*

T25-17B Nogo-A Stabilizes the Architecture of Hippocampal Neurons
*Marta Zagrebelsky, Martin E. Schwab, Martin Korte*

T25-18B Observation of network dynamics in mouse hippocampal slices using genetically-encoded fluorescent Ca2+ sensors
*Susanne Reichinnek, Alexandra von Kameke, Eckehard Freitag, Anna Hagenston, Hilmar Bading, Mazahir Hasan, Andreas Draguhn, Martin Both*

T25-19B Odour-mixture perception in *Drosophila*
*Claire Eschbach, Bertram Gerber*

T25-20B Olfactory memories are intensity-specific in larval *Drosophila*
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*Kathy Rether, Uli Müller*

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*Seidenbecher*

T25-22C Towards the understanding of complex human decision and learning processes across the life-span
*Aki Naito, Wendelin Boehmer, Andreas Marschner, Tobias Sommer, Christian Buechel, Klaus Obermayer*

T25-23C What impact do varying reward magnitudes have on associative strength, memory formation, and extinction in classical conditioning of harnessed honeybees (*Apis mellifera*)?
*Katharina Grauel, Kathrin Marter, Dorothea Eisenhardt*

T25-24C Where does Potein-Kinase A support odor memory?
Functional memory maps based on a RNAi knockdown approach.
*Antje Richlitzki, Marina Efetova, Martin Schwärzel*

T25-25C Why feed-forward structure fails to propagate in plastic recurrent networks
*Susanne Kunkel, Markus Diesmann, Abigail Morrison*

# A behavioural odour-similarity 'space' in larval Drosophila

Yi-chun Chen[1], Dushyant Mishra[1], Linda Schmitt[1], Michael Schmuker[2], Bertram Gerber[1]

[1]Department of Genetics, Institute of Biology, Universität Leipzig, Leipzig, Germany
[2]Neuroinformatics and Theoretical Neuroscience, Institute for Biology, Freie Universität Berlin, Berlin, Germany

To provide a behaviour-based estimate of odour similarity in larval Drosophila, we use four recognition-type experiments: (i) We train larvae to associate an odour with food, and then test whether they would regard another odour as the same as the trained one. (ii) We train larvae to associate an odour with food, and test whether they prefer the trained odour against a novel, non-trained one. (iii) We train larvae differentially to associate one odour with food, but not the other one, and test whether they prefer the rewarded against the non-rewarded odour. (iv) In an experiment like (iii), we test the larvae after a 30min-break. This yields a combined, task-independent estimate of perceived difference between odour-pairs. Comparing these perceived differences to published measures of physico-chemical difference reveals a weak correlation. A notable exception are 3-octanol and benzaldehyde, which are distinct in published accounts of chemical similarity, and in terms of their published sensory representation, but nevertheless are consistently regarded as the most similar of the ten odour pairs employed. It thus appears as if at least some aspects of olfactory perception are 'computed' in post-receptor circuits on the basis of sensory signals, rather than being immediately given by them.

# A critical role for PKA in the acquisition of gregarious behaviour in the Desert Locust

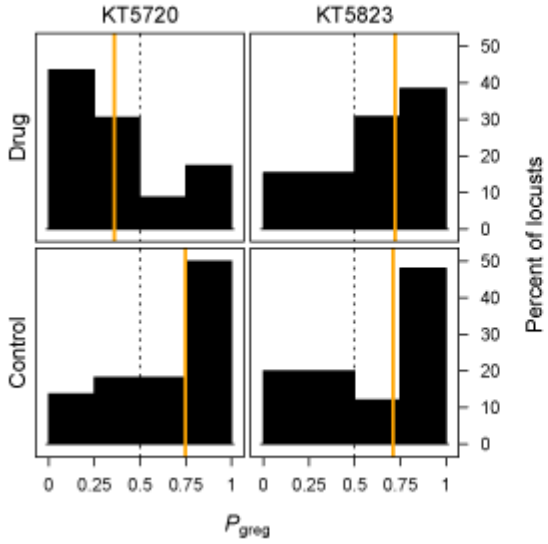
Swidbert Roger Ott[1], Heleen Verlinden[2], Stephen Mark Rogers[1]

[1]University of Cambridge, Department of Zoology, Downing Street, CB2 3EJ Cambridge, United Kingdom
[2]Katholieke Universiteit Leuven, Zoological Institute, Naamsestraat 59, B-3000 Leuven, Belgium

***Background***
The molecular mechanisms that coordinate transitions between alternative integrated phenotypes are little understood. To what extent are alternative phenotypes controlled by orthologous genes in different species? We investigated the roles of two protein kinases in the acquisition of the gregarious behavioural syndrome that underlies swarm formation in Desert locusts, *Schistocerca gregaria*: the *foraging* gene product, which is a protein kinase G (PKG) implicated in behavioural phenotypic switching in other insects, and protein kinase A (PKA), an ubiquitous mediator of plastic responses to environmental cues.

***Results***
Pharmacological inhibitors of PKA but not of PKG interfered with the acquisition of the gregarious behavioural phenotype through crowding. The behaviour of long-term gregarious-phase locusts was unaffected by either inhibitor. RNA interference (RNAi) against *foraging* caused a strong reduction in mRNA and protein expression in the CNS but affected neither the acquisition nor the manifestation of gregariousness. By contrast, locusts with an RNAi-induced reduction in the expression of PkaC1 catalytic subunit protein showed reduced gregariousness before and during the transition. RNAi against the inhibitory PkaR1 subunit had the opposite effect, causing a significant shift towards increased gregariousness.

***Conclusions***
PKA is critical for the transition from solitarious to gregarious behaviour. Thus, a ubiquitous mediator of cellular plasticity has been evolutionarily recruited into implementing the wide-ranging reconfigurations of the neural circuitry that are required for transitions between alternative integrated phenotypes.




Solitarious-phase locusts were injected with an inhibitor of PKA (KT5720), with an inhibitor of PKG (KT5723), or with vehicle alone (matched controls). The histograms summarize the probability of gregarious behaviour ($P_{greg}$) after 1 hour of crowding with 30 conspecifics (orange lines indicate the group medians; parametric analysis on logits, i.e., log-odds of gregariousness). The control-injected locusts gregarized strongly. Locusts injected with KT5823 were no different from their matched controls ($p = 0.554$), whereas locusts injected with KT5720 remained solitarious ($p = 0.00443$). The two KT compounds did not show a common effect on behaviour ($F_{1,91} = 1.113$, $p = 0.294$): only injection with KT5720 was effective in preventing gregarization (interaction "drug *versus* control" : "KT5720 *versus* KT5823," $F_{1,91} = 7.677$, $p = 0.00678$).

# A MODEL FOR INHERITANCE OF HIPPOCAMPAL PHASE PRECESSION: FROM CA3 TO CA1

Jorge Hernan Jaramillo[1], Robert Schmidt[1], Richard Kempter[1]

[1]Humboldt Universitaet zu Berlin/Bernstein Center for Computational Neuroscience, Theoretical Neuroscience, Invalidenstr. 43, 10115 Berlin, Germany

Hippocampal place cells exhibit phase precession which describes the advancement of a neuron's firing phase with respect to theta oscillations (4-12 Hz) in the local field potential. Phase precession has been observed in the CA1 and CA3 regions of the hippocampus during exploratory behavior, and its functional role has been linked to memory and spatial navigation. An open question is the origin of phase precession. Previous models of phase precession have assumed that it is generated intrinsically in the CA1 region. Through computational modeling, we investigate here the possibility of the CA1 region inheriting phase precession from the CA3 region. The model of inheritance consists of a feedforward excitatory input from a subset of the CA3 place cell population onto the CA1 region via the Schaffer Collaterals. Taking into account the firing rate within a CA3 place field and the parameters characterizing excitatory postsynaptic potentials, we are able to simulate the CA1 membrane potential and calculate its analytical form as a function of the model parameters. The resulting membrane potential trace is similar to recent experimental recordings (Harvey et al., 2009), suggesting that CA1 can inherit phase precession from CA3. Furthermore, we show how a signal-to-noise ratio analysis constrains the parameters of the model, particularly on the minimal amount of neuronal input necessary.

References:

Harvey C., Collman F., Combeck D., and Tank D. Intracellular dynamics of hippocampal place cells during virtual navigation. Nature vol. 461, 941-949, 2009

# A virtual environment for insects: extracellular brain recordings in walking honeybees

Nora Vanessa de Camp[1], Ruth Bartels[1], Sören Hantke[1], Randolf Menzel[1]

[1]FU Berlin, Neurobiology, Königin-Luise-Str.28/30, 14195 Berlin, Germany

Our goal is to characterize the neural correlates of behavioral decision making in honeybees.

We have developed a setup that allows the bee to control the visual environment when walking on a floating ball. The bee is able to control different three dimensional cues with her walking movements on a Styrofoam ball. The balls movements are detected by computer mice to control the virtual environment in order to simulate rotatory and translatory movements in a virtual 3D environment. Furthermore we have developed a method for extracellular long-time recordings from central neurons in the walking bee for a period of up to one week. We are recording from single mushroom body extrinsic neurons including the PE1. The neuron responses are correlated with the animals walk through the environment. The virtual environment setup gives us the possibility to observe the activity of multiple neurons while performing an operant conditioning paradigm.

# Acetylation-dependent modulation of memory in the honeybees: towards the identification of the regulated genes

Katja Merschbächer[1], Uli Müller[1]

[1]Saarland University, Dept. 8.3 Bioscience - Zoology and Physiology, post office box 151150, 66041 Saarbrücken, Germany

Regulation of gene expression plays an important role in learning and memory formation. In addition to the regulation of genes by learning induced activation of the transcription machinery gene expression is at the same time modulated by the chromatin structure, which comprises DNA and histones. The latter modulation mainly depends on DNA methylation and histone modifications. Hyperacetylation of histone tails is catalyzed by histone acetyltransferases (HATs) and leads to an open chromatin structure facilitating the transcription process. Histone deacetylases (HDACs) catalyze hypoacetylation and lead to a closed chromatin structure. Although it has been shown in both, mammals and invertebrates, that elevation of acetylation by the HDAC inhibitor TSA (trichostatin A) facilitates memory formation, only little is known regarding the genes implicated in this process. Most work in mammals focuses on the acetylation-dependent regulation of a BDNF gene (brain-derived neurotrophic factor) that critically contributes to learning and memory formation. Since the modulation of gene expression by acetylation is a conserved mechanism, and since BDNF is not found in invertebrates, other genes will have to carry out a homologous function. Moreover it is very likely that acetylation-dependent processes regulate other genes implicated in learning.
We aim for a direct identification of mRNAs the expression of which is affected by the acetylation status *in vivo*. *In vivo* labelling of newly synthesized mRNA followed by its isolation, identification, and quantification allows for detection of genes potentially involved in acetylation-dependent modulation of memory.

# Analysis of a Working Memory for Visual Orientation in Walking *Drosophila*.

Sara Kuntz[1], Burkhard Poeck[1], Marla Sokolowski[2], Roland Strauss[1]

[1]Johannes Gutenberg-University, Department of Zoology III - Neurobiology, Coronel-Kleinmannweg 2, 55099 Mainz, Germany
[2]Department of Biology, University of Toronto, Mississauga, Ontario L5L 1C6, Canada

Navigation in a complex environment requires memory formation to exert goal driven behaviour. Orientation towards a target that gets temporarily out of site has to be memorized to pursue navigation in the proper direction. We have developed the so called *detour paradigm* for walking *Drosophila* flies to analyze this type of visual orientation memory (Neuser et al., 2008). Flies confronted with two opposing, inaccessible black stripes in a circular arena will walk back and forth for a considerable amount of time. If the target stripe disappears as the fly crosses a virtual midline of the platform it will keep its orientation, suggesting a memory for the position of the stripe or its own trajectory (*persistence of orientation*). But even if the fly is simultaneously lured out of the direct path by a lateral distracter stripe, it still remembers the position of the original stripe and resumes walking to its original target, after the distracter has also disappeared.
Mutant analysis has revealed that the ellipsoid body of the fly's central complex is required to accomplish this task (see also Thran J, Poeck B, Strauss R, this issue). Moreover, the learning and memory mutant *ignorant* ($ign^{58/1}$) is deficient in this type of working memory. $ign^{58/1}$ represents a null-allele for the gene encoding the protein-kinase enzyme S6KII. Differential rescue experiments in a sub-type of ellipsoid body ring neurons (R3; Renn et al., 1999) of $ign^{58/1}$ flies rescues the orientation memory. In contrast, the cAMP/PKA signaling pathway doesn't seem to be required because mutants for the phosphodiesterase *dunce* behave wild-type like (Neuser et al., 2008).
To extent our study we tested mutants affecting other signaling pathways involved in learning and memory formation. We have identified the cGMP-dependent protein kinase encoded by the *foraging* (*for*) gene to be required for the orientation memory. Differential rescue experiments using the UAS/GAL4-system (Brand and Perrimon, 1993) will be provided to identify the neuronal networks utilizing this signaling pathway.

Brand & Perrimon (1993): Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development. 118(2); pp.401-415.
Neuser et al., (2008): Analysis of a spatial orientation memory in Drosophila. Nature. 453(7199); pp.1244-7.
Renn et al., (1999): Genetic analysis of the Drosophila ellipsoid body neuropil: organization and development of the central complex. J Neurobiol. 41(2); pp. 189-207.

# Associative Olfactory Learning in *Schistocerca gregaria*

Patricio Manuel Simoes[1], Swidbert R. Ott[1], Jeremy E. Niven[1]

[1]University of Cambridge, Zoology, Downing Street , CB2 3EJ Cambridge, United Kingdom

Locusts can learn associations between olfactory stimuli and rewards, using the acquired memories to choose between nutrients they require. They are a model system for both the study of olfactory coding and insect nutritional regulation. Previous studies have used operant paradigms for conditioning freely-moving locusts, restricting the study of the neural mechanisms underlying the acquisition of olfactory memories, which requires restrained preparations for electrophysiological recordings.

Here we present two complementary paradigms for the classical conditioning of olfactory memories in restrained desert locusts (*Schistocerca gregaria*). These experimental paradigms allow precise experimental control of the parameters influencing learning, including satiation state and the number and timing of the odour stimuli and food rewards.

The first paradigm is based on classical (Pavlovian) appetitive conditioning of maxillary palp opening response. We show that the opening of locusts' maxillary palps provides a behavioural measure of memory acquisition. Maxillary palp opening in response to odour presentation is significantly higher in locusts trained with a paired schedule, in which odour presentation is paired with a food reward, than in locusts trained with either an unpaired schedule or the odour alone. The memory formed by this conditioning paradigm lasts for 24 hours or more.

In a second paradigm, we show that classical conditioning of an odour memory in restrained locusts influences their decisions in a subsequent operant task. When locusts that have been trained previously to associate an odour with a food reward are placed in a Y-maze, they choose the arm containing that odour significantly more often compared with naïve locusts or locusts trained with an unpaired or odour-only schedule. A single conditioning event is sufficient to induce a significant bias for that odour for up to 4 hours. Multiple and block-trial training can induce a significant bias that lasts for at least 24 hours. Thus, locusts are capable of forming long-term appetitive olfactory memories in classical conditioning paradigms and can use these memories to modify their decisions in a context that requires choices similar to those encountered during foraging.

The comparison of the Pavlovian and the operant read-out of memories acquired in the same training paradigm reveals a striking difference: The increase in the Pavlovian response is already significant after a single training trial but continues to increase further over successive trials. By contrast, following a single training trial the operant preference is already indistinguishable from that after multiple trials. A strong memory is thus already present after a single trial, but the coupling of this memory to the behavioural output is task-dependent.

# AVERSIVE VISUAL LEARNING IN *DROSOPHILA MELANOGASTER*

Katrin Vogt[1], Christopher Schnaitmann[1], Simon Triphan[2], Hiromu Tanimoto[1]

[1]Max-Planck-Institute of Neurobiology, Am Klopferspitz 18, 82152 Martinsried, Germany
[2]Department of Neurobiology and Genetics, University of Würzburg, Am Hubland, 97074 Würzburg, Germany

To understand neurobiological mechanisms for visual perception and memory, we developed a new conditioning assay for aversive visual associative learning in adult flies. Flies are trained en masse to associate one of two different visual stimuli (e.g. blue and green light) with electric shock punishment. In the test phase, both punished and control visual stimuli were presented, and the difference in flies' visual preference was measured as associative memory. The trained flies showed significant conditioned avoidance of a punished cue. We analysed critical parameters for the formation and expression of visual memory, such as training repetition and strength of reinforcement. Since the setup is compatible with an existing appetitive conditioning assay we can directly compare appetitive and aversive visual memories. Furthermore we started to identify neurons underlying aversive and appetitive memories. We will discuss about the contribution of prominent brain structures (i.e. mushroom body and central complex) to visual associative memory.

# Avoidance training in infancy blocks adult avoidance learning in mice: The impact of age and underlying epigenetic mechanisms

Almuth Maas[1], Katharina Braun[1]

[1]Otto von Guericke University, Institute of Biology, Department Zoology/Developmental Neurobiology, Leipziger Str. 44, 39120 Magdeburg, Germany

Cognitive training during early childhood plays an important role in shaping neural circuits and learning capacity in adulthood. We have shown in rats that avoidance training in a two-way active avoidance (TWA) task during infancy accelerates avoidance learning in adulthood. The aim of the present study was i) to replicate these studies in mice (C57BL/6), examine ii) the age-dependency of TWA learning, and iii) search for epigenetic mechanisms involved in avoidance learning. The number of phosphoacetylated histone H3 immunoreactive neurons [P(Ser10-Ac(Lys14)-H3$^{+}$] were quantified in serial sections of the dentate gyrus and amygdala in 3 and 12 weeks old mice with or without TWA training.

We found that i) young mice (age: 3, 4, 5 or 6 weeks) showed attenuated avoidance performance compared to adults; ii) 6 weeks old mice learned better than the younger age groups iii) only mice pretrained at the age of 6 weeks showed accelerated avoidance learning in adulthood compared to non pretrained adults, whereas iv) mice pre-trained at the age of 3 weeks completely failed to learn an active avoidance strategy in adulthood. The immunohistochemical analysis revealed that the number of P(Ser10)-Ac(Lys14)-H3$^{+}$ neurons in the dentate gyrus and amygdala was lower in infant (3 weeks old) than in adult (12 weeks old) brains, and ongoing analysis will reveal possible differences of H3-labelled neurons between trained and naive mice in both examined age groups.

The different cognitive outcome of infant avoidance training on adult learning observed in the two rodent species suggests that the yet to be identified epigenetic mechanisms and the presumed neuronal/synaptic changes in learning-relevant brain circuits may differ between rats and mice.

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# BDNF modulates the postnatal extension of dendrites in the CNS in an area-specific way

Anita Remus[1], Marta Zagrebelsky[1], Sabrina Schild[1], Martin Polack[1], Martin Korte[1]

[1]Technische Universität Braunschweig, Cellular Neurobiology, Spielmannstrasse 7, 38106 Braunschweig, Germany

Brain-Derived Neurotrophic factor (BDNF) elicits potent effects on neurons to regulate their differentiation, synaptic plasticity as well as dendritic arborization. However, as the bdnf null mutation leads to death soon after birth, its role during central nervous system (CNS) development has remained difficult to assess in vivo. In addition, the anterograde axonal transport of BDNF complicates the interpretation of areas-specific gene deletion. This question is even more crucial as BDNF levels markedly increase in the postnatal brain.

We used a new conditional mouse mutant in which BDNF is lacking throughout the CNS through the use of the Cre-loxP recombination system (Rauskolb et al. 2010). These BDNF-depleted animals survive for several months after birth and while the size of their brain is reduced, the effect of BDNF deprivation is surprisingly area-specific. The volume of the hippocampus is not significantly affected, while the size of the cortex is reduced by about 20% and the volume of the striatum is even smaller by about 35%. Accordingly, detailed analysis of the morphology of the length of dendrites and spine density in medium spiny neurons of the striatum showed a highly significant reduction. On the contrary, the analysis of the morphology of CA1 pyramidal neurons revealed only minimal effects on dendritic length and branching as well as on spine density, while the proportion of mushroom-type spines is significantly decreased (Rauskolb et al., JNS 2010).

In the current study we expanded the previous data by comparing excitatory neurons in the cortex and inhibitory synapses in different brain areas. The cortical pyramidal neurons of layer IV showed no effect on dendritic complexity, while the pyramidal neurons in layer II/III revealed a reduction in dendritic length. However, the spine density and the spine types of cortical pyramidal neurons of layer II/III and layer IV showed the same reduced proportion of mushroom-type spines as seen for the CA1 pyramidal neurons. Preliminary experiments indicate a layer dependent loss of inhibitory synapses in the hippocampus. The morphological analysis of other inhibitory neurons (e.g. Purkinje cells) is still in progress.

According to previous reports in the full bdnf knockout mice (Korte et al. 1995, Patterson et al. 1996) the long term potentiation is heavily impaired in 2 weeks old conditional knockout mice, while 8 weeks old conditional bdnf knockout mice showed milder defect in the long term potentiation. Accordingly, we are investigating if inhibitory or compensatory effects are involved in 8 weeks old conditional bdnf knockout mice.

Taken together, these results reveal surprising, area-specific requirements for BDNF in modulating the postnatal extension of dendrites. Future experiments will be addressing whether such area-specific requirements for BDNF may be due to intrinsic differences in the responsiveness of diverse neuronal populations to BDNF.

# Behavioral and synaptic plasticity are impaired upon lack of the synaptic protein SAP47

Timo Saumweber[1,3], Annika Weyhersmueller[2], Stefan Hallermann[2], Birgit Michels[1,3], Daniel Bucher[3,4], Natalja Funk[3,5], Dietmar Reisch[6], Georg Krohne[6], Stephanie Wegener[3,7], Erich Buchner[3], Bertram Gerber[1,3]

[1]Universität Leipzig; Institut für Biologie II; Lehrstuhl für Genetik; Talstr. 33, 04103 Leipzig, Germany

[2]Universität Leipzig; Carl-Ludwig-Institut für Physiologie, 04103 Leipzig, Gemany

[3]Universität Würzburg; Lehrstuhl für Neurobiologie und Genetik, Biozentrum am Hubland, 97074 Würzburg, Germany

[4]Present address: Universität Heidelberg; Lehrstuhl für Neurobiologie, Im Neuenheimer Feld 364, 69120, Heidelberg, Germany

[5]Present address: Eberhard Karls Universität Tübingen; Institut für Humangenetik, Calwerstr. 7, 72076 Tübingen, Germany

[6]Universität Würzburg; Abteilung für Elektronemikroskopie, Biozentrum am Hubland, 97074 Würzburg, Germany

[7]Present address: Neurowissenschaftliches Forschungszentrum (NWFZ, Charité der Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

The synapse associated protein of 47 kDa (SAP47) is a member of a phylogenetically conserved gene family of hitherto unknown function. In Drosophila, SAP47 is encoded by a single gene and is expressed throughout all synaptic regions of the wild-type larval brain; specifically, electron microscopy reveals anti-SAP47 immuno-gold labeling within 30 nm of presynaptic vesicles. To analyze SAP47 function, we use the viable and fertile deletion mutant *Sap47*$^{156}$, which suffers from a 1.7 kb deletion in the regulatory region and the first exon. SAP47 cannot be detected by either immuno-blotting or immuno-histochemistry in *Sap47*$^{156}$ mutants. These mutants exhibit normal sensory detection of odorants and tastants as well as normal motor performance and basic neurotransmission at the neuromuscular junction. However, short- term plasticity at this synapse is distorted. Interestingly, *Sap47*$^{156}$ mutant larvae also show a 50 % reduction in odorant-tastant associative learning ability; a similar associative impairment is observed in a second deletion allele (*Sap47*$^{201}$) and upon reduction of SAP47 levels using RNA-interference. In turn, transgenically expressing the most abundant 47-kDa isoform of SAP47 partially rescues the defect of the *Sap47*$^{156}$ mutant larvae in associative function. This report thus is the first to suggest a function for SAP47; it specifically argues that SAP47 is required for proper behavioral and synaptic plasticity in flies- and potentially its homologues in other species.

# Behavioural Phenotyping of Shank1 Null Mutant Mice: Social and Non-Social Cognition

Stephan Röskam[1,2], Isabelle Fülber[2], Richard Dodel[2], Rainer Schwarting[1], Albert Hung[3], Morgan Sheng[3], Markus Wöhr[1]

[1]Department of Psychology, Experimental and Physiological Psychology, Philipps-University of Marburg, 35032 Marburg, Germany

[2]Department of Neurology, Neurological Therapy Research Unit, Philipps-University of Marburg, 35043 Marburg, Germany

[3]The Picower Institute for Learning and Memory, Department of Brain and Cognitive Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

Autism is a neurodevelopmental disorder with a strong genetic component. Core symptoms include abnormal social behaviour, communication impairments, and repetitive and stereotyped patterns of behaviour with restricted interests. Genome-wide and pathway-based association studies led to the identification of several susceptibility genes for autism, including the SHANK gene family. Mutations in SHANK2 and SHANK3 have been detected in several autistic individuals. SHANK genes code for a family of scaffolding proteins located in the postsynaptic density of excitatory synapses. Recently, we disrupted the Shank1 gene in mice to investigate its function in vivo. Shank1 null mutant mice are characterized by high levels of anxiety-like behaviour, impaired contextual fear memory, slightly increased spatial memory as well as reduced dendritic spines and weaker excitatory synaptic transmission.

In the present study we investigated spatial and non-spatial memory using either social or non-social objects in male and female Shank1 null mutant mice. Shank1 null mutant mice and their wildtype littermates of both genders were submitted to a novel object recognition task and a novel object location task, using non-social objects. Furthermore, they were tested in two new behavioural paradigms, namely novel social object recognition and novel social object location, using urine samples from the opposite sex as social stimuli.

Preliminary results indicate intact object recognition and object location memory in Shank1 null mutant mice when using non-social stimuli. Shank1 null mutant mice spent more time interacting with a novel object than with a familiar one in the novel object recognition task and displayed a preference towards an object moved to a new location in the novel object location task. Although Shank1 null mutant mice did not differ significantly from their wildtype littermates, performance was slightly better. During the novel social object recognition task, Shank1 null mutant mice spent more time in proximity to a novel urine spot as compared to a familiar one, indicating intact social recognition. In the novel social object location memory, however, Shank1 null mutant mice spent nearly the same amount of time in proximity to a urine spot relocated to a novel position as they spent in proximity to a non-relocated urine spot. In contrast, wildtype littermate controls showed a preference towards the urine spot in the novel position. This indicates a deficit in social spatial memory in Shank1 null mutant mice.

Our results support previous findings of intact non-social spatial learning and memory in Shank1 null mice, and extend existing data of behavioural phenotyping on Shank1 null mutant mice showing intact social and non-social object-related learning and memory. Results of the novel social object location memory task indicate a potential deficit of spatial learning and memory in Shank1 null mutant mice when using social stimuli.

# Brain proteome changes after memory-enhancing dopamine agonist treatment

Nicole Reichenbach[1], Thilo Kähne[2], Horst Schicknick[1], Daniela C. Dieterich[1], Eckart D. Gundelfinger [1], Karl-Heinz Smalla[1], Wolfgang Tischmeyer[1]

[1]Leibniz Institute for Neurobiology, Lab. Mol. Biol. Tec., Magdeburg, Germany
[2]Inst. Experimental Internal Medicine, OvG University, Magdeburg, Germany

In Mongolian gerbils, the auditory cortex is critical for discriminating frequency-modulated tones. In this paradigm, long-term memory formation is enhanced by local infusion of the D1/D5 dopamine receptor agonist SKF38393 into the auditory cortex shortly after or even one day before the learning event. This effect is sensitive to rapamycin and anisomycin, suggesting that dopaminergic activity in the auditory cortex may induce mTOR-dependent translational changes supporting memory consolidation for hours or days.
To identify proteins differentially expressed one day after injection of SKF-38393 into the auditory cortex of naïve gerbils, we performed a set of proteomic analyses. Two-dimensional gel electrophoresis of subcellular fractions revealed changes in the protein profiles of the auditory cortex itself, and of distant cortical and subcortical brain structures known to receive direct or indirect projections from the auditory cortex. These include the hippocampus, frontal cortex, and striatum, i.e., brain structures implicated in long-term memory formation through novelty computation, salience attribution, and reward processing. At the molecular level, dopamine agonist-induced changes involved, in part, proteins with putative functions in the regulation of synaptic signaling (e.g., a-synuclein, 14-3-3) and of local protein synthesis (e.g., RNA-binding proteins).
The functional relevance of a-synuclein, a primarily presynaptic, mTOR-regulated protein, for FM discrimination learning was analyzed further using mice of an a-synuclein deficient C57BL/6 substrain. These mice were able to learn the discrimination task but with different kinetics when compared to mice of two a-synuclein expressing C57BL/6 substrains. Specifically, during the initial days of training, a-synuclein deficient mice learned the FM discrimination faster than non-deficient mice. Later on, however, at the asymptotic part of the learning curve, non-deficient mice reached a higher final level of discrimination performance than a-synuclein deficient mice. Augmentation and attenuation of these differences by dopamine agonist and antagonist treatment, respectively, point to an involvement of a-synuclein in dopaminergic mechanisms of memory modulation. Together, our findings suggest that dopaminergic neurotransmission in the gerbil auditory cortex may induce changes in the expression, modification and/or distribution of brain proteins, such as a-synuclein, which are involved in the modulation of long-term memory required for the discrimination of complex sounds.

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# Cellular Site and Molecular Mode of Synapsin Action in Associative Learning

Birgit Michels[1], Yi-chun Chen[2], Timo Saumweber[1], Dushyant Mishra[2], Hiromu Tanimoto[3], Benjamin Schmid[2], Olivia Engmann[2], Bertram Gerber[1]

[1]Universität Leipzig, Institut für Biologie, Lehrstuhl für Genetik, Talstrasse 33, 04103 Leipzig, Germany
[2]Universität Würzburg, Lehrstuhl für Neurobiologie und Genetik, Biozentrum am Hubland, 97074 Würzburg, Germany
[3]Max Planck Institut für Neurobiologie, Am Klopferspitz 18, 82152 Martinsried, Germany

We ask where and how Synapsin functions in associative behavioural plasticity. Synapsin is an evolutionarily conserved, presynaptic vesicular phosphoprotein. Upon loss or reduction of Synapsin in a deletion mutant or via RNAi, respectively, Drosophila larvae are impaired in odour-sugar associative learning. Acute global expression of Synapsin and local expression in only the mushroom body, a third-order 'cortical' brain region, fully restores associative ability in the mutant; no such rescue is found by Synapsin expression in mushroom body input neurons or by expression excluding the mushroom bodies. On the molecular level, we find that a transgenically expressed Synapsin with dysfunctional PKA sites cannot rescue the defect of the mutant in associative function, providing the most compelling argument to date to assign Synapsin as a behaviourally-relevant effector of the AC-cAMP-PKA cascade. We therefore argue that Synapsin acts in associative memory trace formation in the mushroom bodies, as a downstream element of AC-cAMP-PKA signaling. These analyses provide a comprehensive chain of explanation from the molecular level to an associative behavioural change.

# Common Requirement of Synapsin in Punishment- and Pain Relief-learning

Thomas Niewalda[1], Birgit Michels[1], Ayse Yarali[2], Jennifer Bretzger[1], Sören Diegelmann[1], Bertram Gerber[1]

[1]Universität Leipzig, Institut für Biologie, Lehrstuhl für Genetik, Talstrasse 33, 04103 Leipzig, Germany

[2]Max Planck Institut für Neurobiologie, 821 52 Martinsried, Germany

Shock can induce 'negative' memories for stimuli preceding it, but also 'positive' memories for stimuli presented upon its cessation: After odour - shock training, fruit flies (*Drosophila*) subsequently avoid the shock-predicting odour, whereas after presentations of the odour at a moment of 'relief' (shock - odour training), they subsequently approach the odour. We ask whether these associative processes, which we call punishment- and pain relief-learning, respectively, share molecular determinants. We focus on the role of Synapsin, an evolutionarily conserved presynaptic phosphoprotein regulating the balance between the reserve - and readily releasable-pool of synaptic vesicles. We find that a lack of Synapsin in the $syn^{97CS}$ deletion mutant leaves all sensory and motor faculties required to perform in these learning tasks unaffected. In contrast, punishment-learning is reduced by about 30 %, and pain-relief learning is fully abolished in the mutant. Both these defects (i) are also observed upon an RNAi-mediated partial knock-down of Synapsin, and (ii) are fully rescued by transgenically restoring Synapsin in mutant flies. We conclude that punishment - and relief-learning, despite their opposing effects upon behaviour, both require the Synapsin protein, and in this sense share genetic and molecular determinants. Corresponding molecular commonalities between punishment- and relief-learning in humans would constrain pharmacological attempts to selectively interfere with excessive punishment-memories e.g. after trauma.

# Cortical neurodynamics during audiovisual category transfer in rodents

Antje Fillbrandt[1], Frank W. Ohl[1, 2]

[1]Leibniz-Institut for Neurobiology, Auditory Learning and Speech, Brenneckestr. 6, 39118 Magdeburg, Germany
[2]Otto-von-Guericke University Magdeburg, Germany

A basic process in the build up of conceptual knowledge is the formation of categories involving the abstraction of shared features from the specific sensory experiences. Our previous work in the rodent (gerbil) auditory system has demonstrated that the formation of auditory categories is accompanied by the emergence of category-specific spatiotemporal activity patterns in auditory cortex (Ohl et al., Nature, 2001). Here, the investigation of the formation of category-specific activity patterns was extended to the multisensory domain. We examined whether perceptual categories, after being formed in the auditory modality, can be transferred to the visual modality, and have suggested a physiological basis for this audiovisual category transfer.
We trained Mongolian Gerbils (Meriones unguiculatus) to associate a slow (0.5 Hz) and a fast (5 Hz) presentation rate of auditory tone pips with the Go response and NoGo response, respectively, in an active avoidance paradigm (shuttle box). After a predefined performance criterion was reached for the discrimination task in the auditory modality, a second training phase was initiated in which the sensory modality of the stimuli was changed from auditory to visual. For one animal group (congruent group) the contingency of the two stimulus presentation rates with the Go/Nogo- responses stayed the same irrespective of the modality of stimulation, for a second group (incongruent group) it was reversed across modalities.
After the modality switch, the congruent groups showed a higher acquisition rate of the conditioned responses than the incongruent groups indicating a crossmodal transfer of the rate-response association.
During training, the electrocorticogram was recorded from two 16-electrode arrays chronically implanted onto the epidural surface of primary auditory and the visual cortex.
Cortical activity patterns in the ongoing electrocorticogram associated with the Go- and the NoGo stimuli were determined in the spatial distribution of signal power using a multivariate pattern classification procedure (Barrie et al., J. Neurophysiol., 1996; Freeman, J. Neurophysiol., 2000).
For animals showing correct discrimination already during the first visual training sessions we suspect that they transferred the rate-response association learned during auditory training to the visual training. In these animals activity patterns detected in the auditory and the visual cortex during visual training were similar to those observed during auditory training.

# Discrimination learning depends on the arrangement of training stimuli

Rodrigo J. De Marco[1], Shi-Bin Li[2], Tatiana Oviedo[2], Georg Köhr[2], Mario Trevino[2]

[1]Max Planck Institute for Medical Research, Developmental Genetics of Nervous System, Jahnstrasse 29, 69120 Heidelberg, Germany

[2]Max Planck Institute for Medical Research, Department of Molecular Neurobiology, Jahnstrasse 29, 69120 Heidelberg, Germany

Training improves the ability to make discriminations in a wide variety of sensory modalities, and the repetition of distributed practice increases the rate of learning (Baddeley and Longman, 1978). In nature, sensory stimuli are organized into diverse combinations that exchange salience and produce different neuronal representations. The salience of an item refers to its quality of 'standing-out' from its surroundings and it is unclear whether and how discrimination learning is affected by variable salience. To explore this, we adapted a water maze (Prusky et al., 2000, 2004) and trained adult mice to visually discriminate pairs of images from a set of 300 black-&-white, isoluminant, low-pass filtered, 'natural' scenes. Our working hypothesis assumes that the difficulty of each training trial depends on the relative salience given by structural differences between reinforced (CS+) and non-reinforced (CS-) images, simultaneously present. Accordingly, the rate of discrimination learning should depend on how the subject is exposed to steady variations in CS+ salience, as defined by either increasing or decreasing structural differences between CS+ and CS- stimuli. To quantify image structure, we apply a parametric description from image quality metrics. The structural similarity index (SSIM; Wang et al., 2004) compares local patterns of pixel intensities (covariance/standard deviation) that have been normalized by luminance and contrast. Our preliminary results show that visual discrimination in the mouse is negatively correlated to CS+/CS- similarity, suggesting that it can be approximated by a subtraction operation (Romo et al., 2003). They also show how learning rate depends on variations in stimulus salience, and thus provide a basis for successful training design.

# Effects of chronic and acute BDNF deficiency on fear learning

Thomas Endres[1], Ayse Özge Sungur[1], Anne Petzold[1], Volkmar Lessmann[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipziger Str. 44, 39120 Magdeburg, Germany

Beyond its trophic function, the neurotrophin BDNF (brain-derived neurotrophic factor) is well known to be an important mediator of synaptic plasticity and is crucially involved in memory formation. Recently, it has been shown by several studies that amygdala-dependent fear learning also depends on BDNF-TrkB-signaling. In these studies BDNF-signaling was impaired either by acute pharmacological blockade of Trk-receptors, or by a region-specific overexpression of a non-functional (truncated) TrkB receptor. Interestingly, studies using heterozygous BDNF knockout mice ($BDNF^{+/-}$-mice) reported no impairment of amygdala-dependent fear learning. The main aim of the present study is to further analyze these seemingly discrepant observations between chronic and acute BNDF depletion in amygdala-dependent learning.

Therefore, in the first part of this study, we tested $BDNF^{+/-}$-mice between one and ten months of age for their ability of fear learning. We observed an age-dependent impairment in fear learning when animals were older than three months of age. However, basal anxiety of these animals was unaltered at any tested age as measured in the elevated plus-maze and open field tests. In parallel to the behavioral experiments the electrophysiological properties of amygdala neurons of these mice were analyzed (see poster of Meis et al.). The amygdalae of all behaviorally tested animals were dissected and the BDNF protein content is currently determined by ELISA-measurements.

In the second part of this study we started to acutely block the BDNF-signaling within the amygdala, e.g. by local infusion of the tyrosinkinase-inhibitior K252a, in order to further analyze the contribution of BDNF-signaling on the acquisition and consolidation of fear memory. Preliminary results indicate an important role of BDNF-TrkB-signaling during the acquisition (injection of K252a 30 minutes before training) as well as in the early consolidation (injection 30 minutes after training) of fear memory. Interfering with BDNF-signaling in between these two time windows did not affect fear memory formation.

In conclusion, our results show an age dependent learning deficit in $BDNF^{+/-}$-mice, when these animals become older than three months of age. We hypothesize that this age-dependent learning decline is due to a diminution of BDNF within the amygdala, resulting in a relevant reduction of fear learning only when BDNF-levels fall below a critical threshold. Furthermore, our experiments provide evidence for the existence of distinct time windows for TrkB signaling in different phases of fear learning, suggesting an important role of BDNF-TrkB-signaling in the acquisition and consolidation of fear memory.

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# Establishment of food vectors by desert ants, *Cataglyphis fortis*

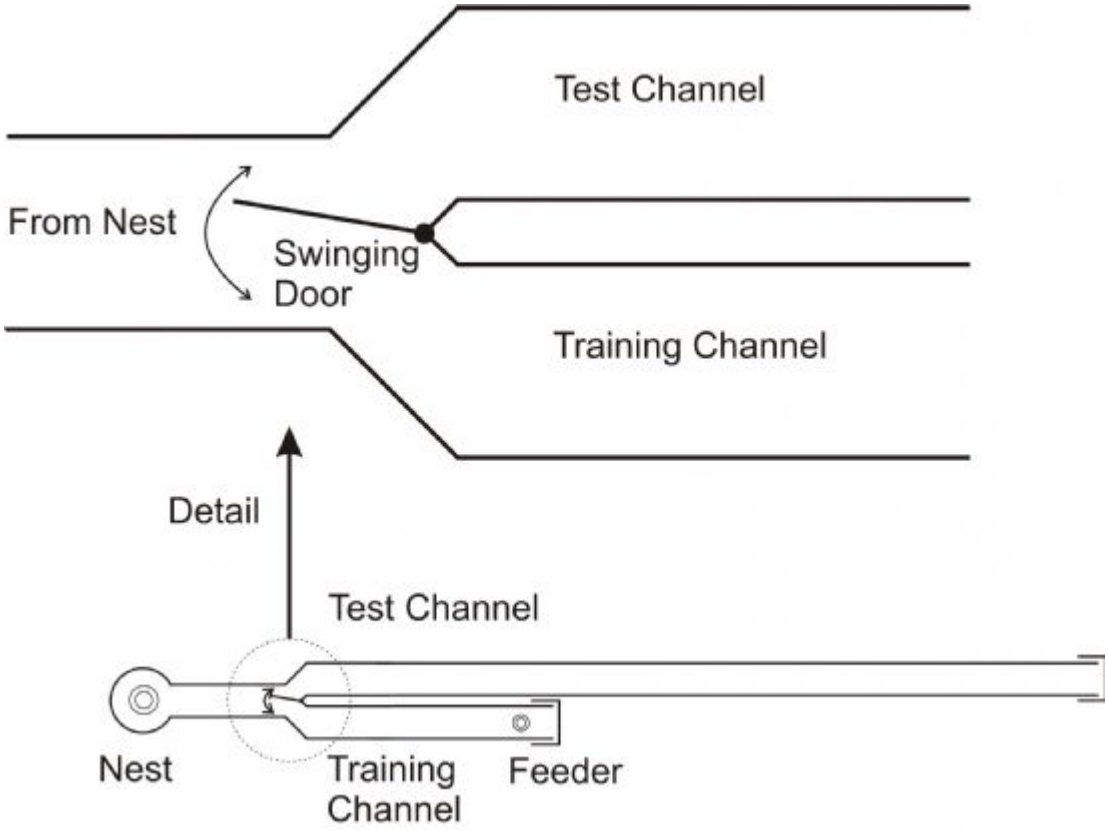
Siegfried Bolek[1], Kathrin Judith Schwannauer[1], Harald Wolf[1]

[1]Ulm University, Institute for Neurobiology, Albert-Einstein-Allee 11, 89081 Ulm, Germany

The desert ant *Cataglyphis fortis* is a central place forager whose habitat are the salt pans of North Africa. This species proved to be a most suitable object in navigation research (Wehner 2003, J Comp Physiol A 189, p. 579). *C. fortis* foragers search the surroundings of their nest for food, mostly dead insects, for distances of up to several hundred meters. After finding a food item, foragers return to the nest on a direct path rather than following their meandering outbound search trajectory. This form of navigation is independent of external landmarks (though these may be used if present). The animals rather rely on a celestial compass and an odometer to integrate their outbound journey into a straight home vector.

The desert ants' means of homing by vector navigation are well studied. What has received less attention is the question of when, and if so, how the vector towards a reliable and repeatedly visited food source is acquired. Here we demonstrate that *C. fortis* averages the lengths of outbound and inbound navigation vectors when assessing the distance of a food source. Our experimental setup was derived from a channel setup commonly used in Cataglyphis navigation research (e.g. Wittlinger & al. 2006, Science 312, p. 1965). Previous setups consisted of a training channel (U-shaped aluminium sheets, channel width 7cm) leading from the nest to a feeding station. Nest and feeder were surrounded by circular barriers connected to the channel walls, thus keeping the ants in the experimental setup. After training, i.e. after a few visits to the feeder, the ants were captured at the feeder and transferred to a test channel arranged in parallel to the training channel. The test channel bypassed the nest and thus allowed examination of nest search behaviour and homing distance. To examine food searching (rather than homing) behaviour, we redirected the outbound (rather than homebound) ants into the test channel arranged immediately next to the training channel. This was achieved by a swinging gate that allowed access from the nest to either training or test channels (Fig.).

Initial control experiments verified that ants showed similar food search behaviour in test channel and open terrain (except for the reduction to one dimension of search space). A first group of ants was trained to a feeder located at 10m distance from the nest, and these ants searched for food in the test channel around a distance of 10.60m - as was to be expected. Corresponding results were obtained for 20m nest–feeder distance, with search distances of about 22.20m. In the critical experiment, a third group of ants was also trained to a feeder 20m distant from the nest. After having picked up a food crumb, the animals were captured and displaced homeward in the training channel to 10m distance from the nest. These animals had thus covered an outbound (search) distance of 20m and a homing distance of 10m. After this training, the ants re-emerging from the nest were directed into the test channel and their search behaviour examined. Their search was centred on a distance about 17.00m from the nest, suggesting that food vectors in *Cataglyphis* are created by averaging the last outbound and inbound travel distances. This conclusion agrees with experiments in *Apis mellifera* (Otto 1959, J Comp Physiol A 42, 303).


Test Channel
From Nest
Swinging
Door
Training Channel
Detail
Test Channel
Nest
Training
Channel
Feeder

# Evidence for one-shot learning in the honeybee

Evren Pamir[1,2], Nicola Stollhoff[3], Katrin Gehring[3], Neloy Kumar Chakraborty[3], Randolf Menzel[3], Dorothea Eisenhardt[3], Martin Nawrot[1,2]

[1]Freie Universität Berlin, Neuroinformatics & Theoretical Neuroscience, Institute of Biology, Freie Universität Berlin, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

[2] Bernstein Center for Computational Neuroscience Berlin, Berlin,Germany

[3] Institute of Biology - Neurobiology, Freie Universität Berlin, Germany

Associative olfactory learning in honeybees ( *Apis melifera*) can be studied by classical conditioning of the proboscis extension response (PER) (Bitterman, 1983). Traditionally, the mean behavioral performance of a population of identically treated animals is regarded as an estimate for the amount of learning and memory formation in individual bees .Here we asked if the gradually increasing *population* PER probability across trials represents the behavioral performance of *individual* animals as implicated by the traditional population analysis. We tested the null-hypothesis that for a certain conditioning or retention trial, the PER probabilities of subgroups of animals that were selected on the basis of their behavior in the previous trial are equal to the population PER probability. Analyzing data from 19 independent experiments with a total of 1486 bees we found that the null-hypothesis had to be rejected in the vast majority of testable cases. In order to formulate an alternative hypothesis we asked if the behavioral data might be correctly described by a Hidden Markov Process. We therefore evaluated the likelihood of 3 generative models by a cross -validation algorithm: (i) the Recorla -Wagner (RW) Model (Rescorla and Wagner, 1972) that represented the traditional null-hypothesis, (ii) a two-state, and (iii) a three-state Hidden Markov Model. The results of our analyses provide strong evidence for an abrupt and rapid learning process and dispute a gradually increasing learning process as formalized by the RW model. Interestingly, Gallistel et al. (2004) reported the same result for the learning process of individual animals in several well-known vertebrate conditioning paradigms.

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# Fear extinction learning in heterozygous BDNF knockout mice

Laura Psotta[1], Volkmar Lessmann[1], Thomas Endres[1]

[1]Otto-von-Guericke University, Institute of Physiology, Leipziger Str. 44, 39120 Magdeburg, Germany

The neurotrophin brain-derived neurotrophic factor (BDNF) is abundantly expressed throughout the mammalian brain. A large number of studies have shown that BDNF is crucially involved in synaptic plasticity, learning and memory formation. Recently, several studies showed that interfering with the BDNF-TrkB-signaling within the amygdala leads to an impairment of amygdala-dependent fear learning (e.g. Ou & Gean; Neurobiol. Learn Mem, 93(3), 372-82, 2010). Beyond fear learning also the extinction of fear memories seems to be dependent on BDNF-signaling (compare Peters et al., Science 328(5983), 1288-1290, 2010): acute administration of BDNF into the infralimbic prefrontal cortex was sufficient to induce fear extinction, even without extinction training. The main aim of the present study is to analyze the age dependence of fear extinction learning in aged heterozygous BDNF knockout mice ($BDNF^{+/-}$-mice), which chronically lack approximately half of the BDNF levels of wild type animals.

Since our group observed an age-dependent impairment in fear learning in these mice when they became older than three months of age (see poster by Endres et al.), we first tested fear learning in aged $BDNF^{+/-}$-mice in response to different classical fear conditioning protocols. We observed that increasing the strength of the fear conditioning protocol by raising the number of tone-shock pairings, restores fear learning even in aged $BDNF^{+/-}$-mice. These data indicate that fear learning in $BDNF^{+/-}$-mice is only impaired but not abolished during aging. After identifying a fear conditioning protocol which reliably induces conditioned fear also in aged $BDNF^{+/-}$-mice, we began to test for fear extinction learning. Therefore we exposed the animals on the consecutive days only to the conditioned stimulus (CS) and quantified the conditioned freezing in response to the CS. After conditioned fear was extinguished the animals underwent a renewal test where the CS was presented in the same context where the conditioning training took place. We observed already in younger animals (two months old) an impairment in the late extinction (2nd extinction session) whereas short- term extinction (within session extinction) performance seems to be unaltered, suggesting an impairment in extinction memory consolidation. Furthermore, in the renewal test $BDNF^{+/-}$-mice performed worse than their wild type littermates. Experiments analyzing fear extinction learning in aged $BDNF^{+/-}$ animals are currently in progress. After finishing the behavioral testing the brains of the tested animals were dissected and the BDNF protein content of the amygdala and the medial prefrontal cortex will be quantified by using ELISA technique. Best to our knowledge, this is the first study analyzing the extinction learning in mice which have a chronic deficit of endogenous BDNF.

In conclusion, our results demonstrate that heterozygous BDNF knockout mice posses an age dependent impairment in fear learning in response to a weak fear conditioning protocol (i.e. three tone-shock pairings). This deficit can be restored by using stronger conditioning protocols. Furthermore, $BDNF^{+/-}$-mice show impaired extinction learning which might be due to decreased extinction memory consolidation.

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# Fear Learning and Extinction in an Automated Home Cage (DualCage) Environment

Torben Hager[1], René Jansen[1], Anton Pieneman[1], Dawid Eckert[2], Stephan Schwarz[2], Christian Gutzen[2], Oliver Stiedl[1]

[1]VU University Amsterdam, Behavioral Neuroscience Group, De Boelelaan 1085, 1081 HV Amsterdam, Netherlands
[2]Biobserve GmbH, Königswinterer Str. 418, 53227 Bonn, Germany

Fear conditioning is an important test in behavioral neuroscience to investigate the neural systems and molecular basis of various aspects of emotional learning across a wide range of species including rodent models. Dysfunction of the fear circuits is implicated in mechanisms of affective disorders. Traditional fear conditioning methods require frequent handling of the animals, which confounds the measurement of fear-related parameters such as the behavioral response and autonomic responses (e.g. heart rate) of the animal. We therefore developed a novel fully automated home cage environment (DualCage) to explore the dynamics of fear responses during deliberate and/or motivated choice of singly housed male C57BL/6J mice. The DualCage consists of a home compartment (HC) attached to a shock compartment (SC) separated by an automatically operated sliding door. A separation of compartments is crucial to exploit the novelty-seeking behavior of mice at distinct times after training to investigate short- and long-term memory.
Mouse behavior is monitored by two cameras using specific tracking software (Viewer©, Biobserve) with a software script controlling the actions of the hardware. This includes the opening and closing of doors and shock delivery at specific times and/or depending on the position of the mouse in an operant fashion, e.g. after an instrumental responses such as entering the SC. The behavior is monitored for several days throughout the light and dark phases and the video is stored digitally. Mice are not handled during the entire experiment to monitor spontaneous exploratory behavior during training and testing.
A mouse is initially habituated to the HC for 3 days. For acquisition of conditioned fear the sliding door is opened and the mouse can deliberately enter the SC. As soon as it has fully entered the SC, the door is closed. The mouse then explores the SC for 30 s before a single/series of tone/shock stimuli are used to condition the mouse to the tone and the SC (context). After another 30 s, the door is opened and the mouse can return to its HC.
Retention tests are performed at different times after the training session to explore the dynamics of memory formation, its expression and extinction. Tone retention tests can be performed in the HC or in the SC. The latter obviously works only if and when the conditioned mouse voluntarily returns to the SC. The spontaneous behavior elicited by contextual fear memory is characterized by risk assessment when access to the SC is granted including peeking through the door in a stretch-attend posture before eventually revisiting the SC after considerable time. Revisits of the SC eventually occur despite the lack of e.g. rewarding reinforcement indicating the natural motivation of mice to explore their environment if given a chance despite previous negative experiences. This resembles predator avoidance behavior of rats in a visible burrow system [1]. Behavioral measures are now being complemented by autonomic [2] and neural measures of conditioned contextual fear and its extinction.
This DualCage system exploits naturalistic-like behavior of mice that is characterized by trade-offs between avoidance and novelty-seeking (curiosity) in conflict situations with quantification on long time scales based on deliberate choice. The importance of deliberate choice for the temporal organization of behavior has recently been demonstrated in open field exploration in mice [3]. The DualCage system with a separation of HC and novel compartment is equipped with versatile add-on components designed to integrate any commercially available hardware for multi-purpose use depending on the scientific questions and the behavior of interest. This approach will avoid any unspecific interference by the experimenter and may increase the replicability of findings.

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# Functional analysis of the octopaminergic/tyraminergic system in *Drosophila* larval classical olfactory conditioning

Dennis Pauls[1], Mareike Selcho[1,2], Christian Wegener[2], Reinhard Stocker[1], Andreas Thum[1]

[1] University of Fribourg, Department of Biology, Zoology, Chemin du Musée 10, 1700 Fribourg, Switzerland; [2]Philipps-University Marburg, Department of Biology, Neurobiology/Ethology, Karl-von-Frisch-Str. 8, 35032 Marburg, Germany

The *Drosophila* larva is able to associate an odor with salt punishment or sugar reward, respectively [1]. Simple neuronal circuits facilitate the accessibility on the genetic, molecular and physiological level. Yet, detailed analysis on the olfactory pathway is available up to third-order neurons in the mushroom bodies including detailed reports on the anatomy and physiology of olfactory receptor neurons, the antennal lobes and the projection neurons [1]. Learning and memory is based on experience-dependent neuronal plasticity within the mushroom bodies. For this purpose the simultaneous arrival of conditioned and unconditioned stimulus information is required at the level of the Kenyon cells [2]. So far, it is shown that dopaminergic neurons are required to mediate sugar reward and salt punishment information within the brain [3]. As former studies suggested that octopaminergic/tyraminergic neurons are both necessary and sufficient to form appetitive memories [4,5] we revisited their role in larval chemosensory learning in more detail. Preliminary results suggest that octopaminergic/tyraminergic neurons are necessary for normal locomotion. Our functional approach will hopefully demonstrate whether these neurons are also required for chemosensory learning in the *Drosophila* larva.

*[1] Gerber and Stocker 2007; [2] Gerber et al., 2004; [3] Selcho et al., 2009; [4] Schroll et al., 2006; [5] Honjo and Furukubo-Tokunaga, 2009*

# Generalization and transfer in honeybee navigation

Konstantin Lehmann[1], Uwe Greggers[1], Randolf Menzel[1]

[1]Freie Universität Berlin, Institut für Biologie - Neurobiologie, Königin-Luise-Str. 28/30, 14195 Berlin, Germany[11]

Honeybees learn about the environment by exploratory orientation flights and during their foraging flights. Local and long ranging landmarks are used as navigation cues. We addressed the question whether the geometry of long ranging landmarks (e. g. tree lines, irrigation canals, roads) learned in one environment (the home environment) is used in navigating in a different (the test) environment. Experienced bees were transferred into the test environment which resembled partially landmark features of their home environment. The search flights of single bees were monitored with the harmonic radar. We found that bees generalized between landscapes according to the similarity of long ranging landmarks. The patterns of their search flights indicated that they navigated according to the memory of the geometry of long ranging landmarks in their home environment embedded in the sun compass. These findings are interpreted as indicating a kind of bearing map that bees are able to retrieve even under conditions that did not provide the respective landmark features. Our data support a model of a map-like representation composed of isolated local and integrated long ranging landmarks as put forward by Jacobs and Schenk (2003)[1].

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# Differential dopaminergic circuits for the formation of olfactory memory in Drosophila

Hiromu Tanimoto[1], Yoshinori Aso[1], Igor Siwanowicz[1], Chang Liu[1]

[1]MPI of Neurobiology, NWG Tanimoto, Am Klopferspitz 18, 82152 Martinsried, Germany

A paired presentation of an odor and electric shock induces aversive odor memory in Drosophila melanogaster. Electric shock reinforcement is mediated by dopaminergic neurons, and it converges with the odor signal in the mushroom body (MB). Dopamine is synthesized in ~280 neurons that form distinct cell clusters and is involved in a variety of brain functions. Recently, one of the dopaminergic clusters (PPL1) that includes MB-projecting neurons was shown to signal reinforcement for aversive odor memory. As each dopaminergic cluster contains multiple types of dopaminergic neurons with different projections and physiological characteristics, functional understanding of the circuit for aversive memory requires cellular identification. We here show that MB -M3, a specific type of dopaminergic neurons in cluster PAM, is preferentially required for the formation of labile memory. Strikingly, flies formed significant aversive odor memory without electric shock, when MB-M3 was selectively stimulated together with odor presentation. We additionally identified another type of dopaminergic neurons in the PPL1 cluster, MB-MP1, which can induce aversive odor memory. As MB-M3 and MB-MP1 target the distinct subdomains of the MB, these reinforcement circuits might induce different forms of aversive memory in spatially segregated synapses in the MB.

# Hebbian plasticity combined with Homeostasis shows STDP-like behavior

Christian Tetzlaff[1], Christoph Kolodziejski[2,3], Florentin Wörgötter[1,3]

[1]University of Goettingen, III. Physics - Computational Neuroscience, Friedrich-Hund Plastz 1, 37077 Goettingen, Germany
[2]Max-Planck Institute for Dynamics and Self-Organization, Bunsenstrasse 10, 37073 Goettingen, Germany
[3]Bernstein Center for Computational Neuroscience, Goettingen, Germany

Memory formation as well as other functional properties of neural networks are dependent on the network connectivity. Connections between neurons are formed by synapses (synaptic weights) and are modified by different biological mechanisms. In this study, we considered the following three mechanisms: Hebbian, Homeostatic, and Spike-Timing-Dependent (STDP) plasticity. In Hebbian plasticity (Hebb, 1949; Dayan and Abbott, 2001) weight change depends on the activity rate of pre- and post-synaptic neuron and, thus, is a local learning rule, whereas in Homeostatic plasticity (Van Ooyen, 1994; Turrigiano and Nelson, 2004) weight change depends only on the rate of the post-synaptic neuron. This mechanism adjusts all synapses of the incoming inputs of the neuron and, therefore, is a global plasticity process. STDP, like Hebbian placticity, is a local process, however, differently from Hebbian plasticity (which can only produce long-term potentiation) it does not depend on the rates of pre- and post-synaptic neuron, but on the timing of the neuronal activity, and can either lead to long-term potentiation or long-term depression (LTP and LTD, Bi and Poo, 1998; Caporale and Dan, 2008). However, STDP can be reformulated in a rate description (Bienenstock et al., 1982; Izhikevich and Desai, 2003).
In this study we are interested in the behavior of these three mechanisms with respect to the change of the dynamics when they interact with each other. To test this we analyzed single and two neuron systems analytically and numerically by looking at their fixed points in weight development. We found out that these fixed points change their position and stability state (from stable to unstable or vice versa) dependent on the parameter space related to these mechanisms. We show that an interaction of Homeostatic plasticity with both other mechanisms leads to a stable weight development. We also demonstrate that Hebbian together with Homeostatic plasticity shows qualitatively the same behavior as the rate description of STDP. This means that STDP, which has both LTP and LTD, can be a mixture of Hebbian and Homeostatic plasticity.
In general, we demonstrate that dynamically different plasticity mechanisms change their behavior dramatically when they interact with each other.

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# Homing pigeons with navigational experience show a more lateralised brain than pigeons without navigational experience

Julia Mehlhorn[1], Gerd Rehkämper[1]

[1]University of Düsseldorf, C.&O. Vogt Institute of Brain Research, Universitätsstrasse 1, 40225 Düsseldorf, Germany

Homing pigeons (Columba livia f.d.) are well-known for their homing abilities which are based on a genetical predisposition, multimodal learning and spatial cognition. Their brain seems to be functionally adapted to homing as exemplified, e.g. by larger hippocampi and olfactory bulbs. As in other birds, functional specialization of the two hemispheres of the brain ('lateralization') occurs as well in homing pigeons.
In a recent study of the influence of experience on hippocampus volume, an increased hippocampus volume in pigeons with navigational experience had been observed. To show in what way lateralization is reflected in brain structure volume and whether some lateralization is caused by experience, we compared brains of homing pigeons with and without navigational experience referring to this. Fourteen homing pigeons were raised under identical constraints. After fledging, seven of them were allowed to fly around the loft and participated successfully in pigeon races. The other seven stayed permanently in the loft and thus did not share the navigational experiences of the first group. After reaching sexual maturity, all individuals were sacrificed and morphometric analyses were carried out to measure the volumes of five basic brain parts and eight telencephalic brain parts. Measurements for the telencephalic brain parts and optic tectum were done separately for the left and right hemispheres.
The comparison of left/right quotients of both groups reveal that pigeons with navigational experience show a smaller left mesopallium in comparison to the right one and pigeons without navigational experience a larger left mesopallium in comparison to the right one. Additionally, there are significant differences between left and right brain subdivisions within the two pigeon groups namely a larger left hyperpallium apicale in both pigeon groups and a larger right nidopallium, left hippocampus and right optic tectum in pigeons with navigational experience. Pigeons without navigational experience did not show more significant differences between their left and right brain structures.
The results of our study confirms that the brain of homing pigeons is an example for mosaic evolution and indicates that lateralization is correlated with individual life history (experience) and not exclusively based on heritable traits.

# Honeybees integrate learned and communicated flight vectors in navigation

Andreas Kirbach[1], Jacqueline Fuchs[1], Konstantin Lehmann[1], Uwe Greggers[1], Randolf Menzel[1]

[1]Freie Universität Berlin, Department of Neurobiology, Königin-Luise-Str. 28-30, 14195 Berlin, Germany

Honeybees share their knowledge about a feeding place with their nest mates. By means of a ritualized behavior, the waggle dance, they communicate locations by encoding vector information. Since many of the recruited bees will have visited another feeding place before they follow a dancing bee they will have to decide between the communicated vector and the flight vector they have learned before. We carried out an experiment in which we tracked the flights of recruits with harmonic radar. We asked whether recruits compare these two vectors and whether they decide between them. Recruits indeed chose between the two vectors. If the directional components of the two vectors in the 600m experiment were more similar (30° rather than 60° difference) the learned vector was more frequently used. Decision for the communicated vector requires more information (more waggle runs were followed). In the 30° condition some bees performed novel shortcut flights between the communicated and learned location in both directions, indicating that bees integrate communicated and experienced information in a common spatial representation. To determine whether the bees use the angle difference or the absolute distance between the feeding place and the dance indicated place we repeated the experiment with a distance of 300m between the hive and the feeding place or the dance indicated place. In this situation recruits performed novel shortcut flights also when the angle was 60°. This shows that the decision to fly directly between the two places depends on the absolute distance between these two places.

# How outcome expectations organize learned behaviour in larval *Drosophila*

Michael Schleyer[1], Wiebke Nahrendorf[2], Benjamin Fischer[2], Timo Saumweber[1], Bertram Gerber[1]

[1]Universität Leipzig, Institut für Biologie, Lehrstuhl für Genetik, Talstraße 33, 04103 Leipzig
[2]Universität Würzburg, Lehrstuhl für Neurobiologie und Genetik, Biozentrum Am Hubland, 97074 Würzburg

*Drosophila* larvae combine a numerically simple brain, a correspondingly moderate behavioural complexity and the availability of a rich toolbox for transgenic manipulation. This makes them attractive as a study case when trying to achieve a circuit-level understanding of behaviour organization. From a series of behavioural experiments, we here suggest a circuitry of chemosensory processing, odour-tastant memory trace formation and the 'decision' process to behaviourally express these memory traces - or not. The model incorporates statements about the neuronal organization of innate versus conditioned chemosensory behaviour, and the kinds of interaction between olfactory and gustatory pathways during the establishment and behavioural expression of odour-tastant memory traces. It in particular suggests that innate olfactory behaviour is responsive in nature, whereas conditioned olfactory behaviour is captured better when seen as an action in pursuit of its outcome. It incorporates the available neuroanatomical and behavioural data and thus should be useful as scaffold for the ongoing investigations of the chemo-behavioural system in larval *Drosophila*.

# Increase of fearlessness and latency behavior of Prnp0/0 mice

Matthias Schmitz[1], Catharina Greis[1], Walter Schulz-Schaeffer[2], Andre Fischer[3], Inga Zerr[1]

[1]National TSE Reference Center, Department of Neurology, Georg-August University Goettingen, Robert-Koch-Str. 40, 37075 Goettingen, Germany

[2]Department of Neuropathology, 3Department of Clinical Chemistry, Medical Center Georg-August University, Göttingen, Germany

[3]Laboratory for Aging and Cognitive Diseases, European Neuroscience Institute, Grisebach Str. 5, D-37077 Goettingen, Germany.

The cellular prion protein (PrPc) is a multifunctional protein that may be implicated in cellular adhesion, neurite outgrowth, excitability and maintenance, differentiation, synaptic plasticity and cognition. However, the exact physiological function of PrPc is not completely understood. Mouse models in which PrPc gene (PRNP) was ablated have been used in several studies to examine behavior and cognition. First observations on PrPc knockout mice (PrP0/0) did not show any important behavioral abnormalities (Bueler et al., 1992). However some recent studies had pointed out some differences between PrP0/0 and wildtype mice (WT). PrP0/0 mice exhibit an altered circadian rhythm and alterations in sleep (Tobler et al., 1996).Furthermore, locomotor performance, latent learning and long term-memory or synaptic transmission (Martins 2002; Wong et al., 2001) can be influenced by PrPc.

In our study we used male knockout mice homozygous for the disrupted PrPc gene, Prnp null mice (Zrch I, 29B6) produced as previously reported (Bueler et al., 1992). We examined age related behavioral changes in PrP0/0 mice in comparison to WT PrP+/+ mice. Three, nine and twenty months old mice were submitted to a battery of behavior tasks such as Elevated Plus-Maze, Rotarod and Morris Water Maze. The Elevated-Plus-Maze test is based on rodent's aversion of open spaces. Here, we found age and PrPc dependent changes regarding the general anxiety behavior. In addition, PrP0/0 mice showed a markedly higher latency to explore a new environment compared to WT mice. Moreover, we investigated the spatial learning, the ability to be adapted on a new environment and the motor coordination and the ability to keep the balance.

All together PrP0/0 animals exhibited significant behavioral differences, which may explain, why PrPc is highly conserved in the evolution.

# Increased pupil size distinguishes familiar from novel images

Marnix Naber[1], Ueli Rutishauser[2], Wolfgang Einhäuser[1]

[1]Philipps-University Marburg, Neurophysics, Karl-von-Frisch-Str. 8a, 35032 Marburg, Germany
[2]Max Planck Institute for Brain Research, Neural Systems and Coding, 60528 Frankfurt, Germany

Pupil size has been associated with cognitive processes such as decision making, arousal, attention, and emotional valence. Here we examined whether the pupil size relates to recognition memory. In a series of experiments, we recorded pupil size while observers viewed sequences of images of different difficulty (man-made objects, dissimilar natural scenes, similar natural scenes). First, observers were asked to remember 100 novel and unique images shown in sequence (memorization phase). After a delay (during which active rehearsal was precluded by a distracter task), observers' recognition memory was tested by randomly intermixing the target images with 100 novel images (rehearsal phase). For each image, observers indicated whether the image was old or new together with a confidence (sure, less sure, guessing) judgment. We found that, independent of the image set and task difficulty, observers had larger pupils after the presentation of familiar images compared to novel images. Furthermore, pupil size was larger for intermediate levels of confidence (less sure) than for high and low levels of confidence (sure and guessing). Since behaviorally relevant stimuli (i.e., targets) affect pupil dynamics, the increased pupil size for familiar images could, however, be a consequence of target selection rather than of memory. In a second series of experiments, we presented each image up to four times during the memorization phase. This procedure manipulated familiarity during memorization without creating explicit recognition targets. Again, the pupil was larger for familiar images (2nd to 4th presentation) than for novel images (1st presentation). To test whether this effect depended on explicit memorization, we repeated the experiment with different instructions: rather than memorizing the images, observers were asked to rate their aesthetic quality. The pupil increase for familiar images remained even without an explicit memory component of the task. In conclusion, we found that pupil size reflects image familiarity irrespective of task difficulty and explicit memorization.

# Interaction of genetics and environmental influences during fear extinction in 5-HTT knock-out mice

Venu Narayanan[1], Jörg Lesting[1], Rebeca Heiming[2], Frederica Jansen[2], Nobert Sachser[2], Klaus-Peter Lesch [3], Hans-Christian Pape[1], Thomas Seidenbecher[1]

[1]Westfälische Wilhelms-University Münster, Institute for Physiology I, Münster, Germany
[2]Westfälische Wilhelms-University Münster, 2Institute of Neuro and Behavioural Biology I, Münster, Germany
[3]University Würzburg, Unit for Molecular Psychiatry. Würzburg, Germany

Introduction: Emotional behaviors such as fear and anxiety can be modulated by both, environmental and genetic factors. Thereby the serotonin transporter (5-HTT) plays a key role in the regulation of central 5-HT neurotransmission. The 5- HTT-deficient mouse is an established model for emotional dysregulation (anxiety, depression etc.). The aim of this study was to evaluate the consequences of genotype specific disruption of 5-HTT function and repeated social defeat for fear-related behaviors and corresponding neurophysiological activities in 5-HTT wild type ($^{+/+}$), homo- ($^{-/-}$) and heterozygous ($^{+/-}$) mice.

Methods: Male 5- HTT naïve mice and experienced losers (generated in a resident -intruder paradigm) were unilaterally equipped with stainless steel electrodes in the lateral amygdala (LA) and medial prefrontal cortex (mPFC). All animals underwent a Pavlovian fear conditioning. One day after conditioning, animals run through an extinction training (R1- R6); 24 hours later, recall of extinction was tested (E1, E2). Tone - and fear-related behaviors (e.g. freezing) and field potential activities (with respect to theta frequency synchronization) in LA and mPFC were simultaneously recorded before and during the presentation of the conditioned stimuli.

Results: Compared to controls, 5-HTT$^{-/-}$ naïve mice showed significantly impaired recall of extinction, (see A, % freezing). In contrast, 5-HTT$^{-/-}$ losers showed delayed extinction learning and impaired recall of extinction. Furthermore, the impaired behavioral responses were accompanied by increased theta synchronization between the LA and the mPFC during extinction learning in 5-HTT$^{-/-}$ losers and extinction recall in 5-HTT$^{-/-}$ naïve and 5-HTT$^{-/-}$ loser mice (B).
Conclusion: Extinction learning and memory of conditioned fear can be modulated by both the 5-HTT gene activity and social experiences in adulthood.

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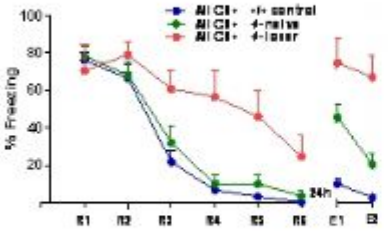
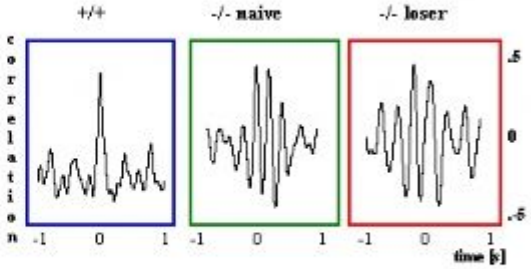



A. The 5-HTT$^{-/-}$ naïve mice exhibited impaired recall of extinction (high level of freezing during E1). The 5-HTT$^{-/-}$ losers showed delayed extinction and impaired extinction recall.




B. LA-PFC theta synchronization during E1. Note, increased theta synchronization in 5-HTT$^{-/-}$ naive and $^{-/-}$ loser.

# Learning from positive and negative rewards

Wiebke Potjans[1], Abigail Morrison[3,4,2], Markus Diesmann[1,2,5]

[1]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Jülich, Germany
[2]RIKEN Brain Science Institute, Wako-shi, Saitama, Japan
[3]Functional Neural Circuits Group, Faculty of Biology, Albert-Ludwigs-University of Freiburg, Germany
[4]Bernstein Center Freiburg, Albert-Ludwigs-University of Freiburg, Germany
[5]Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako-shi, Saitama, Japan

Animal learning is highly driven by reward and punishment. However, it is still an open problem in the field of computational neuroscience how reward learning is carried out on the neuronal level. One influential hypothesis in this context is that the brain implements temporal-difference (TD) learning, an algorithm capable of solving complex tasks with sparse reward. This hypothesis is mainly based on experimental findings involving the dopaminergic system. In particular, it has been found that the phasic dopaminergic activity resembles the TD error during reward learning [1] and that cortico-striatal synaptic plasticity is modulated by dopamine [2]. However, the phasic dopaminergic signal can only realize an imperfect TD error due to the low baseline firing rate. In addition, synaptic weights are limited in their ability only to represent estimates of future rewards, due to their inherent lower bound.

To analyze the consequences of a dopaminergic system on TD learning, we develop a spiking neuronal network model that integrates multiple experimental results. Our model generates a phasic dopaminergic signal with realistic firing rates which is in turn exploited by dopamine-dependent plasticity that is consistent with experimental data with respect to pre- and postsynaptic activity and dopamine concentration. Our analysis shows that the deviation of the neuronal error signal from the theoretical TD error results in a slightly modified TD learning method with self-adapting learning parameters.

We demonstrate that the spiking neuronal network is able to learn a grid-world task with sparse reward with similar speed and equilibrium performance to a traditional TD learning implementation. However, differences in the learning behavior between the neuronal and the traditional algorithm become apparent in cliff-walk tasks, where the agent is punished when stepping into a certain region. If an external reward is present in addition to punishment, we find that the neuronal agent is still able to learn the cliff-walk task, albeit with a slightly different learning strategy than the traditional TD learning agent. However, if learning is driven exclusively by punishment, we find that the task can no longer be learned by the neuronal agent, as in this case all synaptic weights reach their minimal allowed values.

Our results show that dopamine-dependent plasticity modulated by a phasic dopaminergic error signal enables TD learning when learning is predominantly driven by reward, but not by punishment. In the literature, two main hypotheses have been proposed to account for how negative errors are represented in the mammalian brain. Whereas it has been suggested that negative error could be encoded in the duration of the phasic activity of the dopamine neurons [3], our results support the second hypothesis, namely that negative errors are represented by a different neuromodulator such as serotonin [4].

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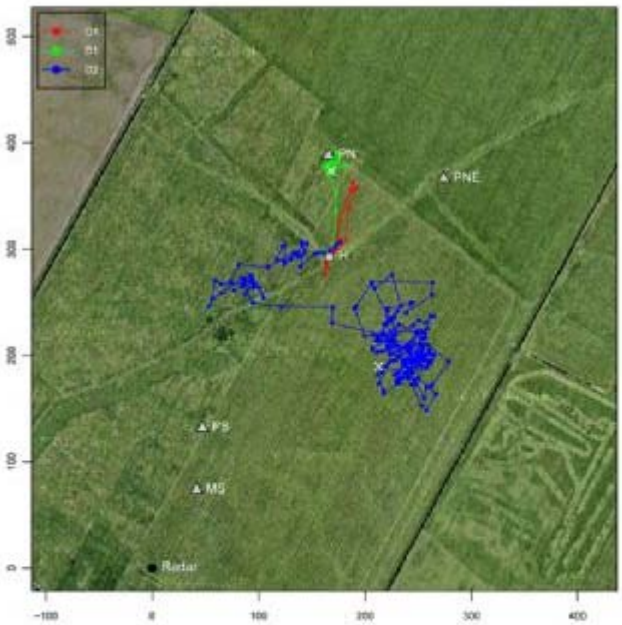
# Learning to navigate: orientation flights of young honeybees

Jacqueline Fuchs[1], Andreas Kirbach[1], Konstantin Lehmann[1], Uwe Greggers[1], Randolf Menzel[1]

[1]Freie Universität Berlin, Department of Neurobiology, Königin-Luise-Str. 28-30, 14195 Berlin, Germany

Honeybees perform exploratory orientation flights after leaving the hive for the first time. Path integration and learning of landmarks are thought to contribute to their save return to the hive. We monitored the first exploratory flight of young bees of known age with the harmonic radar which enables us to track the complete flight path of bees. After their return to the hive we transported these bees to the explored and then to the unexplored area and vice versa. Bees found their way back to the hive significantly faster when displaced to the explored rather than to the unexplored area. If bees apply only path integration they should not have been able to return to the hive after displacement. Hence we conclude that bees learn about the spatial relations of landmarks during their first orientation flight.




Flights of an individual bee plotted on the landscape where the experiments were performed. The legend indicates the order of the flights (O = Orientation flight; D = Displacement). H marks the position of the hive, X the release sites, PN, PNE and PS landmarks on the ground (plastic tarps) and MS the weather station

# Mapping of regional brain activity during two-way active avoidance behavior using 2-FDG autoradiography and in-vivo SPECT imaging

Anja Mannewitz[1], Jürgen Goldschmidt[2], Anett Riedel[1], Michael Gruss[1], Jörg Bock[1], Katharina Braun[1]

[1]Otto von Guericke University, Institut of Biology, Zoology/Developmental Neurobiology, Leipziger Str. 44, Bldg. 91, 39120 Magdeburg, Germany

[2]Leibniz Institute for Neurobiology, Department Auditory Learning & Speech, Brenneckestraße 6, 39118 Magdeburg, Germany

Using 2-Fluoro-deoxy-glucose (2-FDG) autoradiography, we recently described regional metabolic brain activity and functional connectivity in rats undergoing two-way active avoidance (TWA) training in the shuttle box. In contrast to adolescent rats (P38-P42), infant rats (P17-P21) do not learn the TWA task despite intensive training. This difference in the behavioral output is reflected by the degree of functional coupling of a number brain regions related to emotional-autonomic circuits. Thus, in adolescent rats, the metabolic activities of the cortico-limbic, hippocampal, amygdaloid, striatal and brain stem regions as well as primary sensory and motor areas measured significantly correlate among each other; the only regions that are functionally not coupled are the infralimbic cortex and central nucleus. In infant rats displaying a poor TWA performance, the metabolic activities of the caudal hippocampus, rostral subiculum, central and medial nuclei of the amygdala are not coupled with the other brain regions.

A disadvantage of 2-FDG autoradiography is that patterns of neuronal activity can be mapped only once. Thus, e.g. developmental learning aspects cannot be followed up within one individual animal. In order to circumvent this problem, we employed small animal single photon emission computed tomography (SPECT)-imaging of regional cerebral blood flow. Rats were catheterized via the external jugular vein and injected with the blood flow tracer 99m-Technetium HMPAO at different training stages i.e. during the 1$^{st}$ (acquisition phase) and 5$^{th}$ (retrieval phase) day of TWA training in the shuttle box.

This makes it possible to monitor individual and ontogenetic changes in regional brain activity related to acquisition and retrieval of the TWA task and compare these results with the 2-FDG measurements.

The study was supported by the German Research Foundation (Sonderforschungsbereich 779), the EU (EFRE) and the CBBS (Center for Brain and Behavioral Sciences, Magdeburg).

# Mapping the Individual Body-Size Representation Underlying Climbing Control in the Brain of *Drosophila melanogaster*

Tammo Krause[1], Roland Strauss[1]

[1]Johannes Gutenberg University, Zoology III Neurobiology, Colonel Kleinmannweg 2, 55099 Mainz, Germany

Wild-type *Drosophila* flies limit their climbing-effort only to surmountable gaps (Pick and Strauss 2005). Small flies systematically try to overcome only smaller chasms in the walkway whereas big individuals try to surmount also wider gaps. The initiation of a climbing attempt depends on the own body size in relation to the chasm's width, which is visually estimated. But body size critically depends on the food and temperature conditions during the larval stages and cannot be genetically determined in the brain. Indeed, the decisions of learning defective *dunce*[1]- and *rutabaga*[1]- flies are independent of their individual size. They try to overcome chasms which are clearly impossible to cross suggesting that the information on body size depends on the cAMP cascade. In this study, we rescued the climbing adaption in a *rutabaga*[1]- mutant background via expressing UAS-*rutabaga* with GAL4-lines. The *rutabaga* encoded adenylyl cyclase is not needed during the developmental stages. It is sufficient for the rescue of the behavioral phenotype to provide *rutabaga* just acutely via GAL80$^{ts}$-constructs during the adult stage. Furthermore, we tried to find out when and how wild-type flies learn about their body size. Trial and error learning in the paradigm can be excluded, because the frequency of climbing-attempts does not decrease during the approaches. Flies, which had been raised in the dark until the experiment showed significantly more climbing attempts than their siblings which were raised under normal 10/14h dark-light cycles. Moreover, flies that had been kept in isolation since the pupal stage but under normal dark-light cycles for three days after hatching, performed similar to those flies raised in complete darkness. We conclude that the own body size is learned from visual experience as well as interactions with other individuals.

This work is supported by the German Science Foundation under STR590/2-4.

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# Memotaxis: An Advanced Orientation Strategy in Fruit Flies and its Consequences in Visual Targeting and Temperature Orientation

Christian Berg[1], José A. Villacorta[2], Valeri Makarov[2], Manuel G. Velarde[2], Paolo Arena[3], Luca Patane[3], Pietro S. Termini[3], Roland Strauss[1]

[1]Johannes Gutenberg-University Mainz, Dept.of Zoology III - Neurobiology, Col.- Kleinmannweg 2, 55099 Mainz, Germany
[2]Universidad Complutense de Madrid, Spain
[3]DIEES - University of Catania, Italy

Animals need to find food, mating partners, nesting sites or a comfortable environment. Therefore, an effective orientation strategy provides a huge benefit for those who move in a complex environment. A way to optimize orientation is analyzed here, shown representatively in visual object approach and in temperature gradient orientation of fruit flies. This orientation strategy is called "memotaxis". It benefits from the integration of past events, leading to a robust path towards the desired goal. Although memotaxis is perfectly suited for noisy environments, its existence is conveniently proven in situations with low noise. In landmark approach experiments, fruit flies show that the longer they approach a particular target, the longer it takes for them to abandon it after its disappearance. This holds true even in the presence of a distracting landmark that comes on only after disappearance of the first-visited target. The strategy was found again in temperature orientation, in which flies are over-running a temperature optimum: distances travelled after crossing the optimum scale with the distances walked towards the optimum. Memotaxis is assumed to exist in many animals, but the genetic tools available in *Drosophila melanogaster* allow localizing the relevant brain centers and pathways. Several mutants are tested for the presence of Memotaxis. The same orientation strategy proves useful also for autonomously roving robots, which find targets in a noisy environment more efficiently.
The project is funded by the EUFP7-programme (ICT – 216227) SPARK II.

# Neural correlates of combined olfactory and visual learning in mushroom body extrinsic neurons of the honeybee (Apis mellifera)

Ina Klinke[1], Randolf Menzel[2]

[1]Freie Universität Berlin, Institute for Neuobiology, Königin-Luise Str. 28-30, 14195 Berlin, Germany

[2]Bernstein Center for Computational Neuroscience (BCCN) Berlin, Germany

The mushroom body (MB) is a central neuropil structure in the protocerebrum of the honeybee brain and plays a crucial role in sensory integration and memory formation.
In order to elucidate the role of higher-order feedback neurons at the output region of the mushroom body, we applied extracellular electrophysiological recordings while the bee performed a classical conditioning task. The aim was to detect correlated changes of spike rate, spiking patterns, and spike timing among different neurons measured simultaneously, and collect these activities for longer periods of time (up to three days and nights) searching for neural correlates of learning and memory consolidation.
The learning task was composed of compounds of odors and colors in order to study especially the multisensory features and context dependencies of feedback neurons during classical conditioning and memory retrieval. The bee was first preconditioned differentially to two colors, one rewarded, the other not. Subsequently, a compund training was applied with the same rewarded color followed by an odor, both were rewarded, the other combination was not rewarded. All stimuli were tested seperately, in the combinations trained, and in the "wrong" combinations that were not trained.
In a first step, units were categorized as responsive or non-responsive towards the colors and/or odors, and grouped by their response polarity. Based on the motor output of the bees (correct, incorrect and omission of response) in the test towards the stimuli alone and towards the different combinations, spike rate and spike timing of single units were analyzed. Also, the time frequency spectra of the local fiel potentials were investigated.
Increased power in the frequency band between 1 and 20 Hz was found only in the training of the rewarded combination after onset of the color, lasting for the whole stimulation, and an increase in power of 50 Hz after odor onset was detected, additionally, suggesting a crucial role in the acquisition of multisensory stimuli of MB output neurons.
We found increasing and decreasing rate responses towards the learned odors and colors. Some units started to respond towards both modalities after learning, others were unimodal. Another fraction of units stopped responding towards the learned stimuli or were newly recruited. The correct visual context amplified the response towards the odor.
MB output neurons thus show memory-related plasticity in different time windows with dynamic rate changes, and recruitments of neurons, suggesting a change in neuronal ensemble activity.

# Neurobehavioral changes in mice offspring induced by prenatal exposure to lipopolysaccharides

Hossam Ebaid[1], Jamaan Ajarem [1], Gasem Abu-Taweel [1]

Department of Zoology,College of Science, King Saud University, P. O. Box 2455, Riyadh 11451, Saudi Arabia

Sepsis with its complications is still a major challenge since it is a syndrome representing a systemic inflammatory response to microbial infection or severe injury. Animal models are used as an approach to mimic multi-organ dysfunction typically seen in septic patients. This study was aimed to determine the neurobehavioral changes resulted from the prenatal exposure of mice to LPS during fetal and early postnatal development. The developmental observations as well as the behavioral tests, such as sensory motor reflexes, and learning and memory test in automatic reflex conditioner (shuttle box) (Active avoidance responses) were applied. Adult mice were assigned into two groups: the first group was remained as a control group given phosphate buffered saline; the second group (Pregnant mice) was intraperitonealy injected with LPS at a dose of 0.2 mg/kg of body weight. Appearance of body hair and opening of eyes of the pups from treated mothers were significantly delayed. The body weight gain came significantly lower than those of the control. LPS also inhibited the sensory motor reflexes in all elements of acts and postures in a dose dependent manner. The active avoidance training-test indicated that LPS exposure was associated with learning impairment.

# Neuromodulation of avoidance learning by the ventral tegmental area and lateral habenula: Differential effects on acquisition, retrieval, long-term retention and extinction.

Anton Ilango Micheal[1], Jason Shumake[3], Wolfram Wetzel[1], Henning Scheich[1], Frank Ohl[1,2]

[1]Leibniz Institute for Neurobiology, Bio future Group-Neuroprostheses, 6, Brennecke strasse, 39118 Magdeburg, Germany
[2]Institute of Biology, Otto-von-Guericke University, Magdeburg, Germany
[3]Department of Psychology, The University of Texas, Austin, USA

Dopamine release is essential for the development of avoidance learning, and stimulation applied to the ventral tegmental area (VTA) evokes dopamine release in terminal regions. Previous studies indicated that rewarding VTA stimulation facilitated two-way active avoidance learning. We further investigated the parameters determining the efficacy of VTA stimulation, in particular whether VTA self-stimulation was more effective if delivered before vs. after the avoidance training sessions, and whether the addition of stimulation during avoidance trials was more effective than self-stimulation alone. The results show that self-stimulation before or after avoidance learning was equally effective in improving acquisition, and the addition of stimulation during avoidance trials resulted in an even greater improvement. However, the latter was detrimental to performance in a long-term retention test which was conducted 10 days later in the absence of VTA stimulation, which may reflect a frustration effect from the omission of expected reward. This drop in performance was prevented by training animals under a schedule of partial, rather than continuous, reinforcement. Electrical stimulation of the lateral habenula, which was previously shown to inhibit midbrain dopaminergic neurons, impaired acquisition of avoidance but not memory retrieval, and this effect was dependent on the frequency of stimulation.

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# Newborn cells after cortical spreading depression – proliferation, survival and functional contribution to hippocampus dependent learning and memory

Eileen Baum[1], Otto W. Witte[1], Anja Urbach[1]

[1]University Hospital Jena, Hans Berger Clinic for Neurology, Erlanger Allee 101, 07747 Jena, Germany
[2] Leibniz Institute for Age Research-Fritz Lipmann Institute, Jena, Germany

The dentate gyus (DG) of the hippocampus is one out of two regions that generate new neurons throughout life. Preliminary data of our and other labs imply that adult neurogenesis is a finely tuned process that, under physiological conditions, contributes to at least some forms of hippocampus-dependent learning and memory. However, when hippocampal neurogenesis is increased far above physiological levels (e.g. due to cortical spreading depression, CSD), the excess of new neurons appears not to lead to the expected increases in cognitive performance. The current study was designed to evaluate the significance of our previous findings and particularly to figure out whether there exists a specific time window when the excessively newly born neurons become functionally integrated into the hippocampal neuronal network.

Therefore, we used 10-12 week old male C57Bl/6 mice which were trained in a Morris water maze (MWM) starting either 14 days (tp1), 28 days (tp2) or 42 days (tp3) after the induction of CSD (induced by epidural application of 1.5 mol/l KCl / sham 1.5 mol/l NaCl). Animals were sacrificed immediately after the probe trial which was performed 1 week after the last training trial. We applied two different thymidine analogs to label proliferating cells at distinct time points along the experimental course. To identify cells born immediately after CSD/sham surgery mice received one daily i.p. injection of iododeoxyuridine (IdU) for six consecutive days thereafter. To investigate the influence of spatial learning on proliferation, the animals received a single daily injection of chlorodeoxyuridine (CldU) during the period of MWM acquisition. Mice that underwent CSD/sham surgery but not behavioural tests served as controls.

Independently of the time point of analysis, the number of $IdU^+$ cells was significantly increased in the ipsilateral DG of CSD animals. However, albeit the number of newborn $IdU^+$ cells did not change between the sham groups over time, that of ipsilateral $IdU^+$ cells in the CSD groups appeared to accumulate with increasing time ($tp1_{CSD}$ vs. $tp3_{CSD}$: $p = 0.009$,). That accumulation was not observed in the DG of CSD animals without MWM training.

This suggests that spatial learning selectively supports the survival of the excessively generated $IdU^+$ cells that were 5-6 wks old at the begin of training, in CSD animals. The comparison of animals with and without MWM training further revealed that spatial learning also affects proliferation in the DG. We found significantly more $CldU^+$ cells in the DG of CSD animals that started spatial learning 2 or 4 wks after surgery, and similarly in sham mice that started learning 2 wks after surgery. Analysis of the MWM revealed no significant differences in learning performance or spatial memory between sham and CSD animals.

From our study we conclude that 1) CSD are a reliable stimulus to induce proliferation in the adult DG; 2) The excessively newly born cells are selectively supported to survive by training the CSD animals in a hippocampus dependent spatial learning task. The time window for this effect is very specific with an age of the newborn cells of 5-6 wks at start of learning; 3) Spatial learning modulates cell birth in the DG; and 4) Numbers of newborn DG cells far above the physiological level not necessarily increase cognitive performance.

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# Nogo-A Stabilizes the Architecture of Hippocampal Neurons

Marta Zagrebelsky[1], Martin E. Schwab[2], Martin Korte[1]

[1]TU Braunschweig, Cellular Neurobiology, Braunschweig, Germany

[2]Brain Research Institute, University of Zurich and ETH Zurich

Although the role of myelin-derived Nogo-A as an inhibitor of axonal regeneration after CNS injury has been thoroughly described, its physiological function in the adult, uninjured CNS remains less well known. We address this question in the hippocampus, where Nogo-A is expressed by neurons as well as oligodendrocytes. We used 21 d in vitro slice cultures of neonatal hippocampus, and applied different approaches to interfere with Nogo-A signaling/ expression and analyzed their effects on the dendritic and axonal architecture of pyramidal cells. Neutralization of Nogo-A by function-blocking antibodies induced a major alteration in the dendrite structure of hippocampal pyramidal neurons. Although spine density was not influenced by Nogo-A neutralization, spine type distribution was shifted toward a more immature phenotype. Axonal complexity and length were greatly increased. Down-regulation of NgR1 replicated the alterations observed after Nogo-A neutralization indicating that Nogo-A plays a major role in stabilizing and maintaining the architecture of hippocampal pyramidal neurons mostly by a receptor-mediated mechanism involving NgR1.

In the current study we expanded the previous results applying a gain of function approach by treating mature hippocampal neurons with the Nogo-A-Δ20 peptide, which is part of the Nogo-A-specific inhibitory domain. Indeed, an acute (10 minutes) Δ20 treatment induces changes at dendritic spines opposite to those observed following Nogo-A neutralization. Significantly more mushroom and less stubby spines are present in Δ20 treated versus control neurons. On the other hand a chronic (4 days) Δ20 application induces a major alteration in the dendrite structure of hippocampal pyramidal neurons, reproducing the one observed after Nogo-A neutralization suggesting that a long-term Δ20 treatment results in a Nogo-A loss of function, possibly due to the internalization of the Δ20-receptor complex.

In an ongoing set of experiments we are comparing the effects of both the loss and gain of function approaches for the Nogo-A specific inhibitory domain (Δ20) with similar approaches for the Nogo66 inhibitory domain. By this set of experiments we expect to better characterize the molecular mechanisms by which Nogo-A regulates the stability of the architecture of mature hippocampal pyramidal neurons.

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# Observation of network dynamics in mouse hippocampal slices using genetically-encoded fluorescent Ca2+ sensors

Susanne Reichinnek[1], Alexandra von Kameke[3], Eckehard Freitag[2], Anna Hagenston[2], Hilmar Bading[2], Mazahir Hasan[4], Andreas Draguhn[1], Martin Both[1]

[1]Institut für Physiologie und Pathophysiologie Heidelberg, Germany
[2]Department of Neurobiology, Universität Heidelberg, Germany
[3]University of Santiago, Spain
[4]MPI für medizinische Forschung, Heidelberg, Germany

Higher cognitive functions involve complex patterns of distributed activity in neuronal networks, thereby forming neuronal assemblies of information coding neurons. An important example is the formation of declarative/spatial memory which requires that assemblies can be formed during learning, subsequently stabilized and re-activated in a highly reproducible way. The hippocampus is important for spatial and declarative memory formation and expresses different functional states for different steps of assembly formation and re-activation, respectively. Memory consolidation has been suggested to rely on the re-activation of place-encoding cells during sleep-associated network states called sharp wave-ripple complexes (SPW-R).

We analyze network activity resembling sharp wave-ripple complexes in acutely prepared and in cultured mouse hippocampal slices. In previous work we found that different waveforms of individual SPW-R at the field potential level reflect different underlying assemblies of CA1 neurons (Reichinnek et al., in press). However, it is still unknown how these network activity patterns are generated, stabilized, and how they encode information. This deficient understanding is mainly due to the lack of appropriate methods to monitor activity within large neuronal networks.

Our approach was to develop an easy-to-build and an easy-to-use imaging setup, which allows us to monitor single cell activity and field potentials of spontaneously occurring oscillations in mouse hippocampal slice cultures at the same time. Therefore, we mounted a custom-built epi-fluorescence microscope to an interface chamber. To monitor single neuron activity, we used a recombinant adeno-associated virus (AAV) to express a calcium-sensitive fluorescent protein in CA1 and CA3 neurons (GCaMP3). The expression level and dynamics of GCaMP3 were investigated 4 to 14 days after virus transfection. Besides optical signals, spontaneously occurring field oscillations and single unit activity were recorded simultaneously with custom made tetrodes.

With this setup we are able to monitor a field of view of 410 µm x 410µm with sub-cellular optical resolution (20x objective, 0.4 NA). Propagation of network activity could be monitored in a field of view of 2 mm x 2 mm with single cell optical resolution (4x objective, 0.1 NA). With the high-end camera used (Hamamatsu Image EM C9100-13) the fluorescence signal is strong enough to identify calcium transients accompanying single action potentials in identified neurons with frame rates of up to 62 Hz. Within the field of view, more than 200 cells can be identified and their network dynamics can be studied with respect to assembly formation and stability. At the same time, the activity of multiple cells (i.e. the output pattern of the network) can be correlated to the local field potential which appear to reflect mainly the input to the same networks.

Our approach allows to monitor complex spatio-temporal patterns of activity in neuronal networks. It provides an adequate tool to investigate oscillation patterns in the hippocampus and other circuits.

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# Odour-mixture perception in *Drosophila*

Claire Eschbach[1], Bertram Gerber[1,2]

[1]Universität Würzburg, Lehrstuhl für Neurobiologie und Genetik, Biozentrum Am Hubland, 97074 Würzburg, Germany
[2]Universität Leipzig - Institut für Biologie , Lehrstuhl für Genetik , Talstrasse 33 , 04103 Leipzig , Germany

Using olfactory associative recognition experiments, we investigate mixture perception in fruit flies using 4 odours and their respective binary mixtures: benzadehyde (B), n-amylacetate (A), 4-metylcyclohexanol (M) and 3-octanol (O). The odour dilutions have been adjusted so that all odours support equal conditioned avoidance after odour-shock associative learning. Two experiments are performed: (i) flies are trained with a pure, 'elemental' odour, e.g. A and tested with a mixture, e.g. AB; (ii) flies are trained with a mixture, e.g. AB and tested with one of its elements, e.g. A. In both cases, the rational is that the more similar the flies regard element and mixture, the stronger conditioned avoidance should be. We thus ask the following questions:
(i) Is generalization between an element and a binary mixture containing it symmetrical, in the sense that conditioned avoidance is equal if the element is trained and the mixture is tested as when the mixture is trained and the element is tested?
(ii) Is an element, e.g. A, equally similar to all mixtures containing it, i.e. to AB, AO, and AM?
(iii) Is a mixture, e.g. AB, equally similar to A as it is to B?
(iv) Does the similarity between element and mixture depend on the similarity of the elements to each other, i.e. does the similarity between A and AB depend on the similarity between A and B?

# Olfactory memories are intensity-specific in larval *Drosophila*

Dushyant Mishra[1], Yi chun Chen[1], Ayse Yarali[3], Bertram Gerber[1,2]

[1]Universität Würzburg, Department of Neurobiology and Genetics, Biozentrum Am Hubland, Würzburg, Germany
[2]Universität Leipzig, Department of Genetics, Institute of Biology, Leipzig , Germany
[3]Max Planck Institut für Neurobiologie, Martinsried, Germany

Learning can rely on stimulus quality, stimulus intensity or a combination of these. Regarding olfaction, the coding of odour quality is often proposed to be combinatorial along the olfactory pathway, and clear working hypotheses are available concerning short-term associative memory trace formation of odour quality. However, it is less clear how odour intensity is coded and whether olfactory memory traces include information about the intensity of the learnt odour. Using odour-sugar associative conditioning in larval *Drosophila*, we first describe the dose-effect curves of learnability across odour intensities for four different odours (n-amyl acetate, 3-octanol, 1-octene-3-ol, benzaldehyde). We then choose odour intensities such that larvae are trained at intermediate odour intensity, but are tested for retention with either that trained intermediate odour intensity, or with respectively HIGHer or LOWer intensities. We observe a specificity of retention for the trained intensity for all four odours used. This adds to appreciate the richness in 'content' of olfactory memory traces even in a system as simple as larval *Drosophila*, and to define the demands on computational models of associative olfactory function.

# Phase precession of entorhinal grid cells in two-dimensional environments

Eric T. Reifenstein[1], Martin B. Stemmler[2], Andreas V.M. Herz[2], Susanne Schreiber[1]

[1]HU Berlin, Institute for Theoretical Biology, Invalidenstr. 43, 10115 Berlin, Germany
[2]LMU Neurobiology, Grosshadernerstr. 2, 82152 Martinsried, Germany

When a rat explores its environment, grid cells in the medial entorhinal cortex show increased activity at specific locations that constitute a regular hexagonal grid. As the rat enters and progresses through one of these

# Phase-dependent neuronal coding of objects in short-term memory

Markus Siegel[1], Melissa R. Warden[1], Earl K. Miller[1]

[1]The Picower Institute for Learning and Memory, Massachusetts Institute of Technology, Cambridge, MA 02139, USA
[2]Centre for Integrative Neuroscience, University of Tübingen, Paul-Ehrlich-Str. 17, 72076 Tübingen, Germany

The ability to hold multiple objects in memory is fundamental to intelligent behavior, but its neural basis remains poorly understood. Here, we show that neuronal information about two objects held in short-term memory is enhanced at specific phases of underlying oscillatory population activity. We recorded neuronal activity from the prefrontal cortices of monkeys remembering two visual objects over a brief interval. We found that during this memory interval prefrontal population activity was rhythmically synchronized at frequencies around 32 and 3 Hz and that spikes carried the most information about the memorized objects at specific phases. Further, according to their order of presentation, optimal encoding of the first presented object was significantly earlier in the 32 Hz cycle than that for the second object. Our results suggest that oscillatory neuronal synchronization mediates a phase-dependent coding of memorized objects in the prefrontal cortex. Encoding at distinct phases may play a role for disambiguating information about multiple objects in short-term memory.

# Phosphorylated CREB is predominantly located in the mushroom bodies of honeybees

Katrin Barbara Gehring[1], Isabella Kersting[2], Kirsten Hoffmann[3], Dorothea Eisenhardt[4]

[1-4]Freie Universität Berlin, Neurobiologie, Königin-Luise-Straße 28/30, 14195 Berlin, Germany

The transcription factor CREB (cAMP response element binding protein) plays an essential role in the formation of long term memory (LTM). CREB is required for the induction of a consolidated LTM and induces gene expression after becoming phosphorylated (pCREB) by several kinases. A huge body of work analyzed this CREB phosphorylation after learning but little is known about its role in the honeybee (*Apis mellifera*), a well known invertebrate model system for learning and memory. Our aim is to analyze the role of *Apis mellifera* CREB (AmCREB) in the formation of LTM in the honeybee.

In order to localize AmCREB dependent processes in the honeybee brain we use an antibody against the phosphorylated form of the vertebrate CREB to detect phosphorylated AmCREB in fixed bee brain slices. Interestingly, strong pAmCREB immunoreactivity is predominantly located in a subgroup of mushroom body intrinsic neurons (Kenyon cells). In contrast to the assumed distribution of pCREB, which, as a transcription factor, should be predominantly located in the nucleus, we find not only signals in the nuclei but also strong immunoreactivity in the axonal and dendritic parts of the Kenyon cell neurons. Counterstainings with pre- and postsynaptic markers show no pAmCREB staining in the presynaptic boutons of the projection neurons whereas pAmCREB signals can be detected in the Kenyon cell dendrites and their postsynaptic sites. Interestingly, another antibody directed against the N-terminal region of several AmCREB splice variants produces a different staining pattern. AmCREB immunoreactivity is predominantly observed in the central complex and in the glomeruli of the antennal lobes whereas no staining can be detected in the mushroom bodies. Based on the dissimilar staining patterns in the mushroom bodies we conclude that the two antibodies detect different AmCREB splice variants.

Since the antennal lobes and the mushroom bodies are involved in processing sensory information during learning, ongoing experiments focus on changes of pAmCREB and AmCREB levels in these neuropils.

# Differences in spatial learning and memory between pubertal and late adolescent male Wistar rats are related to estradiol rather than testosterone

Katrin Meyer[1], Volker Korz[2,3]

[1]Leibniz Institut for Neurobiology, Neuronale und Verhaltensplastizität, Brenneckestraße 6, 39120 Magdeburg, Germany
[2]Otto-von-Guericke Universität, Institut für Biologie, Leipziger Straße 44, 39118 Magdeburg, Germany
[3]Center for behavioral Neurosience, Magdeburg, Germany

Adolescence, defined as the developmental period between child- and adulthood includes the important period of puberty. Puberty is especially characterized by hormonal changes (namely testosterone in males) along with typical behavioral patterns, like intense peer interaction, impulsivity and increased risk and novelty seeking. Although it is conceivable that this phenomenon affect spatial learning, little is known about pubertal learning abilities in dependence on hormonal states and gene expression patterns.
Therefore, we investigated spatial memory performance, in pubertal (8 weeks old) and late adolescent (11-12 weeks old) male Wistar rats. Four groups of animals were tested: vehicle treated (v-pubertal) and testosterone treated (t-pubertal) pubertal rats, and vehicle treated (v-adolescents) and testosterone treated (t-adolescent) late adolescent rats. General activity, average velocity, wall distance, ambulation as well working and reference memory errors were registered. Testosterone and vehicle were applied 24 h before the retention trial. After retention hippocampal, prefrontal cortex and serum concentrations of testosterone, 17-ß-estradiol and corticosterone, as well as the gene expression of different steroid receptors and aromatase, were measured. Unexpectedly, we found no differences for all behavioral parameters and hormone concentrations between the vehicle and the testosterone treated animals within the two developmental groups. However, the v-pubertal and t-pubertal rats were significantly more active than both late adolescent animal groups. Interestingly, these differences were independent of testosterone, since no difference in either tissue or serum testosterone concentrations could be determined. However, we found significantly increased hippocampal estradiol concentrations in v- adolescents as compared to v- pubertal rats, whereas serum and prefrontal cortex concentrations were similar. Furthermore, hippocampal concentrations of estradiol were significantly higher in trained vs. non-trained animals in both developmental groups, increased in v-adolescent as compared to t-adolescent rats, and not different between v-pubertal and t-pubertal animals. The expression of a variety of steroid receptor genes is up-regulated in trained pubertal rats as compared to trained late adolescent rats, however within both developmental groups trained rats showed significantly up- regulated estrogen receptor a mRNA and down-regulated androgen receptor mRNA in as compared to non-trained controls.

Our data suggest common features of hormone and gene expression modulation during training in pubertal and late adolescent rats, but also differences in regulations according to the two phases of maturation. Surprisingly, these differences are based more on estradiol rather than on testosterone functions. The suggested specific functions on hippocampal estradiol concentrations and related receptors remain to be investigated.

# Potential role of pCaMKII in neuronal and behavioural plasticity in the honeybee

Christina Scholl[1], Thomas S. Muenz[1], Elisabeth Pasch[1], Wolfgang Rössler[1]

[1]University of Würzburg, Biozentrum, Behavioral Physiology and Sociobiology, Am Hubland, 97074 Würzburg, Germany

The honeybee *Apis mellifera* is a social insect well known for its complex behaviour. Many aspects of honeybee behaviour are plastic, but in many cases the neuronal and molecular basis of this behavioural plasticity, is unknown. This is particularly true for the transition between different tasks inside and outside the hive. The calcium-calmodulin-dependent protein kinase II (CaMKII) is a protein known to be involved in neuronal and behavioural plasticity mechanisms and therefore, an interesting candidate for molecular mechanisms of neuronal and behavioural plasticity. Our study focuses on the mushroom bodies (MB) - important centres for sensory integration as well as learning and memory processes. The MBs were shown to undergo massive structural plasticity during behavioural maturation during the transition from indoor duties to outdoor foraging. To investigate a potential role of CaMKII during synaptic reorganization in the MBs, we examined the age-related distribution of the activated (phophorylated) form of CaMKII (pCaMKII) in the MBs. Furthermore, potential changes in levels of pCaMKII in the course of learning and memory processes were investigated by olfactory conditioning experiments. The distribution of CaMKII was visualised using either an antibody against the autophoshorylation site of the protein to label the active form of the protein or an antibody against an epitope distant from the phosphorylation site to detect the total amount of CaMKII.
In adult bees CaMKII was found throughout the brain, but with a particularly high concentration in MB intrinsic neurons, the Kenyon cells (KCs). The KC subpopulation of inner non compact cells, forming the olfactory innervated lip and the visually innervated collar showed strong CaMKII immunoreactivity. This holds true for all cellular subcompartments (somata, dendrites and axons). At the level of MB calyx microglomeruli, characteristic synaptic complexes in the MB calyx, CaMKII was strongly accumulated in dendritic spines and colocalized with f-actin. Interestingly, in the population of inner compact cells, forming the bimodally (vision and olfaction) innervated basal ring of the MB calyx, CaMKII immunoreactivity was more or less absent. This general distribution did not differ between the active and inactive form of CaMKII, and high levels of CamKII were detected with a similar distribution throughout adult maturation. This indicates that the protein may play an essential role during all stages of behavioural development. Olfactory conditioning experiments did not seem to drastically change the distribution of CaMKII in the MBs at the level of immunocytochemistry.
We use quantitative western blotting to further investigate behaviourally induced potential changes in the amount of the activated form of the protein.

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# Rapid Processing of Animals in Natural Scenes: Image Features and Anxiety

Maximilian Frank Hilger[1], Marnix Naber[1], Wolfgang Einhäuser[1]

[1]Neurophysics, Phillip University Marburg, 35032, Marburg, Germany

Humans can detect animals in natural scenes extremely rapidly with high accuracy. The question as to which factors allow such rapid processing has recently received increasing interest. Whereas the role of low-level image features (e.g., luminance, contrast, or size) and emotional valence on detection performance has been investigated in considerable detail, it has remained unclear how these factors interact. Furthermore, only few studies have addressed these factors in the context of identification and detection simultaneously. We asked observers to detect animals, chosen from five categories with different degrees of threat, in rapidly presented and masked natural scenes, while we recorded the size of their pupils. Observers had to report the presence or absence of the animal as fast as possible by conducting an eye movement. In case of an "animal present" response, observers had to choose the animal species from a list of 15, and report their confidence in identification. In a separate experiment after the rapid presentation task, observers evaluated the images during prolonged viewing with respect to positive/negative affect and rated their level of anxiety when encountering the depicted animal. We found that detection, identification, and reaction time improved with animal luminance (but not image luminance), animal contrast, and animal size. In addition, detection improved for animals associated with positive emotions and low anxiety. Pupil size was negatively correlated with anxiety, indicating that threatening animals induced a smaller pupil size. In a similar vein, observers with higher mean anxiety rating had generally smaller pupil sizes throughout the experiment. In conclusion, target-related bottom-up and top-down factors affect rapid processing of complex scenes. Target features – but not image features – affect detection and identification; emotions - in particular anxiety, as reflected by the pupil size - modulate detection.

# Rescue of a Spatial Orientation Memory and Characterization of the *Drosophila* Mutant *ellipsoid-body-open*

Julia Thran[1], Burkhard Poeck[1], Roland Strauss[1]

[1]Johannes Gutenberg-University Mainz, Institute of Zoology III, Colonel-Kleinmann-Weg 2, 55099 Mainz, Germany

*Drosophila melanogaster* wild-type flies are capable of forming a visual orientation memory during walking. They remember the position of or the path to their target object even if it disappears from sight. In this project we are analysing the behavioural and histological phenotypes of three different *ellipsoid body ope*n (*ebo*) mutant alleles (*ebo*EY, *ebo*KS263 and *ebo*678), because Neuser et al. (2008) demonstrated that this structural mutant of the central complex is lacking a spatial orientation memory. All three *ebo* alleles are characterized by either a ventral cleft within the ellipsoid body, or a kidney-formed or completely split ellipsoid body (EB), a structure that is perfectly toroid-shaped in wild-type flies. Via the GAL4/UAS system (Brand & Perrimon, 1993) we drove the expression of EBO in spatially defined tissues in an *ebo* defective background so as to rescue the spatial orientation memory. We used both the pan-neural GAL4 line *Appl* and the tissue specific GAL4 lines c232, c547, c305, c42 and 189Y, which drive expression in subtypes of EB ring neurons (Renn et al., 1999). The orientation behaviour was examined in the detour paradigm (Neuser et al., 2008), where two inaccessible landmarks vanish for a short time. The locomotor parameters (walking activity, walking distance, walking speed and orientation capacity) were recorded in Buridan's paradigm, where flies walk continuously between two landmarks for 15 minutes (Bülthoff et al., 1982). The pan-neural expression of an *ebo* cDNA recovered the wild-type spatial orientation behaviour as well as the EB structure of the defective UAS-lines. There was no rescue with the driver lines c232, c547 and c42, whereas 189Y and c305 did restore the orientation phenotype to the full extent. Only the R3 specific 189Y-GAL4 line restored the anatomical defect. Moreover, we used the heat shock-inducible hsp70-GAL4-System, but let the flies grew up at 17.5°C without functional EBO and defective EB until day 3 of the adult stage. Thereafter, the flies were incubated for 24h at 30°C and tested in the detour paradigm. The acute expression of EBO was sufficient to rescue the behavioural phenotype without a rescue of the structure of the EB. Thereby, we showed that regarding the spatial orientation memory EBO is important in an acute and not developmental way in the EB.

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# Rescue of rugose, the fly homologue of Neurobeachin.

Sabrina Scholz[1], Karolien Volders1[1], Marina Efetova[2], Martin Schwärzel[2]

[1] Department of Human Genetics, KU Leuven, 3000 Leuven, Belgium.
[2] Department of Biology, Free University Berlin, D-14195, Berlin, Germany.

The vertebrate neurobeachin gene encodes for a neuron-specific protein containing a BEACH (beige and chediak-Higashi) domain implicated in synaptic vesicle trafficing as well as A-Kinase-Anchoring-Protein (AKAP) domains linked to localization of cAMP dependent Protein-Kinase (PKA) activity. In humans, dysfunction in neurobeachin is linked to autism, a developmental disorder of the CNS characterized by impairments in social interaction, communication and a restricted repetitive and stereotyped behaviour. The fly homologue of neurobeachin is rugose encoding for a 550 kDa neuronal protein containing BEACH and AKAP domains. Mutants affected in rugose exhibit rough eyes, abnormal brain morphology and impaired associative odor learning.

Here we report functional rescue of rugose based on targeted expression of either (a) a Drosophila wild type rugose cDNA or (b) a mouse neurobeachin cDNA. The rough eye phenotype of rugose mutants could be rescued via eye-specific expression of either transgene, proving functional homolgy between Drosophila rugose and mouse neurobeachin. Pan-neuronal expression of rugose cDNA also rescued the brain morphology phenotype, as well as the learning phenotype.

This study will improve the understanding of BEACH proteins and will establish rugose as fly model to study functional asapects of these proteins in a simple genetic organism.

# Rescue of the dunce learning mutation.

Lisa Scheunemann[1], Antje Richlitzki[1], Eva Jost[2], Andreas Thum[3], Shireen Davies[4], Johnathan D. Day[4], Marina Efetova[1], Martin Schwärzel[1]

[1]Free University Berlin, Neurogenetic, Takustr. 6, 14195 berlin, Germany. [2]Faculty of Veterinary Medicine, LMU Munich, D-80539 Munich, Germany. [3]Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland. [4]Division of Molecular Genetics, University of Glasgow, Glasgow G11 6NU, U.K.

The second messenger cAMP plays a pivotal role in memory formation and its concentration is tightly regulated by adenylyl cyclases (ACs) and phosphodiesterases (PDEs). It is these "checkpoints" of cAMP that are affected in the mutants dunce (a PDE) and rutabaga (an AC), originally isolated due to their poor learning performance. Functional rescue of the rut-dependent learning defect identified rut-AC function within Kenyon Cells (KCs) of the Mushroom Bodies to be sufficient for odor learning, placing cAMP dependent plasticity at the KC layer at a central position for this type of behavioral plasticity.

Here, we have performed functional mapping of the neuronal circuit that requires dnc-PDE function. We identified two neural layers of the olfactory pathway, GABAergic local neurons (LNs) at level of the antennal lobes and KCs at level of the Mushroom Bodies to require dnc-PDE for odor learning. At either layer dnc-PDE functions in a non-redundant fashion and together LNs and KCs define a minimum circuit where dnc-PDE function is necessary and sufficient for odor learning.

The discrepancy in overlap between the requirement for either dnc-PDE and rut-AC function on level of neuronal circuits lead us to investigate performance of rut-dnc double mutants and we show that either mutation affects a distict aspect of odor memory. This result shows that two distinct forms of cAMP dependent plasticity are involved in odor learning: One rut-AC depenent mechanism localized at the KC layer, and a dnc-PDE dependent mechanism localized at the level of KCs and LNs.

# Responses to social stimuli in the rat hippocampus

Moritz von Heimendahl[1], Michael Brecht[1]

[1]Humboldt University of Berlin, Bernstein Center for Computational Neuroscience, Philippstr. 13 Haus 6, 10115 Berlin, Germany

Even though lesion studies implicate the rodent hippocampus in the formation of social memories, we know little about how hippocampal neurons in rats respond to and represent conspecifics.

To address this question, we conducted chronic extracellular recordings from single neurons of the CA1 region of dorsal hippocampus while rats were interacting with conspecifics. We found that a subset of cells did react to stimulus rats with an enhancement or a suppression of their firing rate by, on average, +68% or -40%, respectively. While these effects were not significant at the single-cell level, they could be shown to be significant through a population analysis. We did not, however, find cells that varied significantly in their responses to different individual rats. As expected, almost half of the neurons displayed spatially modulated discharge patterns. Some cells showed conjunctive responses, i.e. firing that was enhanced or suppressed in specific locations only.

To our knowledge, this is the first report of hippocampal responses during interactions with conspecifics. While social stimuli clearly are represented in rat CA1, they seem to play a less prominent role than the well-described spatial representations.

# Retrieval of long-term memory after unilateral olfactory conditioning of the honeybee proboscis extension reflex

Johannes Fischer[1], Bernd Grünewald[1]

[1]Goethe-Universität Frankfurt am Main, Institut für Bienenkunde, AG Grünewald
Karl-von-Frisch-Weg 2, 61440 Oberursel, Germany

Classical conditioning of the proboscis extension reflex (PER) is a powerful tool to investigate the neurophysiological mechanisms of learning and memory in the honey bee Apis mellifera. Here we investigated the dynamics and localization of the information transfer and memory retrieval of long term memory using local injections of the local anesthetic procaine.

For this, bees were stimulated with an odor as the conditioned stimulus (CS) with the to only one antenna paired with a sucrose reward. Such unilateral conditioning leads to a lateralized memory that can exclusively be retrieved on the trained side for the first few hours after the acquisition. After a day, the conditioned response (CR) can be triggered by stimulating either, the antenna of the trained or the untrained hemisphere.

The neuroanatomy of the olfactory pathways within the bee brain suggests that the odor information of both hemispheres may first converge at the level of the mushroom body (MB). Between the bilaterally symmetrical MB neurons of the a-lobe interconnect its main output regions. Furthermore, the MB is supposed to be essential during the consolidation of long term memory and during non-elemental learning tasks. To investigate if the CS stays on the trained hemisphere or if it is transferred to the contralateral side, we inhibited spike-activity within the a-lobe of the trained or untrained brain side and thus blocked the communication between the MBs.

We tested the effect of procaine injections in the a-lobes 24 hours after the acquisition, assuming that at this time point the learned information is already consolidated. Unilateral procaine injections in the trained side almost completely impaired the CR on the untrained side 24 hours post acquisition. Injections in the untrained side had a similar but not so powerful effect after olfactory stimulation of this side.

It was not possible to eliminate the learned response to a stimulation of the antenna of the trained hemisphere, neither with a procaine injection in the a-lobe of the trained nor in the untrained side 24 hours after the acquisition. An injection one hour after acquisition however impaired the retrieval of the CR on the trained hemisphere.

Our results show that unilaterally learned information is not transferred to the contralateral side, but it remains retrievable as long as either the connections between the a-lobes of the MB are functional. This indicates that unilaterally acquired odor information is stored within the ipsilateral brain hemisphere and this lateralized memory is also accessible by the contralateral side. The finding of a CR even after blocking the ipsilateral a-lobe is very interesting because it has been shown that the retrieval of the CR is almost completely inhibited when the ipsilateral a-lobe is blocked shortly after acquisition. This indicates that there are different neuronal pathways for retrieving either short-term or medium-term or already consolidated long-term memory.

# Reversal learning in honeybees – a behavioral and an electrophysiological study

Ravit Hadar[1], Randolf Menzel[1]

[1]Freie Universitπt Berlin, Institute of Biology – Neurobiology, Free University of Berlin, Germany , Kъnigin-Luise-Str. 28-30, 14195 Berlin, Germany

The honeybee (Apis mellifera) serves as a valid model for the study of the underlying mechanisms of learning and memory. Here, two different approaches were used to study reversal learning: a pharmacological approach which allowed us to manipulate protein synthesis, and an electrophysiological approach enabling us to record from mushroom body extrinsic neurons. In reversal learning honeybees are first trained to respond to a reinforced odor (CS+) and not to respond to a nonreinforced odor (CS -). Once this rule has been successfully learned, the contingencies of the conditioned stimuli are reversed, and animals learn to adjust their response to the new rule. The effect of emetine, a protein synthesis inhibitor, on the memory consolidation after reversal learning was found to yield different results when tested with summer bees or winter bees. Blocking protein synthesis in summer bees inhibits consolidation of the excitatory CS+ learning following reversal learning whereas the consolidation of the inhibitory CS - learning was blocked in winter bees. These findings suggest that in bees excitatory and inhibitory learning may involve different molecular processes, which are also dependent on the season. At the neuronal level, mushroom body extrinsic neurons were found to change their firing rate over the course of reversal learning and may be clustered to different groups: responding either to both odors or just to one of the odors, either by an excitation or depression of the firing rate.

# Short and long-term changes in serotonergic neurones reflect the induction of behavioural phase change in Desert Locusts

Stephen Mark Rogers[1], Swidbert Roger Ott[1]

University of Cambridge, Department of Zoology, Downing St, CB2 3EJ Cambridge, United Kingdom

Background

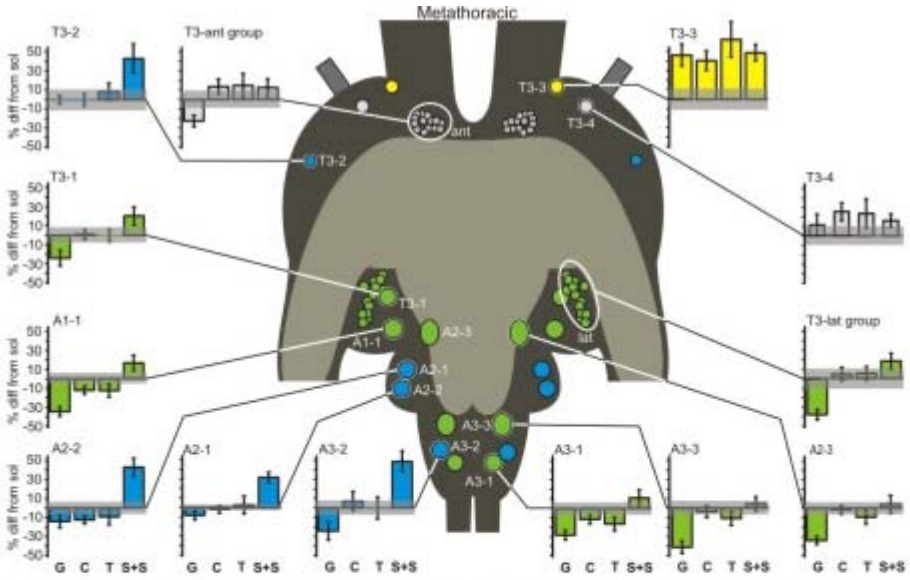
The reversible transformation of Desert Locusts (*Schistocerca gregaria*) between a lone-living solitarious phase and a swarming gregarious phase presents an extreme but experimentally tractable model of phenotypic plasticity. The two phases differ profoundly in morphology, physiology and particularly in behaviour. The transformation to the gregarious phenotype is driven entirely by the presence other locusts through two separate and equally potent sensory pathways: the combined visual and olfactory stimuli emanating from other locusts or mechanosensory stimuli directed specifically to the hind leg. Either alter key behavioural characters from solitarious to gregarious within just 1-4 h. In particular, there is a marked increase in activity and a profound change from locusts being repelled by other locusts towards active mutual attraction. We have recently found that Serotonin (5-Hydroxy Tryptamine, 5HT) is a key effector of this transition. Gregarizing stimuli lead to a dramatic, but short-lived, increase in 5HT within the thoracic ganglia of the CNS. Receptor antagonists or inhibitors of 5HT synthesis prevent the induction of gregarious behaviour, whilst agonists or 5HT itself induce a shift towards gregarious behaviour even in the complete absence of other locusts. We used immunohistochemistry to identify serotonergic neurones within the thoracic ganglia and to analyze how acute gregarizing input and long-term behavioural phase state affect the expression of 5HT.

Methods

We compared 5 groups of locusts (n=12 each): unstimulated solitarious; acutely gregarized for 1 h through one of three treatments: forced crowding with 30 gregarious -phase locusts; stroking the hind leg with a paint-brush; or exposure to the sight and smell of other locusts; and gregarious for many generations. Thoracic ganglia were fixed in situ, dissected free, processed for 5HT immunofluorescence staining, and imaged by confocal microscopy. The relative intensity of staining in the somata was measured. To compensate for random differences in total brightness between preparations, cell body brightness was regressed against the sum neuropil brightness of the ganglion, which showed no significant treatment-related differences.

Results

The relative intensity of 5HT immunofluorescence in individual identified neurones showed strong differences in the pattern of staining across the 5 treatment groups, with most serotonergic neurones showing some difference in staining intensity. There was one group of neurones, which showed strong segmental homology, that differed in intensity between long-term solitarious and gregarious locusts, with those of gregarious locusts being less intensely stained. Another population of neurones was more intensely stained in locusts that had received one or more of the gregarizing stimuli. Neurones that increased in staining intensity in response to tactile stimuli were found only in the metathoracic ganglion, but neurones that were brighter in locusts treated by crowding or being exposed to the sight and smell of other locusts were found in all three thoracic ganglia, with the greatest number found in the prothoracic ganglion. By measuring the increased intensity of staining in solitarious locusts that have been exposed to different gregarizing stimuli for just one hour compared to untreated controls we have been able to identify candidate neurones that may have a key role in producing the behavioural transition from solitarious to gregarious behaviour.




Neurones in the metathoracic ganglion that stained positive for serotonin. The graphs show the percentage difference in mean intensity from long-term solitarious locusts. Green neurones were less intensely stained in long term-gregarious locusts, but were unaffected by the gregarizing treatments. The blue neurones increased in intensity in locusts subjected to the intense sight and smell of other locusts. The yellow neurones were brighter in locusts subjected to two or more of the gregarizing stimuli. The grey neurones did not significantly differ between any of the treatments.

# Spatial Orientation in Japanese Quails *(Coturnix coturnix japonica)*

Tim Ruploh[1], Agnieszka Kazek[1], Hans-Joachim Bischof[1]

[1]Universität Bielefeld, AG Neuroethologie, Morgenbreede 45, 33615 Bielefeld, Germany

To find a given location, for example a feeding area or the nest, can be based on different cues. One of several possible strategies is the use of spatial relations between landmarks. Such *spatial orientation* is attributed as a cognitive skill, demanding a neuronal representation of the environment. We here investigated whether Japanese quails are able to learn the relation of food trays to distant landmarks and tested the longevity of their spatial memory.

Birds were trained to find mealworms in three adjacent food cups that were part of a circle of 20 such cups. The positions of the baited feeders were always constant in relation to four two-dimensional landmarks, each with a different pattern, placed in the corners of the squared test arena. Birds were trained until they showed a significant orientation towards the correct feeders. To examine whether the quails used the given landmarks for orientation, landmarks were then shifted by 90° clockwise and the birds' orientations were measured again. After a period of either three or six weeks without an experiment, the quails were tested again to examine the endurance of spatial memory.

Seven quails showed significant orientation towards the baited food cups after training. After the 90° landmark rotation all birds redirected their search to the new correct direction. In later trials, some of the birds were dithering between the old and the new direction indicating the influence of other, not shifted landmarks. Two out of four quails that were tested again after three weeks and one out of three tested after six weeks still showed significant correct orientation..

Our results demonstrate that Japanese quails are using spatial relationships between distinct visual cues to orientate in space. They are able to remember specific places over a long period. Further research on quails is planned to investigate the role of hippocampus for spatial memory.

# Spikelets in hippocampal CA1 pyramidal neurons are highly correlated with spikes at a near by unit.

Edith Chorev[1], Michael Brecht[1,2]

[1]Bernstein Center for Computational Neuroscience, 10115 Berlin, Germany.
[2]Humboldt Universität, Tier Physiologie, Philippstr. 13, House 6 , 10115 Berlin, Germany

Spikelets in CA1 neurons have been observed both in-vivo and in-vitro. Recently, spikelets have been shown to undergo spatial modulation in a similar fashion as spikes in place cells (1). However, the mechanisms underlying the generation of these events remains unknown. One popular suggestion for the emergence of spikelets is that they are the outcome of dendritic spikes (2). Alternatively, they could be the outcome of ectopic spikes (3). Another theory suggests that they result from spikes in an electrically coupled neuron (4).
In this study we recorded simultaneously intracellular and extracellular signals from anesthetized rats. Extracellular recordings were done in the close proximity of the intracellular neurons (<120 microns), thus picking the same unit as recorded intracellularly. We show that out of 24 neurons recorded 6 neurons displayed spikelets. Out of these 6 recordings in 4 we managed to record a second unit that exhibited a firing that was highly correlated with the occurrence of spikelets intracellularly. Moreover, we show that in those instances the extracellular unit precedes the initiation of spikelets and that the delay is to short (~120 microseconds) to account for a chemical transmission. This supports the theory that spikelets are the outcome of spikes in an electrically coupled neurons and that this neurons is a neighboring one. Further analysis demonstrated that spikes and spikelets appear with similar patterns suggesting that the coupled neurons is likely to also be a pyramidal CA1 neuron.

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# Temporal dynamics of reward prediction in mushroom body output neurons in the honeybee

Tiziano D'Albis[1], Martin Strube-Bloss[2], Randolf Menzel[2], Martin Paul Nawrot[1]

[1]Freie Universität Berlin, Neuroinformatics & Theoretical Neuroscience, Königin-Luise-Str. 1-3, 14195 Berlin, Germany

[2]Freie Universität Berlin, Institut für Biologie - Neurobiologie, Königin-Luise-Str. 28/30, 14195 Berlin, Germany

The proboscis extension response (PER) is a well established paradigm to study classical conditioning in honeybees. Thereby the odor-reward association can be expressed occasionally after a single learning trial (see also companion poster by Pamir et al. on one-shot learning in the honeybee). Learning related plasticity in single honey bee neurons and ensembles were reported for different processing levels (mushroom body extrinsic neurons: Mauelshagen 1993; Menzel 2005, Okada 2007, ventral unpaired maxilar neuron1 *Vummx1*: Hammer 1993). But still the roles of interactions between single neurons and their ensemble and between different neuropiles during memory formation and transformation are little understood.

Mushroom bodies (MBs) of the insect brain are higher order multimodal integration centres involved in learning and memory formation. MB extrinsic neurons (ENs) provide output from the MB. After odor-reward association single ENs changed their odor response profile other ENs increased their response strength. Both changes were dominated by the rewarded stimulus (Strube-Bloss, Nawrot, and Menzel, submitted manuscript).

Here we study the temporal dynamics of the establishment of the odor reward association in the single unit activity of mushroom body extrinsic neurons. Specifically we estimate the time-resolved signal-to-noise ratio (SNR) of the instantaneous firing rates as a measure of the tuning strength of single ENs. We adopt the following definition of the SNR:

$$SNR = (s_s^2 - s_e^2) / s_n^2$$

where $s_s^2$ is the variance of the signal, i.e. the instantaneous tuning curve, $s_n^2$ is the variance of the noise, i.e. the variance of trial-to-trial fluctuations, and $s_e^2$ is the bias due to finite sampling in the case of a flat tuning curve. The SNR takes into account the trial-by-trial variability of responses and has been shown to correlate well with single-trial decoding performances (Mehring et al., 2004).

In this study we show that the SNR increases significantly after learning due to an improved tuning to the reward-related stimulus (Figure 1). This increase occurs within a short time (<200ms after stimulus onset) and the detailed dynamics is stereotyped for two different groups of ENs.

This study suggests that after memory consolidation mushroom body output neurons reliably predict the reward shortly after stimulus onset. We currently study the possibility of decoding odor-reward information from the single-trial activity of the EN ensemble.

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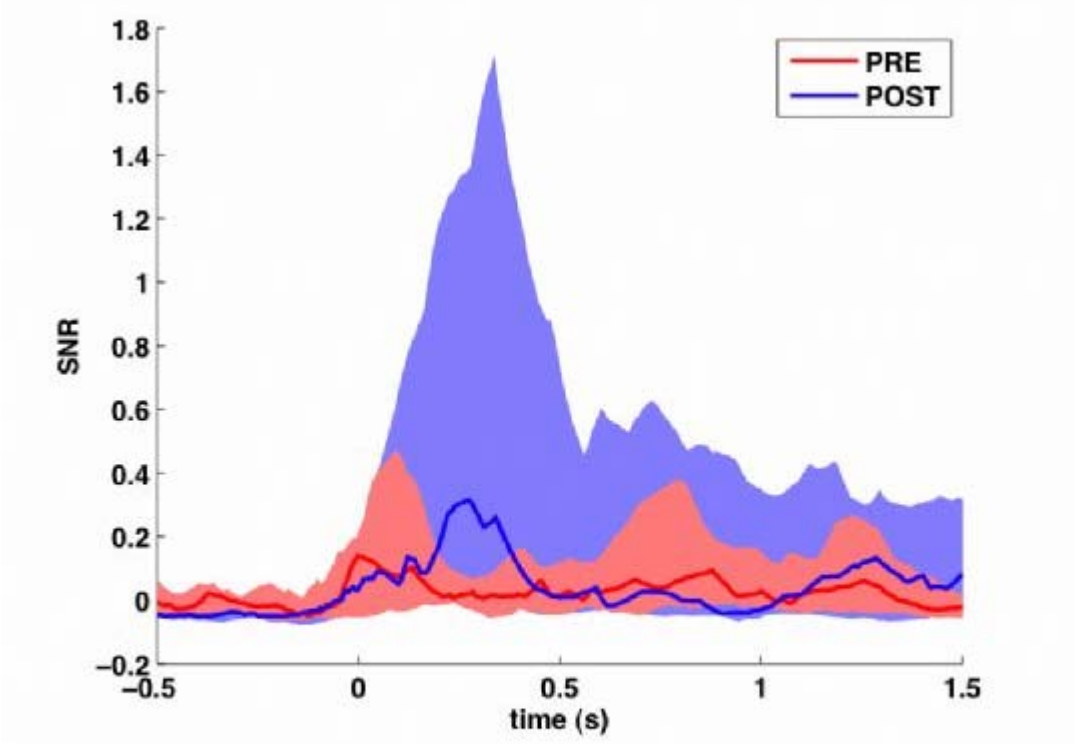


**Figure 1. Time-resolved SNR of the instantaneous firing rate of ENs.** The time-resolved SNR is shown prior to conditioning (in red) and posterior to conditioning (in blue). Lines indicate median values, while color shades span from the 75$^{th}$ percentile to the 25$^{th}$ percentile of the signal distribution. Time is referenced to the stimulus onset.

# THE EFFECTS OF TRANSCRANIAL DIRECT CURRENT STIMULATION OF THE RIGHT DORSOLATERAL PREFRONTAL CORTEX ON PLANNING PERFORMANCE

Colleen A. Dockery[1], Christian Plewnia[3], Niels Birbaumer[2]

[1]Max Planck Graduate School of Neural & Behavioral Sciences,
[2]Institute of Medical Psychology and Behavioral Neurobiology, University of Tuebingen,
[3]Department of Neurophysiology & Interventional Psychiatry,
University Hospital Tuebingen, Tuebingen, Germany

Planning performance has been shown to depend on the integrity of the dorsolateral prefrontal cortex (DLPFC) and to be modifiable by acute transcranial direct current stimulation (tDCS) when applied to the left hemisphere with performance benefits that persist up to a year. Both anodal and cathodal tDCS of the left DLPFC result in phase-dependent, polarity-specific benefits on TOL performance (Dockery et. al 2009). This experiment evaluated the effects of tDCS of the right DLPFC by comparing TOL performance in a within subject, cross-over design of randomly assigned healthy participants (n=24). tDCS (real or sham) was applied for 15 min. over three sessions with a 2 or 6 mos follow-up. Subjects were then grouped according to tDCS sequence, defined as either anodal tDCS before cathodal ($tDCS_{AC}$), or the contrary ($tDCS_{CA}$), while no difference was found for order of sham relative to active tDCS. For both reaction time (RT) and accuracy (ACC), significant interactions of STIMULATION CONDITION X tDCS SEQUENCE [RT: $F(2,44) = 10.148$, $p=.001$; ACC: $F(2,44) = 4.904$, $p=.012$] showed that only the cathodal tDCS group but not sham or anodal tDCS differed according to phase of application. The data indicate that during early acquisition inhibition by cathodal tDCS leads to slower RT and worsened ACC. Further these performance decrements by cathodal tDCS applied early, rather than late in the sessions were specifically apparent for difficult problems from the higher task load (STIMULATION CONDITION X TASK LOAD X tDCS SEQUENCE [RT: $F(2,44) = 4.066$, $p=.042$ GG; ACC: $F(2,44) = 4.098$, $p=.025$ GG]). The known learning effect was shown by differences between sessions ($p=.000$), however a lack of effect of session for anodal tDCS (RT: $p=.143$, ACC: $p=.069$) indicates that anodal tDCS applied during early acquisition leads to faster RT. No effect of tDCS sequence was found in the follow-up session

Our results indicate that the rtDLPFC is essential for TOL planning during early learning, as inhibition by cathodal tDCS impedes performance especially for difficult problems. Increased excitation by anodal tDCS early in performance supports faster processing. This pattern differs from tDCS of the left DLPFC which beneficially influenced performance in late learning and for long-term skill retention. This study elucidates that tDCS can be used to study differences in the functional anatomy of dorsolateral prefrontal cortical circuits and their role in executive function. Since patients with frontal lobe pathologies show impaired executive function, tDCS may provide a tool to improve the planning ability by boosting the connections or compensating for altered excitability in pathways of the compromised hemisphere.

# The Fruit Flies' Basic Strategies of Humidity-Orientation And a New Challenge for the Mushroom Bodies

Bianca Zaepf[1], Constantin Regenauer[1], Roland Strauss[1]

[1]Johannes Gutenberg-University, Dept. of Zoology III - Neurobiology, Col.-Kleinmannweg 2, 55099 Mainz, Germany[1]

*Drosophila melanogaster* spreads as a cosmopolitan across the world and is just limited by the extremes of the biogeographic ranges. Their ability of sensing moist or dry air with two different sensors (LIU ET AL., 2007) gives the basis of humidity orientation. An aversive stimulus like relative humidity above 87% normally causes a turning behavior to drier sites in agitated flies. But in a relaxed and saturated state flies opt for an intermediate humidity and one step further could be the right choice to overcome a local humidity-maximum or an aversive input due to noise. This kind of decision making should be stabilized by means of a kind of positive short-term memory for the parameters of the movement direction. Flies find in this manner their preferred ranges of relative humidity depending on the current needs. The choice for a place with a relative humidity of 87% for example has already proven to be regulated by the thirst level and begins after three hours absence of moisture at 26°C (PERTTUNEN & ERKKILAE, 1952). For the investigation of the fruit flies' strategies of humidity-orientation we planned and designed two different setups. One was based upon an idea of SAYEED and BENZER (1996), who introduced a binary choice apparatus for testing *Drosophila melanogaster* in a simple humidity-choice paradigm. We changed it into a humidity paradigm with a variably adjustable gradient to test orientation behavior in a linear gradient, or a gradient with different ascending slopes on either sides, or a noisy distribution of humidity. The second setup is based on the control of relative humidity in the headroom above aqueous solutions of Glycerin after Braun and Braun (1958). It is used to find the favored humidity ranges of Drosophila at 25°C (±0,5C°) over long-term measurements in a box with an almost linear humidity gradient from 4% to 100% (±3%) relative humidity. The humidity-dependent decision making requires higher integration centers of the brain and likely also spatial orientation capacities. In the case of the humidity orientation we found that the mushroom bodies play a particular role in the termination of an ongoing orientation behavior but not in the final outcome of the preferred humidity ranges in long -term experiments. This gives a new view at the mushroom bodies and their role in orientation behavior. The investigation of relevant centers in the fly brain for the action-oriented perception of humidity is one example, which is inspiring for computational models of the insect brain. Concepts of information processing in autonomously orienting roving robots are based on our results on the flies' orientation strategies in areas with noisy gradients of environmental parameters.

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# The impact of sex, diurnal phase and conditioned stimulus modality on infant and adult two-way active avoidance learning in rats.

Michael Gruss[1], Carolin Rockahr[1], Katharina Braun[1]

[1]Otto von Guericke University Magdeburg, Institute of Biology, Leipziger Strasse 44, 39120 Magdeburg, Germany

A growing body of evidence supports the hypothesis that juvenile cognitive training shapes neural networks and behavior, and thereby determines the adult´s capacity for learning and memory. In particular, we have shown that infant rats are unable to learn an active avoidance strategy in a two- way active avoidance (TWA) task, but nevertheless learn the same learning task faster as adults, indicating that a memory trace was formed in the infant rats, which most likely is recruited during adult training. The objective of the present study was to test the hypothesis that the learning impairment of infant rats might at least in part result from indadequate training conditions. TWA learning was assessed in i.) male and female rats trained ii.) as infants or adults during iii.) the light- or dark-phase using iv.) a tone or light as the conditioned stimulus (CS). Furthermore, v.) rats pre-trained as infants were re-trained as adults to examine the impact of the different training parameters on improved adult learning. Our results revealed that i.) there was no main effect of sex, ii.) infants were poor learners compared with adults. Furthermore, irrespective of sex or age learning performance was improved iii.) during the dark-phase, and iv.) but was not affected by CS modality. Additionally, v.) pre -training accelerated avoidance learning most pronounced in females which were pre -trained during the dark -phase with either light or tone as CS. Taken together, our data clearly show that TWA performance in infant and adult rats is sensitive to the diurnal rhythm. In addition, we demonstrated that the impaired avoidance learning in infants is not the result of inadequate training parameters, but might be related to the immaturity of the brain circuits and an insufficient recruitment of brain regions which are essential to learn an active avoidance strategy.

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# The Protocerebral Bridge holds a Representation for Object Positions – Orientation Studies in *ocelliless*[1] and Wild-Type Flies with Partially Occluded Eyes

Hannah Marie Joger[1], Christina Kauf[1], Ulla Prochazka[1], Roland Strauss[1]

[1]Johannes Gutenberg University, Zoology III Neurobiology, Colonel Kleinmann Weg 2, 55099 Mainz, Germany

The protocerebral bridge is a hemisphere-spanning neuropil in the central complex of insects shaped like a bicycle's handle bar. Based on gap-climbing studies in wild-type and bridge-defective fly strains Triphan et al. 2010 proposed a function model for the protocerebral bridge in *Drosophila melanogaster*. According to this model the bridge holds a representation of the fly's acute visual target whereby the azimuth position of the target is decisive for the latero-lateral position of the representation on the bridge. Medial positions represent the frontal visual field, left and right lateral positions the rear left and right visual field. The bridge is medially disrupted in the structural brain mutants *ocelliless*[1] and *tay bridge*[1] leading to a high angular scatter in targeted motor actions like gap climbing.

If the azimutopic representation holds true, we should be able to create a phenocopy of bridge-defective climbing behaviour in wild-type flies by occluding their frontal eye regions. Moreover, the same occlusions in *ocelliless*[1] flies should not further affect their residual climbing ability. Both proved to be the case. Further evidence comes from orientation experiments in which walking flies are confronted with single landmarks. Tracks of wild-type and *ocelliless*[1] flies and the orientation of their body axes were recorded and compared. *ocelliless*[1] flies ignore the landmark when it is presented frontally (0°) and are attracted when it is presented under 30° to 80°. Wild-type flies decide to walk towards the landmark when it is presented at angles of 0° to 110°. Wild-type flies with occluded eyes act similar to *ocelliless*[1].

To survey whether the frontal visual field of the mutant flies is entirely blind or just fixation blind, *ocelliless*[1] flies with occluded posterior regions of both eyes were tested in an optomotor setup (Strauss et al. 1997). Just the frontal 30° were left intact. The preliminary results allow the assumption that the frontal part of the eye is capable of conveying motion information because flies show optomotor compensation during walking.

This work is supported by the German Science Foundation under STR590/2-4 and by the EUFP7-programme (ICT – 216227) SPARK II.

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# The Role of EPAC in Synaptic and Behavioral Plasticity. - A case study in Drosophila. -

Marina Efetova[1], Sabrina Scholz[1], Kamil Rosiewicz[1], Martin Schwarzel[1]

[1]Freie Universität Berlin, Emmy Noether Nachwuchsgruppe `Biologische Gedächtnisse`, Takustrasse 6, 14195 Berlin, Germany

EPACs (aka exchange protein activated by cAMP) mediate cAMP -dependent signals in parallel and / or additionally to the well established PKA-signaling pathway. In both, vertebrates and invertebrates, EPACs play an inportant role in regulation of synaptic plasticity and associative learning. However, in contrast to PKA signals, the mode of action in regulation of plasticity is not well understood in case of EPAC as genetic model systems are missing.

Here, we initiate a genetic loss-of-function model for EPAC in the fruit fly, Drosophila melanogaster. We have generated a total of six independent deletion mutations (dEPACΔ1 to Δ6) based on FDD-deletion techniques. To demonstrate specificity of appropriate mutant phenotypes we used targetd expression of EPAC-rescue constructs, alternatively encoding either Drosophila EPAC or an EPAC cDNA from mouse.

# The role of haemolymph glucose in honeybee learning and memory

Kathy Rether[1], Uli Müller[1]

Saarland University, Dept. 8.3-Biosciences - Zoology and Physiology (Neurobiology), D-66123 Saarbrücken, Germany; E-mail: kathyrether@mx.uni-saarland.de

When speaking of glucose in memory formation, one tends to think of its role as appetitive reward stimulus. By far more subtle, glucose acts as a modulator of several types of learning and memory formation in humans and rodents. Mechanisms like facilitation of neurotransmitter release and synthesis are suggested. However, the molecular targets of glucose and their role in regulating learning and memory remain unclear. Since all our knowledge bases on studies with humans and rodents there is as yet no evidence of potentially conserved glucose effects on the molecular machinery of learning that may apply to all species.
In our work we addressed this point and investigated whether, in addition to its role as rewarding stimulus, internal glucose contributes to the modulation of learning and memory. Our data revealed a connection between haemolymph glucose levels and the performance in appetitive conditioning. The results in honeybees parallel findings in humans and rodents. Latter studies argue for a conservation of basic features of the internal glucose signalling machinery which participates in the regulation of learning and memory processes. We are now aiming to identify molecular targets and pathways responsible for the effects of internal glucose on learning and memory in the bee.

# The ubiquitin-proteasome system (UPS) mediates the balance between long-term memories for classical conditioning and extinction in the honeybee (*Apis mellifera*)

Johannes Felsenberg[1], Vincent Dombrowski[1], Dorothea Eisenhardt[1]

[1]Freie Universitaet Berlin, Neurobiology, Koenigin-Luise-Strasse 28/30, 14195 Berlin, Germany

The ubiquitin-proteasome system (UPS) is a multi step protein degradation system. Target-proteins are tagged with ubiquitin by an ubiquitin-ligase and then recognized and degraded by the proteasome. The UPS is involved in neuronal processes including synaptic plasticity and synaptic function. Its role in the formation of long-term memory has been demonstrated, but these results are controversial. Also it has been shown that this protein degradation system is involved in memory formation after memory retrieval. It had been concluded that the UPS is necessary for destabilizing processes that are prerequisite for updating the retrieved memories either by reconsolidation or by the consolidation of an extinction memory. Here we examine the influence of the proteasome mediated protein degradation on the formation of memory and on the retrieval induced formation of an extinction memory in the honeybee (*Apis mellifera*). We use an appetitive pavlovian learning paradigm, the olfactory conditioning of the proboscis extension response (PER). We systemically inject a proteasome inhibitor, MG132, after training or after a retrieval session. Our results show that MG132 applied after training enhances the formation of long-term memory depending on the number of training trials and the intertrial interval, leaving the mid-term memory unaltered. Furthermore we show that the expression of the extinction memory is reduced by the injection of MG132 after the retrieval session depending on the training. Our data demonstrate that only if the initially formed memory is affected by the proteasome inhibitor the retrieval induced memory extinction for this memory is as well affected and vice versa.
From these data we conclude that memory formation is regulated by the proteasome activity, based on the number of training trials and the intertrial interval. We hypothesize that this mechanism restricts the formation of an inadequate strong long-term CS-US memory in the honeybee. We postulate that a similar or the same mechanism works on retrieval-induced re-consolidation, restricting the strength of the CS-US memory balancing both memories that are formed after retrieval: the memory for the CS-US association and the extinction memory.

# Theta synchronization and phase distribution of unit activities in amygdalo-hippocampal-prefrontal cortical circuits during fear memory consolidation and extinction.

Jörg Lesting[1], Christian Kluge[1, 2], Rajeevan Therpurakal Narayanan[1], Hans-Christian Pape[1], Thomas Seidenbecher[1]

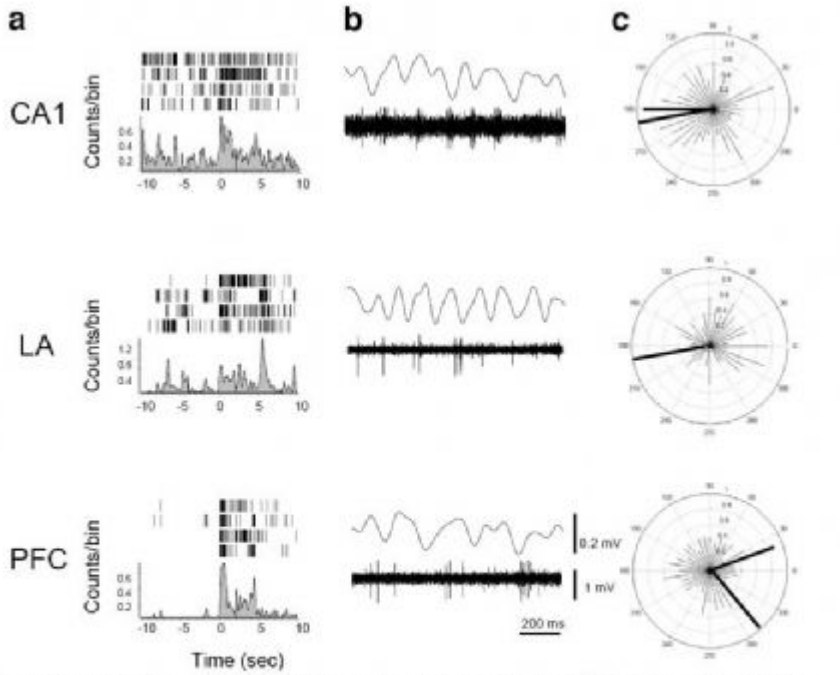
[1]Westfälische Wilhelms-Universität Münster, Institute of Physiology 1, Robert-Koch-Str. 27a, D-48149 Münster, Germany
[2]University College London, Institute of Cognitive Neuroscience, UK

Theta synchronization and phase distribution of unit activities in amygdalo-hippocampal-prefrontal cortical circuits during fear memory consolidation and extinction

Jörg Lesting [1], Christian Kluge [1,2], Rajeevan Therpurakal Narayanan [1], Hans -Christian Pape [1], Thomas Seidenbecher[1]

[1]Westfälische Wilhelms-Universität Münster, Institute of Physiology 1, Münster, Germany
[2]University College London, Institute of Cognitive Neuroscience, UK

It is widely accepted that theta oscillations provide spatiotemporal codes, which, for instance, support the temporal compression from a rather long time scale of behavior into the milliseconds timescale required for synaptic plasticity. In the domain of fear, theta synchrony of hippocampal CA1 and the lateral amygdala (LA) increases during consolidation and reconsolidation of conditioned fear, and returns to baseline at remote stages of fear memory. Moreover, interactions between the medial infralimbic PFC (mPFC), LA and CA1 play an important role in fear extinction.
We used multiple site local field potential (LFP) and single unit recordings in freely behaving mice to experimentally assess theta-entrained activity across this tripartite circuit (LA-CA1-mPFC) during fear memory consolidation and extinction.
Our results showed that conditioned fear and extinction are associated with synchronous theta activity in the lateral amygdala, hippocampal CA1 and infralimbic prefrontal cortex. Furthermore, phase-related unit activity was present at these sites, indicating that synaptic activity contributes to local theta LFP generation in these structures (Fig.).




**Simultaneously recorded LFP and unit activity in CA1, LA and mPFC.** **(a)** Peri-stimulus raster of a conditioned stimulus-related unit activity. **(b)** Theta phase-related unit activity. Shown are original LFP waveforms (upper traces), and simultaneously recorded unit activity (bottom traces). **(c)** Average phase distribution of identified units with significant theta-phase locking.

# Towards the understanding of complex human decision and learning processes across the life-span

Aki Naito[1], Wendelin Boehmer[1], Andreas Marschner[2], Tobias Sommer[2], Christian Buechel[2], Klaus Obermayer[1]

[1]Neural Information Processing Group, TU Berlin, Franklinstr. 28/29, 10587 Berlin, Germany

[2]Institut für Systemische Neurowissenschaften, Universitätsklinikum Hamburg-Eppendorf, Martinistr. 52, 20246 Hamburg, Germany

We present a method of model-based analysis for the behavioural and functional imaging data during learning and decision making tasks. A novel experimental paradigm, which imposes multiple decisions before reaching a goal is introduced. It also incorporates the notion of risk and two types of regret, namely experienced losses and fictive prediction errors. The paradigm is formulated as a Markov Decision Process (MDP), which is used by the Reinforcement Learning (RL) models to analyse and interpret human data. The project plans to analyse individual behaviours across different population groups and establish a model-based analysis method that parameterise the learning behaviour of humans, which could then be used for clinical tests as well as for functional imaging analysis.

# What impact do varying reward magnitudes have on associative strength, memory formation, and extinction in classical conditioning of harnessed honeybees (*Apis mellifera*)?

Katharina Grauel[1], Kathrin Marter[1], Dorothea Eisenhardt[1]

[1]Freie Universität Berlin, Neurobiologie , Königin-Luise-Straße 28/30, 14195 Berlin, Germany

In appetitive classical conditioning an animal learns that a previously neutral stimulus (conditioned stimulus, CS) is associated with a reward (unconditioned stimulus, US). After conditioning, the CS alone elicits a conditioned response (CR). The term associative strength describes the extent to which the CS predicts the reward. It is generally accepted that the associative strength is mirrored in the conditioned response during acquisition (Rescorla & Wagner, 1972).
Repeated retrieval of a CS-US memory by the CS alone leads to a decreasing CR. This phenomenon is termed extinction and is caused by extinction learning: a process during which the animal learns that the CS is no longer associated with the reward (CS-noUS).
Recent data in appetitive conditioning of the proboscis extension response (PER) of harnessed honeybees (*Apis mellifera*) demonstrate a correlation between the US duration during appetitive classical conditioning and the susceptibility of a long-term extinction memory for protein synthesis-inhibition (Stollhoff, N., Eisenhardt, D. (2009) J Neurosci, 29). From this study we concluded that the biochemical identity of a long-term extinction memory depends on the magnitude of the prediction error between the previously experienced reward during the CS-US association and the absence of the reward during memory retrieval. If this holds true, variations in the reward magnitude should result in differences in the associative strength mirrored by a difference in the CR during acquisition. Surprisingly, this was not demonstrated by Stollhoff & Eisenhardt (2009), which might be due to the way the CR (proboscis extension: yes or no) was measured.
Therefore in this study, we ask, if quantifying the CR uncovers an effect of the reward magnitude on the associative strength respectively the CR in harnessed honeybees. In addition we examine the impact of the reward magnitude on the associative strength during extinction.

# Where does Potein-Kinase A support odor memory? Functional memory maps based on a RNAi knockdown approach.

Antje Richlitzki[1], Marina Efetova[1], Martin Schwärzel[1]

[1]Free University Berlin, Neurogenetic, Takustr. 6, 14195 berlin, Germany

PKA is the textbook example of a cAMP downstream target and it is involved in synaptic and behavioral plasticity across the animal kingdom. In Drosophila, the most abundant catalytic subunit of PKA is encoded by the DC0 gene and appropriate mutants show poor odor learning. However, the neuronal substrates are unknown. To map the neuronal substrates of DC0-PKA function, we have used ectopic knockdown of DC0 and analysed the effects on reward- and punishment learning.
Our results revealed a complex involvement of PKA signaling in memory maturation with multiple dissociations between reward and punishment learning. Actually, we define the functional correlation between PKA and separable cAMP pools by simultaneous manipulation of DC0 and either rut-AC or dnc-PDE. As both forms of behavioral plasticity, i.e. reward and punishment learning, share common elements on level of neuronal pathways and molecular signaling, this comparative analysis is ideally suited to define signaling divergences along the cAMP-PKA pathway.

Quality of the unconditioned stimulus (sugar reward or electric shock punishment) determines whether flies acquire either appetitive or aversive odor memories. In either case cAMP signaling is cruical for odor learning as derived from experiments based on the learning mutant rutabaga – affecting an adenylyl cyclase (rut-AC). However, cAMP signaling is also regulated by the dunce phosphodoesterase (dnc-PDE) and further downstream the signaling cascade protein-kinase A (PKA) is required to support aversive and appetitive odor learning.

Here, we ask the question which stages of the olfactory pathway require dnc-PDE or PKA function to support memory. To that end we use tissue-specific rescue of dnc-PDE or RNAi-based knockdown of PKA along the olfactory pathway. These results will allow for a comparison between those neuronal stages requiring cAMP regulation at level of either rut-cAMP or dnc-PDE with those stages requiring PKA.

# Why feed-forward structure fails to propagate in plastic recurrent networks

Susanne Kunkel[1,2], Markus Diesmann[3,4,5], Abigail Morrison[1,2,5]

[1] Functional Neural Circuits Group, Faculty of Biology, Albert-Ludwig University, Freiburg, Germany
[2] Bernstein Center Freiburg, Albert-Ludwig University, Freiburg, Germany
[3] Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako, Japan
[4] Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Jülich, Jülich, Germany
[5] RIKEN Brain Science Institute, Wako, Japan

Spike-timing dependent plasticity (STDP) has traditionally been of great interest to theoreticians, as it seems to provide an answer to the question of how the brain can develop functional structure in response to repeated stimuli. However, despite this high level of interest, convincing demonstrations of this capacity in large, initially random networks have not been forthcoming. Such demonstrations as there are typically rely on constraining the problem artificially. Techniques include employing additional pruning mechanisms or STDP rules that enhance symmetry breaking, simulating networks with low connectivity that magnify competition between synapses, or combinations of the above (see, e.g. [1,2,3]).
Here, we describe a simple model for the propagation of feed-forward structure in plastic recurrent networks. The key prediction of the model is that the number of neurons recruited by a repeated synchronous stimulus protocol is subject to an unstable fixed point. A synchronously firing group of neurons of a size below that of the fixed point recruits a smaller group, leading to a failure of the structure to propagate, whereas a synchronously firing group of a size above that of the fixed point recruits a larger group, causing the whole network to be recruited. In other words, a synchronous stimulus is always either not enough or too much. We demonstrate by simulation that a large-scale network behaves as predicted by the theory. Finally, we investigate biologically motivated adaptations to the balanced random network model that have been proposed to facilitate structure formation in large-scale simulations.

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# Poster Topic

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T26-8B Microcircuits of grid- and head-direction systems in the rat medial entorhinal cortex
*Andrea Burgalossi, Lucas Herfst, Moritz von Heimendahl, Henning Förste, Martin Schmidt, Michael Brecht*

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*Dominik M Endres, Mike W Oram*

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*Yuqiao Gu, Hana Belmabrouk, Antoine Chaffiol, Jean-Pierre Rospars, Dominique Martinez*

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*Nikolay Chenkov, Robert Schmidt, Richard Kempter*

T26-12B Network mechanisms for the modulation of gamma spike phase by stimulus strength and attention
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T26-4C Refractoriness of individual neurons exposed in population spike trains
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*Andreas VM Herz, Carleen Kluger, Alexander Mathis, Martin Stemmler*

T26-16C What correlation coefficients can and cannot tell.
*Tatjana Tchumatchenko, Theo Geisel, Maxim Volgushev , Fred Wolf*

# A Bayesian graphical model for the influence of agency attribution on perception and control of self-action

Tobias F. Beck[1,2,3], Carlo Wilke[2], Barbara Wirxel[2,3], Dominik Endres[1,2], Axel Lindner[2,3], Martin A. Giese[1,2,3]

[1] Section for Computational Sensomotorics
Center for Integrative Neurosciences
[2] Hertie Institute for Clinical Brain Research
Department of Cognitive Neurology
University of Tübingen
Hoppe-Seyler-Str. 3, 72076 Tuebingen, Germany
[3] Bernstein Center for Computational Neuroscience Tübingen
[*] Equal Contribution.

The perception and control of actions depends on sensory information and on internal predictions that are likely produced by internal forward models. Such predictions depend critically on whether the observed consequences of actions are solely associated with one's own actions or with changes in the environment. Likely, the brain continuously updates an estimate of the 'agency' of afferent sensory information as caused by own actions or by the environment. The attributed agency depends on the consistency between predicted sensory consequences of actions and the real sensory input. In an experiment we manipulated the consistency between motor acts and their related sensory consequences and investigated the influence of the consistency between those two variables and attributed agency of the sensory signals (Fig. A), see also [1]. Based on this experiment, we propose a probabilistic model for the relationship between the relevant variables: the internal estimate of the motor consequences, the sensory input and the attributed agency of the sensory input. The model correctly predicts the experimental key result (Fig. B) that the prediction of visual consequences of motor actions results from a cue integration of internal predictions and sensory input, which depends on the attribution of agency to the sensory signals. For small deviations between these cues, the sensory input is attributed as caused by oneself and the predicted sensory consequences result from an integration of sensory input and internal state estimate. However, large deviations between sensory signals and the predicted sensory consequences result in the attribution of the sensory input as not being caused by oneself, strongly reducing the influence of the sensory signals on the internal estimate of the external motor consequences. The relevant variables are modeled by a Graphical Bayesian Network (Fig. C) with the variables: target location $X_T$, internal position information $X_{int}$, internal estimate for motor state consequences $X_{est}$, visual input $X_{vis}$ and binary agency variable Self, with prior distributions $s_e$, $s_v$, $(s_0, \mu_0)$ and $P_{self}$ as estimated from the experimental data.
Bayesian Graphical Models provide an insightful framework for investigating the flexible integration of signals in sensory motor control.

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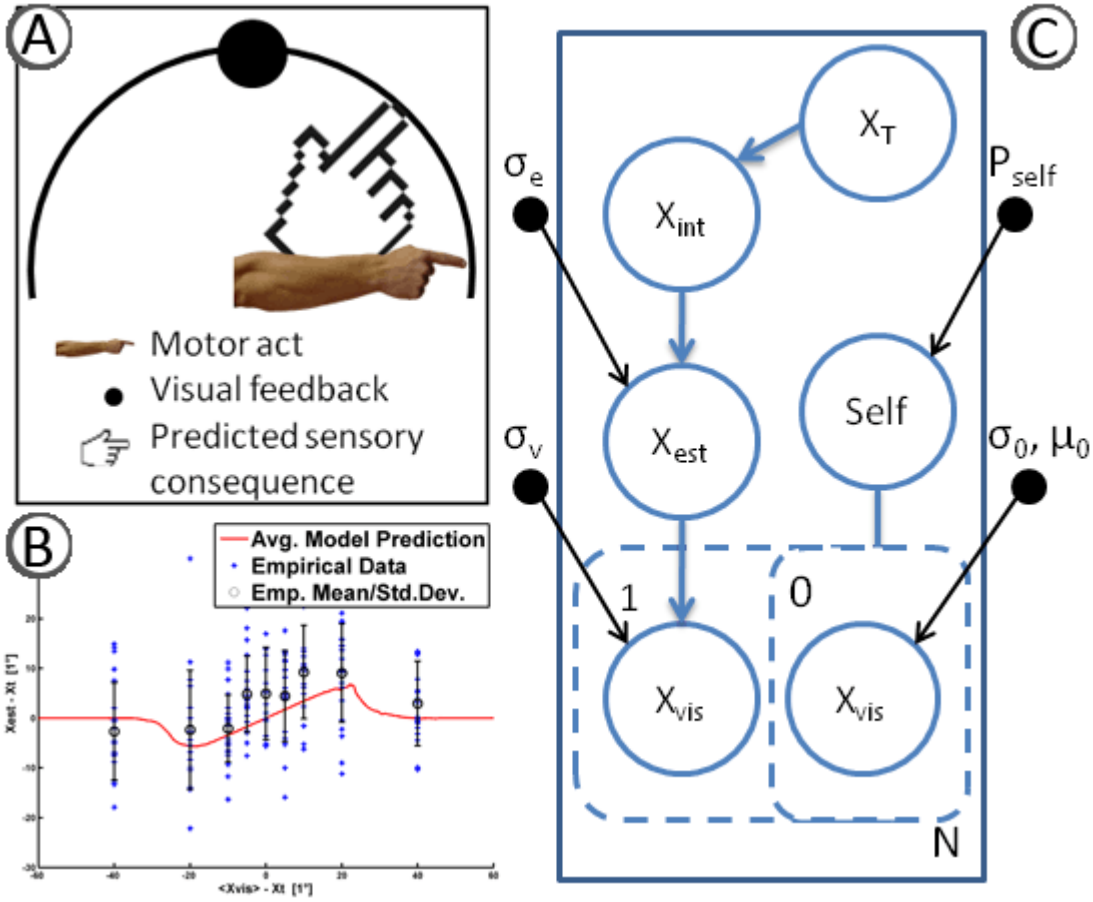
A
Motor act
Visual feedback
Predicted sensory consequence
B
Avg. Model Prediction
Empirical Data
Emp. Mean/Std.Dev.
Xest - Xt [1°]
<Xvis> - Xt [1°]
C
$X_T$
$X_{int}$
$X_{est}$
Self
$X_{vis}$
$X_{vis}$
$\sigma_e$
$P_{self}$
$\sigma_v$
$\sigma_0, \mu_0$
1
0
N

# A learning neural field model of decision making

Christian Klaes[1], Sebastian Schneegans[2], Gregor Schöner[2], Alexander Gail[1]

[1]German Primate Center, Sensorimotor Group, Kellnerweg 4, 37077 Göttingen, Germany
[2]Institute for Neural Computation, Ruhr University Bochum, Universitätsstraße 150, 44780 Bochum, Germany

In order to plan goal-directed reach movements, it is necessary to combine sensory information about possible target locations with information about the current behavioral context to select an appropriate action. The frontoparietal reach network consists of several brain areas, which are involved with value based selection of actions. As an underlying mechanism, it has been proposed that the selection and specification of possible actions are not two distinct, sequential operations, but that instead the decision for an action emerges from the competition between different movement plans, which are specified and selected in parallel[1]. Here, we present a neural field model[2], which is able to learn an arbitrary spatial remapping rule and which simulates the dynamics of action selection in the frontoparietal reach network. The model was developed in parallel with an electrophysiological study in monkeys[3] and can reproduce several findings of that study. The model was trained to perform a task which required rule-based spatial remapping of a motor goal. In this task the motor goal was defined by combining a spatial cue and a remapping rule, which was in indicated by a contextual cue. The model can learn this context-dependent remapping task via an implemented Hebbian-style learning rule (Fig. 1). It is trained from a pre-structured initial state (with default cue-response mapping behavior), using a training procedure that emulates the training procedure of the monkeys. The trained model developed activity patterns and neuronal tunings consistent with the empirical data. We then examined how actions are planned in the absence of an explicit rule, i.e. with no contextual cue. In this case the model showed a decision bias towards one goal or an equal representation of both potential goals, depending on the input statistics during training. The model remained susceptible to later experience and changes of the reward schedule. This matches the observations in monkeys performing the same task and it provides an account for the formation of action plans under ambiguous conditions. The field model provides an integrated account for the operations of sensorimotor transformations, working memory, and action selection required for decision making.

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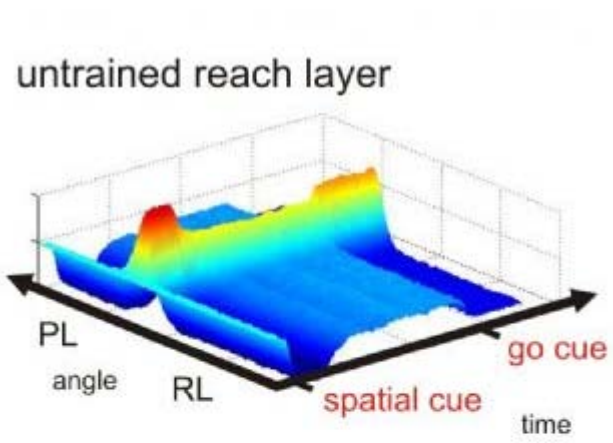
a


untrained reach layer
PL
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RL
spatial cue
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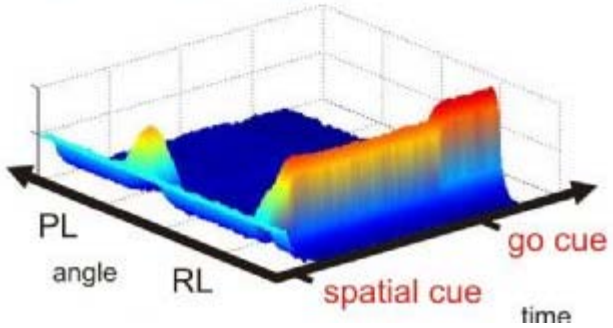
time

b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# A machine learning approach to estimation of auditory spectro-temporal receptive fields

Arne-Freerk Meyer[1], Jan-Philipp Diepenbrock[2], Max Happel[2], Frank Ohl[2], Jörn Anemüller[1]

[1]Carl von Ossietzky University Oldenburg, Medical Physics Section, Carl-von-Ossietzky-Str. 9-11, 26111 Oldenburg, Germany
[2]Leibniz Institute for Neurobiology and Institute of Biology, Otto-von-Guericke University, Brenneckestr. 6, 39118 Magdeburg, Germany

The linear spectro-temporal receptive field (STRF) of a neuron is defined as the linear filter that, when convolved with the spectro-temporal representation of an arbitrary stimulus, gives a linear estimate of the evoked firing rate [1]. Common methods of STRF estimation are the spike-triggered average (STA) that computes the mean stimulus pattern preceding every spike [1] or linear regression between stimulus and estimated spike rate [2].

Here, we present an approach that considers the STRF estimation problem as a binary classification task, not only using stimulus patterns that evoke spikes but also those after which no spikes occur. We show that the linear STRF model is equivalent to the structure of a linear classifier, implemented here using a support vector machine (SVM). After SVM training, the corresponding STRF is inferred from the learned support vectors. A framework for determination of model complexity is presented and the novel method is tested using multi- and single-unit data obtained from primary auditory cortex of anesthetized Mongolian gerbils [3] and compared to linear regression for different stimulus classes.

In general, STRFs estimated using SVM classification and linear regression reliably capture the linear response field of a neuron if both methods produce the same STRF structure. We compared groups of frequency modulated sweeps (FM banks, ~3000 spikes) to dynamic moving ripple stimuli (DMR, ~4000 spikes). It turns out that FM bank-based STRFs show the same structure for different model complexities whereas DMR-based STRF structure changes with model complexity.

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# A minimal model of metabolic energy management in the brain

Florian A. Dehmelt[1], Christian K. Machens[1]

[1]Group for Neural Theory, Laboratoire de Neurosciences Cognitives (Inserm U960), Département d'Etudes Cognitives, Ecole Normale Supérieure, 29 rue d'Ulm, 75005 Paris, France

Models of cerebral blood flow and metabolism have been very successful in reproducing the shape and timing of the haemodynamic response to neural activity. On the other hand, as these models are mechanical or phenomenological in nature, they do not allow for judging the efficiency of the underlying allocation of energy. This would however be crucial for understanding why there is such a response in the first place. Hence, we here describe a complementary approach, suggesting that optimality with respect to resource constraints contributes to the characteristics of the haemodynamic response.

# A Parametric Free Method for Estimating High Dimensional Tuning Curves

Dinu-Mihai Patirniche[1], Alexander Mathis[1,2,3], Martin Stemmler[2,3], Andreas Herz[2,3]

[1]GSN-LMU, BCCN München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany
[2]Bernstein Center for Computational Neuroscience Munich, 82152 Planegg-Martinsried, Germany
[3] Graduate School for Systemic Neuroscience, LMU, 82152 Planegg-Martinsried, Germany

A simple way of characterizing the response of a neuron is to count the number of spikes fired during the presentation of different stimuli [1]. The neuron's tuning curve traces the expected number of spikes as the stimulus changes, projecting the space of continuous stimuli onto the neuron's average response. Yet measuring the neuronal response is typically riddled by intrinsic noise inherent in spiking neurons. Standard methods for estimating tuning curves fall into two classes: the first bins the stimulus space and averages the spike count during repeated trials of identical stimuli [2], while the second assumes that the tuning curve comes from a family of parametrized functions, which reduces the estimation task to fitting parameters [1]. In the first case, the neuronal response must be sampled many times for accuracy [1]; in the second case, prior assumptions are made.

Both standard methods have drawbacks, so we apply an alternative approach based on particle filters. Randomly distributed points, called particles, are added to the measured data (s $R^n$; n N) these points undergo directed motion along the spike count axis based on the gradient of the squared distance between particles and true data points, weighted by distance in stimulus space. At the end of the motion, the particles lie close to or along the true tuning curve. We tested a wide range of theoretical tuning curves subject to different noise models. The particle filter method reconstructs the shape of the tuning curves with a higher accuracy than the standard binning method and needs fewer observations of real data. This method works for arbitrarily high-dimensional stimulus spaces and the computational complexity scales better than the classical methods for the same search space.

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# Beyond local cortical network modeling: linking microscopic and macroscopic connectivity in brain-scale simulations

Tobias C Potjans[1], Susanne Kunkel[3,4], Abigail Morrison[3,4,5], Hans Ekkehard Plesser[5,6], Markus Diesmann[1,2,5]

[1]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany
[2]Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako, Japan
[3]Bernstein Center Freiburg, Freiburg, Germany
[4]Functional Neural Circuits Group, Faculty of Biology, Albert-Ludwigs-University, Freiburg, Germany
[5]RIKEN Brain Science Institute, Wako, Japan
[6]Dept. of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Aas, Norway

What does a single cortical neuron "see"? A single neuron receives on the order of 10,000 synaptic inputs. These inputs originate in various parts of the brain and can be categorized into local, long-range intrinsic and extrinsic inputs. The extrinsic inputs originate in several subcortical structures and also in on the order of 10 other cortical areas [1]. Therefore, a single cortical neuron processes inputs from potentially every other part of the brain. It has been found that the detailed connectivity structure on the microscopic and the macroscopic level depends on the cell type of a neuron, i.e. on its area, its layer etc. [1,2]. The exact ratio of long-range and local inputs a neuron receives is not known [3].

Cortical network modeling typically involves a single scale: either the scope is the local microcircuit on the level of single neurons [2] or exclusively the macroscopic network missing the link to single-neuron activity [4]. Local cortical network models comprising on the order of 100,000 neurons represent the majority of the local synapses (around 1 billion) and treat the extrinsic inputs as external. These models can adequately explain the local interactions based on the microcircuitry and consistently replicate prominent features of the cell-type specific activity observed in awake animals [2]. However, their explanatory power is limited because the origin of a major fraction of the excitatory inputs is left unexplained.

Here, we present our approach to combine local microcircuit modeling with the macroscopic brain network: the figure shows a cartoon of the local cortical microcircuit on the left and the macroscopic connectivity pattern on the right. In the center, our brain-scale model is depicted that combines both network levels. The link between the microscopic and the macroscopic network is the cell-type specificity of the long-range connections. This specificity has been used by others to construct a theory on hierarchical information processing in the brain [1].

Brain-scale simulations require progress in simulation technology as these models comprise millions of neurons with around 10,000 synapses each. Therefore, we review the technical challenges to scale up the simulations software [5] to tens of thousands of processors for the routine investigation of brain-scale models at synaptic resolution.

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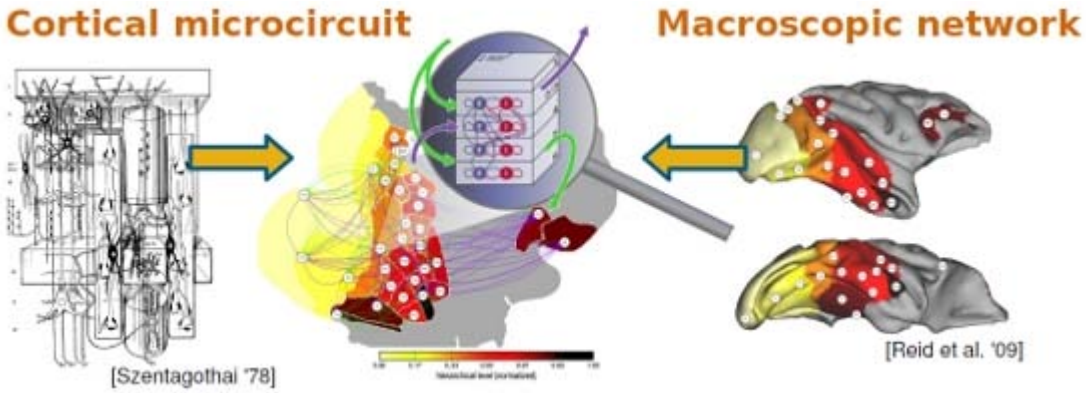


# Bursting dynamics in optically stimulated neuronal networks

Ghazaleh Afshar[1], Ahmed El Hady[1,2,3,4], Walter Stuehmer[2,3,4], Fred Wolf[1,3,4]

[1]Max Planck Institute for Dynamics and Self organization, Göttingen, Germany
[2]Max Planck Institute of Experimental Medicine, Göttingen, Germany
[3]Bernstein focus for Neurotechnology, Göttingen, Germany
[4]Bernstein center for computational neuroscience, Göttingen, Germany

Cultures of channelrhodopsin-2 transfected hippocampal neurons allow simultaneous optical stimulation and electrical recording from neuronal networks. As have been previously reported, after maturation these networks develop an electrical activity that is characterized by synchronized bursts. In this work, we study the influence of whole field blue light illumination on burst dynamics of these cultures. During stimulation the mean firing rate is significantly different than before and after stimulation and the bursting dynamics display characteristic adaptation patterns that we characterize thoroughly. After turning off the stimulus, a silent period follows and then the network gradually switches into an ongoing state of bursting activity. We conclude that light stimulation can be used to influence bursting dynamics in biological neuronal networks.

# Capacity Measurement of a Recurrent Neural Network

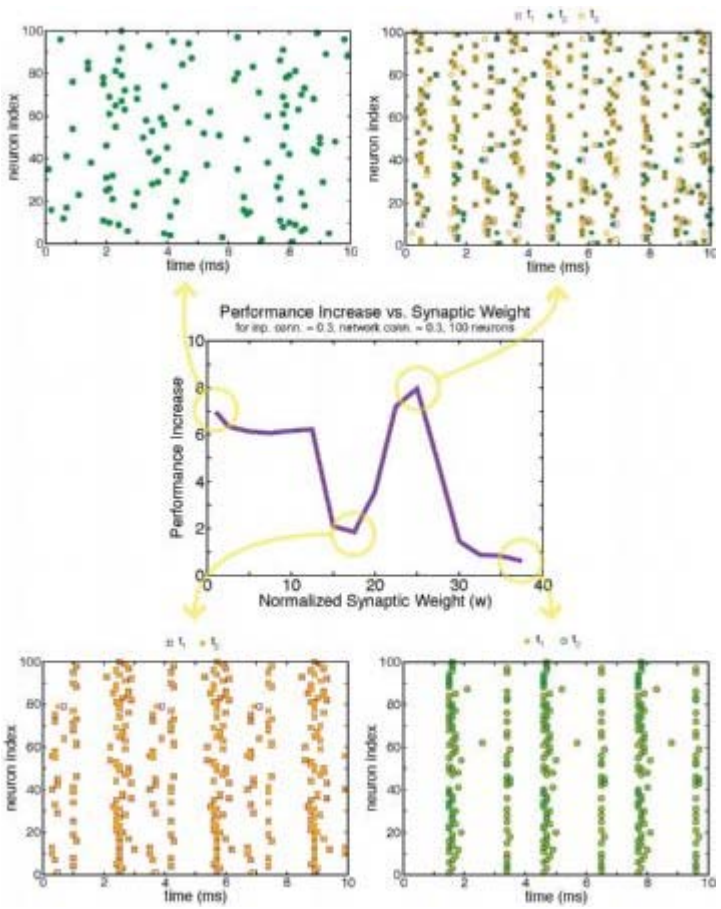
Chun-Wei Yuan[1], Matus Simkovic[1], Nikolay Chenkov[2], Christian Leibold[1]

[1]Ludwigs Maximilians Universitaet, Division of Neurobiology, Department of Biology II, Grosshadernerstr. 2, 82152 Martinsried, Germany
[2]Humboldt Universitaet zu Berlin, Institute for Theoretical Biology, Invalidenstr. 43, 10115 Berlin, Germany

Cortical networks possess highly recurrent connectivity and display complex firing dynamics. It has been postulated that these dynamics serve specific computational purposes, known as reservoir computing. One important feature is the ability to nonlinearly expand a time-varying input into a higher-dimensional spatio-temporal pattern of activations of the network neurons, rendering the possibility to perform a great multitude of computational tasks, such as speech recognition or computer vision.

Our research focuses on quantifying the performance of a randomly connected recurrent network of leaky integrate-and-fire neurons in canonical classification tasks. The implementation includes parallel independent Poisson inputs connected to the recurrent network, with the network output feed-forwardly directed to a read-out perceptron that performs a standard classification task. For benchmarking, another perceptron performs the same task while connected directly to the inputs. The analysis is then conducted by comparing the capacities of both setups, at 90% accuracy, as a function of parameters such as network size, network connectivity, etc.

It is found that, while the performance of network-mediated computation scales linearly with connectivity and network size, it behaves non-monotonically with respect to the normalized synaptic weight. As the weight is increased, the network state goes through phase transitions between asynchronous (high capacity) and synchronous (low capacity) firing modes.



# Combined Control Strategies for Advanced Locomotion Control in a Six-Legged Robot

Martin Biehl[1], Frank Hesse[1,2], Poramate Manoonpong[1], Florentin Woergoetter[1]

[1]Georg-August-Universitaet Goettingen, Third Institute of Physics - Biophysics, Friedrich-Hund-Platz 1 , 37077 Göttingen , Germany

[2]Bernstein Focus Neurotechnology, Bunsenstr.10, 37073 Göttingen , Germany

Walking animals show a variety and self-organization of behaviors leading to effective locomotion over rough terrain as well as complex environments. These achievements of the animals are driven by reactive and self-organized learning mechanisms. Recognizing that, to date, most walking robots employ preprogrammed reactive control leading to a limitation of adaptivity and self-organization. As a consequence, they might have difficulty to locomote over unknown rough terrain. In contrast, we employ neural locomotion control based on a central pattern generator (CPG) [1] and a self-organizing neural learning mechanism (known as homeokinesis [2,3]) for self-organized locomotion generation over rough terrain. Accordingly, this combination allows a hexapod to successfully transverse over the terrain. This is because minimization of the objective function ($E=||v||*||v||$, called time loop error (Fig. 1)) of homeokinesis leads to sensitivity of the motor output with respect to changes in the proprioceptive sensory input and the predictability of future inputs by an internal model. This paradigm shows high exploration abilities enabling the hexapod to appropriately free its legs when getting stuck as well as find footholds based on the self -organization principle. We first evaluate the proposed controller via physical simulation where the simulated hexapod is situated in very rough terrain (Fig. 1). Experimental results show that the hexapod gets stuck and fails to walk through the terrain if only CPG based locomotion control is used. On the other hand, the hexapod successfully walks through the terrain if the combination of CPG and homeokinetic control is employed.
Besides these experiments, we also apply information theoretical measures to analyze and evaluate the performance of homeokinesis in a complex sensorimotor system (i.e., hexapod). In future work, we will implement the controller on the real hexapod platform AMOS II (Fig. 1) and test it in real complex terrains.

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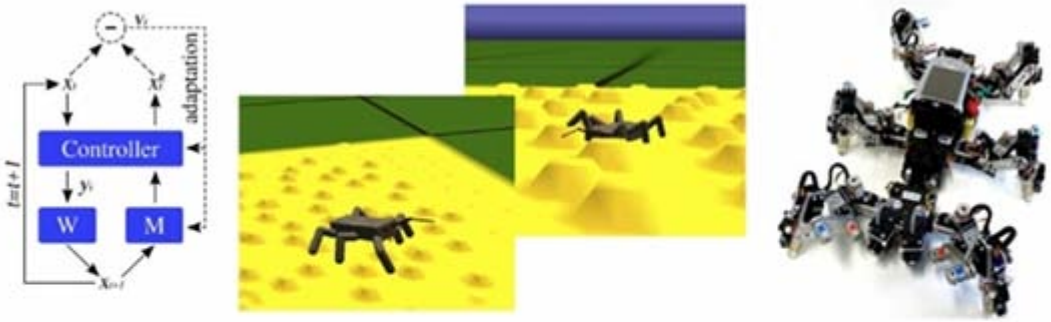


Figure 1: Left: Homeokinetic control structure and indicated learning scheme. The left part shows the sensorimotor loop where a sensor value x is processed by the controller to a motor command y. The execution of the latter in the world (W) leads to a new sensor value in the next time step. In the center part the closing of the time loop is depicted, where the new sensor value is propagated backward in time through an internal model (M) and through the controller, leading to a reconstructed sensor value at time t. The input shift v, measures the difference between the true sensor value and the reconstructed sensor value at time step t. The time loop error E=||v||*||v|| is used for the parameter adaptation of world model and controller. Middle: Simulations of hexapod in rough terrain using the lpzrobots software package [4]. Right: The real hexapod AMOSII.

# Comparison between unsupervised learning algorithms for the extraction of muscle synergies

Enrico Chiovetto[1], Lars Omlor[1], Andrea d'Avella[2], Martin Giese[1]

1-Section for Computational Sensomotorics, Department of Cognitive Neurology, Hertie Institute for Clinical Brain Research, Centre for Integrative Neuroscience, University Clinic Tübingen, Tübingen, Germany.
2-Department of Neuromotor Physiology, Santa Lucia Foundation, Rome, Italy.

Many studies in the last years have provided evidence for the idea that the muscle activation patterns underlying complex movements can be approximated by the combinations of a small number of basis components, also referred to as muscle synergies or movement primitives. To support this hypothesis, a variety of studies has assessed EMG signals from numerous muscles during the execution of a spectrum of motor tasks and has tried to extract low-dimensional components from this data by applying different unsupervised learning techniques. It has been shown that many techniques resulted in good approximations of such EMG data by means of a small number of basis components. However, it has remained largely unclear how to compare the results obtained with different algorithms, and how far certain algorithms are more appropriate than others to answer specific questions in motor control. Here, we systematically compared the results obtained with different methods for the extraction of muscle synergies from EMG data, and tried to quantify and compare their performance.
In order to have data with well-specified statistical properties available, we simulated artificial EMG data. The generative process combined linearly, and partially with time delays, a small number of basic components whose properties were optimized in order to mimic the basic statistical properties or real EMGs. The simulated data were based on three different generative models, inspired by the existing literature: 1) time-invariant synergies: muscle signals were generated by a small number of time-dependent functions that were superpositioned linearly with fixed time-invariant weights. 2) Time-varying synergy: signals were generated by superpositioning a small set of vector-valued time-dependent functions with a fixed set of combination coefficients and fixed time delays. 3) Anechoic synergies: signals were generated by the linear superposition of a small number of time-dependent functions with a fixed set of weights and delays.
We applied the following unsupervised learning algorithms for the extraction of muscle synergies to these simulated data sets: a) Non-negative matrix factorization (NMF). b) A modified version of NMF for the extraction of time-dependent synergies (d'Avella et al., 2006). c) Anechoic demixing (Omlor and Giese, 2007). d) Anechoic demixing with some positivity constraints imposed on the parameters. The results of the application of such methods to the different generated data sets were compared by assessing the accuracy of the reconstruction of the original data sets along with the capability of the algorithms of recovering the structure of the original generative model.
Results showed that all algorithms efficiently reduced data dimensionality, always resulting in a good approximation of the original data by means of a relatively low number of synergies. However, the interpretability of the extracted components was strongly dependent on the type of generative model that underlay the algorithm. In addition, we found substantial differences in the stability of the parameter estimates comparing algorithms whose generative models were consistent with the ones underlying the data.

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# Cortical networks with stable low firing rates and high single-cell stimulation sensitivity: stability versus sensitivity in a network of coupled binary neurons.

Julie Goulet[1], Arthur Houweling[2], Claude Colomer[2], Paul H. E. Tiesinga[1]

[1]Donders Institute for Brain, Cognition and Behaviour, Neuroinformatics, Heyendaalseweg 135, 6525 AJ Nijmegen, Netherlands

[2] Department of Neuroscience, Erasmus Medical Center, Rotterdam, The Netherlands

Recent experimental measurements and modeling studies of rodent somatosensory cortex indicate that both spontaneous as well as stimulus-driven activity is characterized by low neuronal firing rates. A recent study points out that a single action potential in the barrel cortex can generate many post-synaptic action potentials and,therefore, the barrel cortex should employ an encoding mechanism that is robust to a high level of noise (London et al, Nature 2010), which would at first glance preclude codes based on single neuron activity. Nevertheless, rats can be trained to detect in the barrel cortex repetitive electrical stimulation of a single neuron over a short period of time (200ms) (Houweling & Brecht, Nature 2008) indicating sensitivity to weak external perturbations of a single cell. Taken together, these results suggest a trade-off between the stability of the low firing rate state against spontaneous fluctuations in population spike rate and the sensitivity to the fluctuations induced by external stimulation.

We investigated this issue analytically and numerically in a simple network of stochastic binary neurons and a probabilistic spiking response function with a threshold of 10-100 synaptic inputs, modeled as a sigmoid. For this model, stability of the low-firing rate state requires a small derivative of the spiking response function, whereas sensitivity requires a large derivative, because for high sensitivity a small perturbation should lead to a large increase in the firing activity, whereas to maintain the stability of the low firing rate state, which is characterized by large fluctuations, it needs either a small slope of the sigmoid function or dynamic inhibition. We explored the hypothesis that the sensitivity to weak perturbation will be increased if we stimulate a cell projecting to a larger than average number of cells. For this reason, we quantified how structure in the connectivity â€“ either defined by keeping the mean of the number of inputs across cells constant but varying their variance or by incorporating a small subnetwork of fully interconnected neurons into the otherwise randomly connected network â€“ modifies the single-cell stimulation sensitivity as assessed using a ROC-curve analysis. Because of this structure, the stimulation of a cell projecting to a large number of neurons increases the impact of small spontaneous perturbations in the network. To compensate for the corresponding destabilization, we incorporated 20% inhibitory interneurons into the network. These were randomly connected among themselves and randomly with excitatory cells. We varied the relative strength of the excitation/inhibition, to determine under what conditions the sensitivity of the system could be kept high and simultaneously the network could be stabilized at firing rates below 1Hz.

# Decorrelation of neural-network activity by inhibitory feedback

Tom Tetzlaff[1], Moritz Helias[2], Gaute T. Einevoll[1], Markus Diesmann[2,3,4]

[1] Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway

[2] RIKEN Brain Science Institute, Wako City, Japan

[3] Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako City, Japan

[4] Institute of Neuroscience and Medicine Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany

Spatial correlations in spike-train ensembles can seriously impair the en- and decoding of information in the spatio-temporal structure of these spike trains [1,2]. A potential source of correlation in finite neural networks is shared presynaptic input [3]. Recent theoretical and experimental studies have demonstrated that spike correlations in neural networks can be considerably smaller than expected based on the amount of shared presynaptic input in such systems [4,5,6].

Here, we provide an explanation of this observation by means of a simple linear model and simulations of networks of integrate-and-fire neurons. We show that pairwise correlations and hence population-rate fluctuations are actively suppressed by inhibitory feedback. To investigate the role of feedback we compute the response for the intact recurrent system and for the case where the 2nd-order statistics of the feeback channel is perturbed while the shared-input structure and the 1st-order statistics are preserved.

In general, any modification of the feedback statistics causes a shift in the power and coherence of the population response. In particular, the neglect of correlations within the ensemble of feedback channels or between the external stimulus and the feedback can amplify population-rate fluctuations by orders of magnitude. This effect can be observed both in networks with purely inhibitory and in those with mixed excitatory-inhibitory coupling. We show that the observed suppression of response fluctuations by inhibitory feedback in high-dimensional systems can be intuitively understood already by a simple one-dimensional linear model. For the n-dimensional case, we provide analytical solutions of the population-averaged correlations. In purely inhibitory networks, shared-input correlations are canceled by negative correlations between the feedback signals. In excitatory-inhibitory networks, the responses are typically positively correlated. Here, the suppression of input correlations is not a result of the mere existence of correlations between the responses of excitatory (E) and inhibitory (I) neurons, but is instead a consequence of the heterogeneity of response correlations across different types of neuron pairs (EE, EI, II).

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# Development of a salt and pepper organization of orientation preference in visual cortical networks

Juan Daniel Florez Weidinger[1], Fred Wolf[1,2]

[1]Max Planck Institute for Dynamics and Selforganization, Nonlinear dynamics, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen, Germany

Response characteristics of orientation tuned neurons appear to be similar in the visual cortex of long evolutionary separated mammalian linages. The spatial arrangements of tuning properties across the cortex, however, show fundamental differences. While in primates and carnivores orientation preference of neurons varies smoothly and progressively, in rodents and lagomorphs it is randomly distributed (salt and pepper arrangement). What causes this dissimilarity is unclear. It has been proposed that in the mouse similarly tuned neurons are selectively connected in segregated subnetworks. Recent findings however rather indicate that neurons are densely connected independent of orientation tuning. How can the properties of neighboring neurons be so different if they are connected in a dense network? Here we show that a random arrangement of features can naturally emerge within densely connected networks. We study a model in which the developmental dynamics of orientation preferences is modeled by a Landau equation with nonlocal Gaussian interaction kernel. The model has exact spatially ordered (map) solutions, for which the stability of the stationary maps can be analytically studied. Using this approach we examined how the connectivity patterns from the network determine the final organization of preferences. When the excitation range is shorter than inhibition the attractor states of the system are ordered maps. In contrast, with strong short range inhibition and weak wide range excitation, all ordered map solutions are unstable and the attractor state is an apparently random distribution of orientations. Corroborating those results, numerical simulations show that a random arrangement of orientation preferences develops towards an ordered map or stays disordered depending on the connectivity pattern.

# Dynamic effective connectivity: theory and data analysis

Demian Battaglia[1], Annette Witt[1,2,3], Fred Wolf[1,3], Alexander Gail[2,3], Theo Geisel[1,3]

[1]Max Planck Institut for Dynamics and Self-Organization,
[2]German Primate Center,
[3]Bernstein Center for Computational Neuroscience, Göttingen, Germany

Anatomic connections between local cortical areas
constrain neural responses and brain rhythmic activity.
However, structural connectivity does not coincide with effective
connectivity, related to the more elusive question ``Which areas cause
the activity of which others?". Effective connectivity is directed,
task-dependent and can evolve across stages of
a single task. We want to highlight in this poster that symmetric structural connectivity does not imply symmetric effective connectivity. Indeed, directionality in inter-areal interactions can spontaneously emerge even when considering pairs of mutually interconnected areas. Furthermore, dominant directionality and strength of inter-areal interactions are dynamic.

In a first theory part we advance that fast changes in effective connectivity reflect transitions in the organization of coherent neural activity. We use a computational approach to study small network
motifs of interacting cortical areas. Different dynamical collective states are shown to correspond to distinct effective connectivity motifs. Transitions between effective connectivity configurations are achieved via suitably phased pulse perturbations of the ongoing oscillatory dynamics without need for rewiring or plastic changes of the structural connections. Global reorganization of inter-areal oscillatory coherence regulates thus the routing of information through a hard-wired structural network.

In a second experimental part, we analyze paired LFP recordings in areas PRR and PMd during goal-directed reach experiments. We find that effective connectivity in different frequency bands is modulated dynamically by changes of the rule used to map a visual cue into a motor goal.

# Estimation of small-world topology of cortical networks using Generalized Linear Models

Felipe Gerhard[1], Gordon Pipa[2], Wulfram Gerstner[1]

[1]Brain Mind Institute, Ecole Polytechnique Federale de Lausanne, 1015 Lausanne EPFL, Switzerland
[2]Max-Planck Institute for Brain Research, Department Neurophysiology, Frankfurt am Main, Germany

Since the seminal work of Watts & Strogatz and others in the late 90s [1], graph-theoretic analyses have been performed on many complex dynamic networks, including brain structures. Most studies have focused on functional connectivity defined between whole brain regions, using imaging techniques such as fMRI, EEG or MEG [2]. Only very few studies have attempted to look at the structure of neural networks on the level of individual neurons [3,4]. To the best of our knowledge, these studies have only considered undirected connectivity networks and have derived connectivity based on estimates on small subsets or even pairs of neurons from the recorded networks.

Here, we investigate scale-free and small-world properties of neuronal networks, based on multi-electrode recordings from the awake monkey on a larger data set than in previous approaches. We estimate effective, i.e. causal, interactions by fitting Generalized Linear Models on the neural responses to natural stimulation, incorporating effects from the neurons' self-history, coupling terms and modulation by the external stimulus. The resulting connectivity matrix is directed and a link between two neurons represents a causal influence from one neuron to the other, given the observation of all other neurons from the population. We use this connectivity matrix to estimate scale-free and small-world properties of the network samples. For this, the quantity proposed by [5] for quantifying small-world-ness is generalized to directed networks. We find that the networks under consideration lack scale-free behavior, but show a small, but significant small-world structure.

Finally, we show that the experimental design of multi-electrode recordings typically enforces a particular network structure that can have a considerate impact on how the small-world structure of the network should be evaluated. Since for multi-electrode recordings the sampling of neurons is not uniform across the population (one electrode usually captures signals from a small local population), we expect the wiring probability between neurons of the same local spot to be much higher than the wiring probability between neurons from different electrodes. Thus, random graphs that take the different wiring probabilities into account can serve as a more refined null model than the homogeneous Erdös-Renyi random graphs that are usually proposed as reference models to evaluate small-world properties. Moreover, as the set of recorded neurons in a given experiment always represents only a subpopulation of the overall network, we investigate how the evaluation of small-world structure is affected by this undersampling bias.

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# Finite brains see single spikes

Moritz Helias[1], Tom Tetzlaff[2], Markus Diesmann[1,3,4]

[1] Laboratory for Computational Neurophysics, RIKEN Brain Science Institute, Wako City, Japan
[2] Department of Mathematical Sciences and Technology, Norwegian University of Life Sciences, Ås, Norway
[3] Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany
[4] Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako City, Japan

E-mail: helias@brain.riken.jp

Mean-field arguments suggest that the effect of a single spike on the correlation of neurons can be neglected compared to the feed forward component of correlation. However, the recent experimental finding that single spikes have a substantial effect on the rest of the network [1] and the observation that the mean-field component is suppressed in balanced networks [2, 3] require a thorough theoretical assessment of these two contributions.

Here we augment the theory of correlations in purely excitatory recurrent networks presented in [4] by inhibition and delayed spiking interaction. We analytically determine the self-consistent correlation structure of recurrent finite-size random networks of spiking excitatory and inhibitory Poisson neurons with delayed pulse coupling to extend earlier feed-forward approximations [5] and the theory of zero-lag correlation in the asynchronous irregular state [6, 3].

We find that correlation functions generically contain two components: the feed-forward contribution of correlation expected from mean field arguments (Fig. A) and the echo of single spikes in the network (B). For realistic network parameters, both are of similar magnitude (C,D). The additive contribution of spike feedback explains why inhibition seems to lag excitation in recurrent networks, leading to asymmetric cross correlation functions (E). Moreover, our model explains generic features of correlations: the origin of side troughs, the emergence of damped oscillatory correlation functions, and the transition to fast global delay oscillations [7].

The availability of analytical expressions for correlations in finite excitatory-inhibitory networks will facilitate the investigation of their functional implications, in particular in the light of their critical interaction with spike timing dependent plasticity [8].

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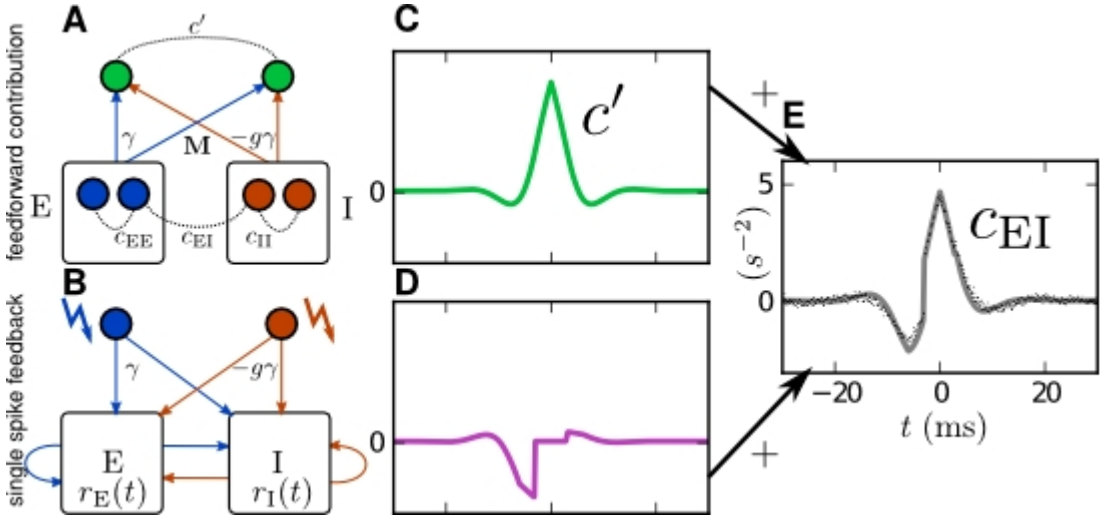

# How local is the local field potential?

Henrik Anders Linden[1], Tom Tetzlaff[1], Tobias C Potjans[2,3], Klas H Pettersen[1], Sonja Grün[4], Markus Diesmann[3,4], Gaute T Einevoll[1]

[1]Mathematical Sciences and Technology,Norwegian University of Life Sciences, Ås, Norway
[2] Institute of Neuroscience and Medicine, Computational and Systems Neuroscience(INM-6), Research Center Jülich, Jülich, Germany
[3]Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako, Japan
[4]RIKEN Brain Science Institute, Wako, Japan

The measurement of local field potentials (LFPs) is becoming increasingly popular as a tool for measuring cortical population activity. The LFP, the low- frequency part of extracellular potentials, is thought to mainly reflect synaptically evoked transmembrane currents in dendrites located in the vicinity of the recording electrode. As the LFP signal stems from the population activity of a large number of cells, it represents a more robust measure of the network dynamics than single-cell recordings. However, despite recent modeling and experimental studies on the origin of the LFP, much of the nature of this signal still remains to be understood. In particular, the literature has contradicting reports on how large the cortical area represented in the signal from an LFP electrode is. Studies have reported estimates ranging from a few hundred micrometers to several millimeters. A possible reason for this discrepancy may be that in direct measurements it is difficult to disentangle LFP correlations between nearby recording sites due to signal conduction from intrinsic correlations in the generators of the signal. Here we address the question using a forward-modeling approach (Pettersen et al, J Comp Neurosci, 2008). We simulate cylindrical columnar populations of up to several thousand morphologically reconstructed neurons receiving synaptic input and calculate the resulting LFP generated by all transmembrane currents in the population. The neuronal populations are designed to have realistic cell densities and geometric arrangements, and contributions to the LFP at different recording positions are calculated. Each neuron receives numerous synaptic inputs with tailored presynaptic spike-train patterns. This enables us to investigate how the population geometry and the statistics of the presynaptic spike trains such as spike-train correlations and spectral properties determine the spatial range of the LFP.

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# How much synchrony would there be if there was no synchrony?

Matthias Schultze-Kraft[1,2], Moritz Helias[3], Markus Diesmann[3,4,6], Sonja Gruen[5,6]

[1]Berlin Institute of Technology, Machine Learning Group, Berlin, Germany
[2]Bernstein Center for Computational Neuroscience Berlin, Humboldt-Universitaet zu Berlin, Berlin, Germany
[3]Laboratory for Computational Neurophysics, RIKEN Brain Science Institute, Wako City, Japan
[4]Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako City, Japan
[5]Laboratory for Statistical Neuroscience, RIKEN Brain Science Institute, Wako City, Japan
[6]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Germany

Ever since the discovery of precisely timed events of cortical neurons [1], their role for information processing has been highly debated. The widespread belief that synchrony is an epiphenomenon caused by shared afferents among neurons [2] has constantly been challenged by reports observing task related modulation of synchrony, lately in primary visual cortex [3] and motor cortex [4]. More so, the recently found decorrelation in cortical networks [5] suggests that the ground state of recurrent balanced networks provides a suitable substrate on top of which synchronized events can represent information. Theoretical insights [6] indicate that even weakly synchronous afferent activity is highly effective to cause synchronized spikes in integrate-and-fire neurons due to non-linear amplification.

In this work we theoretically investigate to what extent common synaptic afferents, fast rate changes, and synchronized inputs each contribute to closely time-locked spiking activity of pairs of neurons [7]. We employ direct simulation and extend earlier analytical methods based on the diffusion approximation [8] to pulse-coupling, in order to answer the question how much synchrony is caused by afferent synchronized events and how much is intrinsic to cortex.

We find that the firing rate dependence of correlation transmission [8] effectively modulates how much synchrony is transferred from the input to the output of pairs of neurons. Rate transitions per se, however, do not contribute significantly, whereas already weakly synchronous inputs are sufficient to cause detectable synchrony in the outgiong spiking activity.

Our quantitative assessment of the different contributions and understanding the underlying mechanisms will support the
interpretation of experimentally observed precisely time-locked events [3,4].

Partially supported by the Helmholtz Alliance on Systems Biology, the Next-Generation Supercomputer Project of MEXT, EU Grant 15879 (FACETS), EU Grant 269921 (BrainScaleS), DIP F1.2, and BMBF Grant 01GQ0420 to BCCN Freiburg. All network simulations carried out with NEST (http://www.nest-initiative.org).

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# Inheritance of behavior by memory-strings

Thomas Kromer[1]

[1]ZfP Südwürttemberg, Münsterklinik Zwiefalten, Hauptstraße 9, 88529 Zwiefalten, Germany

Introduction: We poorly understand, how very complex behavior is inherited in many species. Without learning, many insects for instance show very specific instinctive breeding behavior. It is not possible, that these complex behavioral features are determined by inheritance of specific synaptical weights. Neural nets, working on memory-storage by memory-strings, in biological models as short strings of ribonucleic acid or oligopeptids, which encode by their sequence of triplets or amino-acids according sequences of neural activity, are in principal able to perform all tasks of neural networks(1,2). As a peculiar property, in these models, a set of memory -strings may be determined by the "DNA". This could be the base for evolutionary mechanisms of inheritance.
Methods: Virtual organisms, with a very limited set of neural cells, which form a small neural network, consisting of 50 neural chains. To each neural chain belong 7 neural cells. The wiring within the net is determined first by randomization, but then will be inherited to the next generation, influence by mutational changes and genetic combination. This will represent the evolution of the "hardware". To the neural net belong some sensory, motoric and associative neurons. Each neural cell will be equipped with a set of 100 memory-strings, each with 7 units. The units will represent according neural activity. The sequence of activity in each chain, representing the state of neural activity in it at each moment will be learned by the first neuron of the chain by projecting the activity towards it from one neuron to the other stepwise. The sequence, arriving at the first neuron will be transformed into a memory-string, f.e. a small "RNA"-sequence. Then it will be compared to the strings, available within the neuron by inheritance and memory-history. The best fitting string with the most homologous sequence will be used to reactivate the neural chain, sending the according sequence in reversed direction from the first neuron to the others step by step. The set of memory-strings, each neural cell will contain by inheritance is at the beginning determined by randomization, then by inheriting strings from individuals, which will survive in the virtual environment to the next generation, using mechanisms of evolutionary selection and genetic combination of DNA of different individuals, simulating sexual mechanisms of inheritance. Evolutionary selection is represented by the necessity to find "food" and to avoid "enemies". Individual learning history is represented by deleting strings, causing inappropriate reactions, whilst multiplying strings which proved to be useful. Individuals, which will perform well may pass on their sequence of "DNA".
Results: The study shows, that those principles of neural networking will allow us to explain at least in principle in a simple model the evolution and inheritance of complex behavior. Randomized features of behavior, determined by a set of randomized sequences of the inborn memory-strings of each neural cell at the very beginning are shaped to sets of more adapted and selected sets of memory-strings, inherited from one generation to the other.
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# Linking power laws for microscopic and macroscopic measures of neural activity

Gaute T. Einevoll[1], Klas H. Pettersen[1], Henrik Linden[1], Tom Tetzlaff[1]

[1]Norwegian University of Life Sciences, Mathematical Sciences and Technology, POBox 5003, 1432 Aas, Norway

The cable equation is solved in frequency space for a ball-and-stick neuron with noisy input currents spread homogeneously throughout the dendritic stick. The power spectral densities (PSDs) of the soma potential and the soma current is shown to be directly related to the single-neuron contribution to the electroencephalogram (EEG), and all PSDs are shown to express $1/f^a$ power laws for high frequencies.

For uncorrelated white-noise input the asymptotic high frequency limit gives a-values of 0.5, 1.5 and 2.5 for the soma current, EEG and the soma potential, respectively. For correlated white-noise input the respective values are 1, 2 and 3. However, for the frequency range typically recorded in experiments, i.e., up to one or two hundred Hertz, log-log plots of the corresponding PSDs often express quasi-linear regimes with power about 0.5 less than the asymptotic value.

The theory presented may not only give valuable insight to why neural recordings often obey power laws, but might also be of importance to general 1/f-theory, as this is an example of how a basic physics phenomenon can transfer white noise input to colored $1/f^a$-noise where different physical entities express different powers a.

# Maturation of encoding and action potential onset dynamics in neocortical neurons

Marina Chistyakova[1], Aleksey Malyshev[1,2,3], Elena Kuleshova[3], Fred Wolf[4], Maxim Volgushev[1,2,3]

[1]University of Connecticut, Psychology, 406 Babbidge Road, 06269-2050 Storrs, CT, USA

[2] Department of Neurophysiology, Ruhr University Bochum, Germany

[3] Institute of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia

[4] Max Planck Institute for Dynamics and Self-Organization and Bernstein Center for Computational Neuroscience Göttingen, Germany

The ability of neurons to generate precisely timed sequences of action potentials (APs) underlies basic operations of neural networks, such as coincidence detection, grouping by synchrony and spike-timing dependent plasticity. Temporal precision of the spiking patterns is determined by the ability of neuronal populations to reliably encode high frequency inputs in their firing. Recent experimental studies revealed that in adolescent rats, neocortical neurons can indeed encode high frequency inputs in their firing, as indicated by the high cut-off frequencies of ~200-300 Hz of their frequency response functions (Koendgen et al., 2008; Boucsein et al., 2009) and can respond rapidly to input perturbations (Silberberg et al., 2004). Here we asked if this ability to encode fast varying signals is present already in young neurons, or does it appear during the development? To address this question we made intracellular recordings from layer 2/3 pyramidal neurons in slices from rat visual cortex and assessed their encoding properties using Fourier paradigm. We show that encoding of fast varying signals improves dramatically during early development. Input frequencies >50Hz are encoded significantly better by neurons from adolescent rats (P17-P30) than by neurons from P9-P13 pups. In contrast, frequencies <50 Hz were encoded equally well be neurons from both groups. Based on previous theoretical work (Fourcaud-Trocme et al., 2003; Naundorf et al., 2005) we hypothesized that maturation of encoding properties may be due to the maturation of spike initiation mechanisms. We further show that ability to encode high frequencies is correlated with the onset dynamics of APs in neocortical neurons: mature neurons with fast AP onset dynamics encode high frequencies significantly better than neurons from young animals, with slow AP onsets. These results provide, to the best of our knowledge, the first experimental comparison of encoding by neurons with fast and slow AP onset dynamics, and the first demonstration of the enhanced encoding of high frequencies in neurons with faster AP onset dynamics, predicted by theoretical studies (Brunel et al., 2001; Fourcaud-Trocme et al 2003; Naundorf et al., 2005). From the theoretical perspective, our findings indicate that neuronal operations at different developmental stages are described by different types of models. From the physiological perspective, they show that fast neuronal processing relying on precise timing of spikes requires mature AP generation with fast onset dynamics.

# Microcircuits of grid- and head-direction systems in the rat medial entorhinal cortex

Andrea Burgalossi[1], Lucas Herfst[1], Moritz von Heimendahl[1], Henning Förste[2], Martin Schmidt[2], Michael Brecht[1]

[1]Bernstein Center for Computational Neuroscience, Biology, Philippstr.13, Haus 6, 10115 Berlin, Germany

[2] Institut für Konstruktion, Mikro- und Medizintechnik Fachgebiet Mikro- und Feingeräte,Technische Universität Berlin, Hardenbergstraße 36, 10623 Berlin

Extracellular recordings of place, head-direction and grid cells have elucidated the physiology of spatial representations, but the underlying microcircuits remained unidentified. We analyzed microcircuits of medial entorhinal cortex by labeling grid and head-direction cells in freely-moving rats with a novel friction-based pipette-stabilization system. In novel environments most superficial layer neurons were grid cells, active during exploration. Layer 2 stellate cells were theta-modulated and organized in hundreds of small patches. Deep layer neurons showed little or no activity. Head-direction cells were theta-modulated, polarized neurons residing in about 25 pairs of large patches at the dorsomedial entorhinal border. We identified three long-range axon systems: centrifugal axons from grid cells to single pairs of head-direction patches, centripetal axons from head-direction cells to single grid patches and circumcurrent axons from head-direction cells to many head-direction patches. Thus, the patchy architecture fundamentally shapes entorhinal processing. We hypothesize that spatial alignment and map formation are achieved through the collective or patch-wise rotation of grid orientations. Our results demonstrate that microcircuit analysis during behavior is both feasible and decisive for understanding spatial representations.

# Modeling non-stationarity and inter-spike dependency in high-level visual cortical area STSa

Dominik M Endres[1], Mike W Oram[2]

[1]Hertie Institute for Clinical Brain Research and Center for Integrative Neuroscience and University of Tübingen, Theoretical Sensomotorics, Cognitive Neurology, Frondsbergstr 23, 72070 Tübingen, Germany

[2]Institute for Adaptive and Neural Computation, 10 Crichton Street, Edinburgh, EH8 9AB, UK and School of Psychology, South Street, St Andrews, KY16 9JP, UK

The peristimulus time histogram (PSTH) and its more continuous relative, the spike density function (SDF) are standard techniques in the analytic toolkit of neurophysiologists. The PSTH is obtained by binning spike trains, whereas the SDF is usually computed by smoothing with a Gaussian kernel.
We developed an exact Bayesian, generative model approach to estimating PSTHs (Endres et al, 2010). Our *basic* model encodes a spike generator described by an inhomogeneous Bernoulli process with piecewise constant (in time) firing probabilities. We demonstrated that relevant marginal distributions, e.g. the posterior distribution of the number of bins, can be evaluated from the full posterior distribution over the model parameters efficiently, i.e. in polynomial time. Advantages of our scheme include automatic complexity control, error bars on its predictions and increased predictive performance over standard approaches.

Here, we extend our basic model to investigate the importance of two additional features: firstly, we assumed previously that the generator is constant across trials. This assumption implies a Fano factor <1, which is known to be incorrect for certain types of neural data, e.g. spike trains recorded from high-level visual area STSa. We therefore present a modification of our model where the spike generating probability is allowed to fluctuate between trials, following a Beta distribution. This modification increases both the predictive performance and the Fano factors of the model.
Secondly, we extend our basic model to account for dependencies between consecutive spikes. We choose a *non-parametric* approach, which enables us to determine both the form of the dependency and its timescale. Our approach is more general than e.g. modeling refractory periods with Gamma inter-spike-interval distributions. We find a substantial increase of predictive performance. Moreover, the model discovers both absolute and relative refractory periods on data from high-level visual area STSa.

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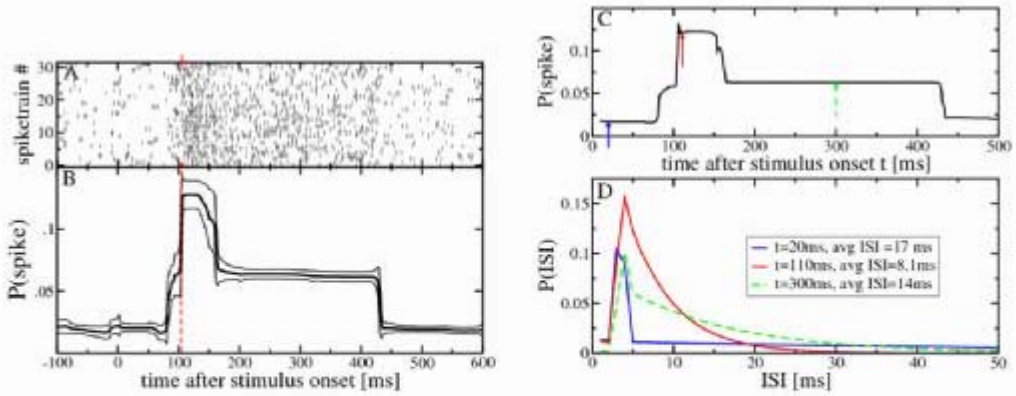


**A**: spiketrains recorded from a STSa neuron, and aligned to the onset of a visual stimulus at time 0. Every row shows the spiketrain from one trial. **B:** predictive PSTH computed with our Bayesian binning method. Thick line: expected firing probability, thin lines: plus/minus one posterior std.dev.. **C:** PSTH from another neuron computed with Bayesian binning. **D:** ISI distributions from this neuron, at different post-stimulus times. The absolute and relative refractory periods, and the exponental tail are clearly visible.

# Modelling study of the cellular mechanisms shaping the multiphasic response of moth pheromone-sensitive projection neurons

Yuqiao Gu[1], Hana Belmabrouk[2], Antoine Chaffiol[1], Jean-Pierre Rospars[1], Dominique Martinez[1,2]

[1]UMR 1272, PISC, INRA, 78000 Versailles, France
[2]UMR 7503, LORIA, CNRS, 54506 Vandœuvre-lès-Nancy, France

In the macroglomerular complex (MGC) of the male noctuid moth Agrotis ipsilon, pheromone-sensitive projection neurons (PNs) exhibit a multiphasic firing pattern when the antenna is stimulated with the pheromonal blend. At low concentrations, the PN response is biphasic and consists of an excitatory phase ($E_1$) followed by an inhibitory phase (I). The duration of $E_1$, but not of I, depends on stimulus concentration and duration. At higher concentrations, the response becomes triphasic with a long tonic excitatory phase ($E_2$) following phase I.

To understand the cellular mechanisms underlying these specific discharge patterns $E_1$/I and $E_1$/I/$E_2$, we developed a biophysical model of a PN receiving cholinergic inputs from olfactory receptor neurons (ORNs) (Fig. 1).

The firing of ORNs was described by a non-homogeneous Poisson process with firing frequencies as measured from ORNs in response to stimulations of different doses and durations. The PN model is based on the Hodgkin-Huxley formalism with realistic ionic currents found in AL neurons (Mercer and Hildebrand, 2002) whose parameters were fitted to whole cell patch-clamp data. These currents are: voltage-gated $Na^+$ (Lapied et al., 1990), $Ca^{2+}$ (Husch et al., 2009, Laurent et al., 1993) sustained $K^+$ and transient (A) $K^+$ currents (Kloppenburg et al., 1999, Mercer et al., 1995), and a $Ca^{2+}$-gated small conductance $K^+$ (SK) current (Roper et al, 2003).

Simulation results show that the multiphasic PN firing pattern is shaped by intrinsic mechanisms in ORNs and PNs: the SK current in PNs can explain the I phase, whereas the $E_2$ phase can result from the long-lasting excitatory response of ORNs.

We then investigated the effects of stimulation and intrinsic parameters on several characteristics of the response such as the duration, onset and ending times of $E_1$ and I phases. The $E_1$ duration increases with the stimulus duration; the mean response frequency of $E_1$ increases with stimulus concentration at low doses but remains almost constant at high concentrations. The ending time of $E_1$ strongly decreases with the peak conductance $g_{SK}$ of the SK current, which has almost no effect on the duration of I phase. The duration of the I phase increases linearly with the time constant of intracellular $Ca^{2+}$ concentration ($t_{Ca}$) and monotonically increases with the peak conductance $g_{Ca}$ of the voltage-gated $Ca^{2+}$ current. These two parameters $t_{Ca}$ and $g_{Ca}$ have almost no influence on the duration of $E_1$ phase. Decreasing the peak conductance $g_A$ of the A current increases the ending time of $E_1$ phase and the onset of I phase in such a way that it does not change the total duration of the $E_1$ phase plus the I phase.

This modelling study revealed specific roles of the intrinsic parameters of intracellular $Ca^{2+}$ and various ionic channels on different characteristics of the multiphasic response patterns of PNs. It gives clues and guidance for further experimental investigations on the mechanisms underlying the PN response.

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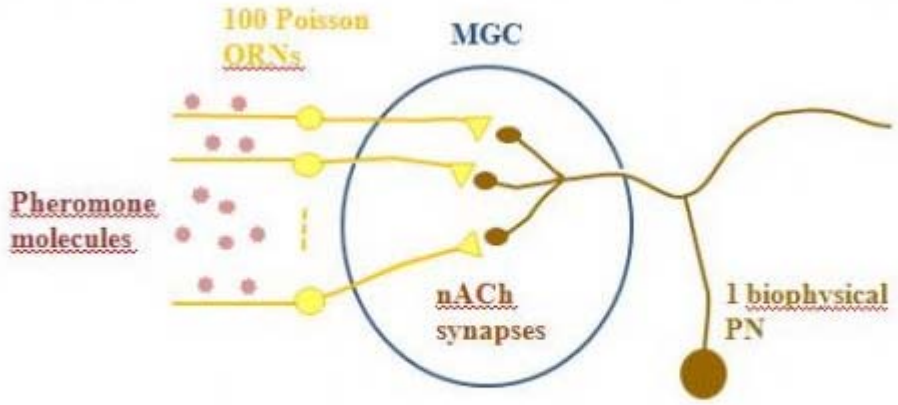


Figure 1. Schematic representation of the MGC. The model is composed of 100 ORNs and 1 PN. The ORNs receive pheromone stimuli and the PN receives inputs from ORNs through cholinergic synapses with nicotinic receptors (nACh) in the MGC.

# Modelling the distal reward problem

Nikolay Chenkov[1,2], Robert Schmidt[1,2], Richard Kempter[1,2]

[1] Bernstein Center for Computational Neuroscience-Berlin, Germany
[2] Theoretical Neuroscience Lab, Institute for Theoretical Biology, HU-Berlin, Germany

More than 100 years ago, Ivan Pavlov was studying the digestive processes in dogs and he accidentally found a phenomenon that he called conditional reflexes (Pavlov 1927). In his classical experiment, he rang a bell (conditioned stimulus) each time before giving a meat powder (unconditioned stimulus) to the dogs. He found that the dogs were able to learn the association between bell and food even when there was a delay between both stimuli. Today this phenomenon is well known as the *distal reward problem* or the *temporal credit assignment problem*. There are many approaches toward modelling the distal reward problem (e.g. Rescorla et al. 1972, Izhikevich 2007) but none of them has solved it in its full aspect. We propose a biologically plausible hippocampal network model that can map the long time scales of the real world to the relatively short time scales at which neurons and induction of plasticity operate.

To bridge the temporal gap on the time scale of seconds between two stimuli, we use a phenomenological model of semilunar granule cells (SGCs) (Larimer et al. 2010). The SGCs are neurons in the inner molecular layer of the dentate gyrus. After a single transient input the SGCs are persistently depolarized for a few seconds. They fire initially with a rate around 10 Hz, which is decreasing exponentially with a time constant of about 7 seconds. In our model, we assume that the SGCs project to the inner areas of the hippocampus via mossy fiber synapses (Gundlfinger et al. 2007). The mossy fiber synapses exhibit presynaptically induced short-term plasticity. They undergo a strong facilitation, where a single pre-synaptic spike can evoke one or more post-synaptic action potentials. Additionally, the recovery from this facilitation is relatively slow, on the order of 10 seconds. In the presence of a hippocampal theta rhythm (6-12 Hz), the mossy fiber facilitation can drive the post-synaptic CA3 pyramidal neurons to undergo phase precession (Thurley et al. 2008). Phase precession is the advancement of the action potential phase relatively to the global theta oscillations. Thus, the modelled network can encode the time since a stimulus onset on a much smaller time scale on the order of tens of milliseconds. After compressing the delay between two stimuli, we apply spike-timing-dependent plasticity between the target CA3 pyramidal neurons. In this way, the synaptic connection between the CA3 neurons is modified according to the sequence and the temporal delay of the stimuli. In this way we solve the problem of how to associate two stimuli that are separated by several seconds, which is an important aspect of the distal reward problem.

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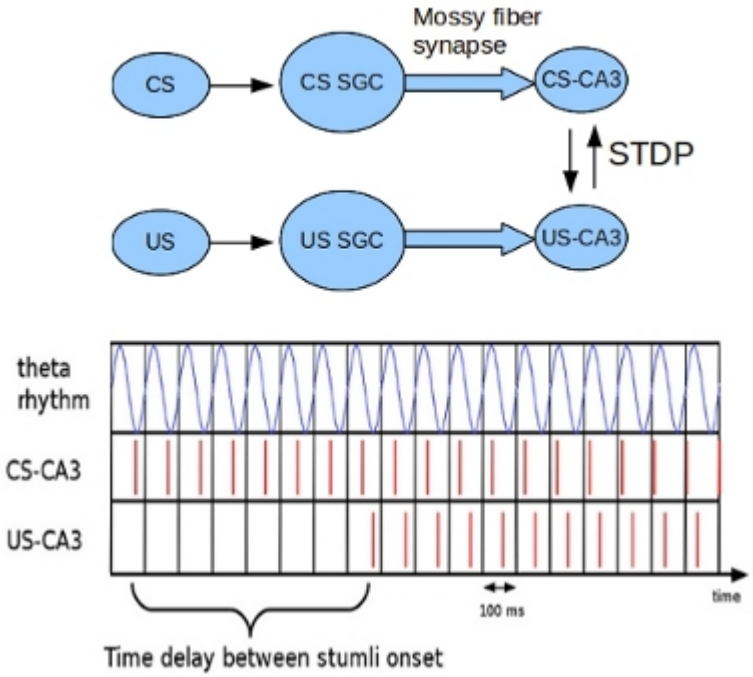

# Network mechanisms for the modulation of gamma spike phase by stimulus strength and attention

Paul Tiesinga[1], Terrence J Sejnowski[3,4]

[1]Radboud university Nijmegen, Neuroinformatics, Heyendaalseweg 135, 6525 AJ Nijmegen, Netherlands
[2]University of North Carolina, Physics & Astronomy, Philips Hall, Chapel Hill, NC 27599-3255, United States
[3]Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, CA 92037, USA
[4]Division of Biological Studies, University of California at San Diego, La Jolla, CA 92093, USA

Recent experiments demonstrate that in prefrontal cortex spikes at a specific gamma (30-80 Hz) phase relative to the local field potential are most informative about stimulus identity, where the specific value of the phase depends on the order of the stimulus within the stimulus sequence (Siegel, M., Warden, M.R., and Miller, E.K. PNAS, 106, 21341 (2009)). Within the context of a model cortical circuit this corresponds to changes in the relative phase between excitatory (E) and inhibitory (I) cells. Gamma oscillations can emerge via the interneuron-gamma (ING) or pyramidal-interneuron gamma (PING) mechanism. We use theoretical arguments and simulation to determine how the relative phase between E and I cells can be modulated in cortical circuits.

In the ING mechanism, I cells synchronize in the gamma frequency range when they are sufficiently depolarized. The resulting synchronous inhibition in turn synchronizes the E cells. Our simulations indicate that there are two scenarios for phase modulation. First, when the E cells have a firing rate much less than the oscillation frequency and when in addition they receive an incoherent excitatory drive, they will fire near the troughs of the inhibitory conductance. Increasing the depolarization of the E cells will increase their firing rate, but will not significantly change their mean phase relative to I cells. Second, when the E cells are sufficiently depolarized they will fire at a rate similar to the oscillation frequency, at a high precision (about 1 ms) and at a specific phase. Increasing E cell depolarization changes the E phase because it decreases the delay between E and I cells. The first scenario is more realistic in the absence of a strong stimulus, whereas the second might hold for circuits directly driven by a stimulus.

In the PING mechanism, gamma oscillations emerge when the E cells are sufficiently depolarized and when a synchronized E cell volley recruits a synchronous I cell volley, which inhibits the E cells for approximately a gamma cycle. When the E cells have recovered, the oscillation cycle starts over. We find that hyperpolarizing the I cells will delay the I cell volley, thus increasing the phase of the I cells relative to the E cells. By contrast, when the depolarization of the E cells is increased, they recover faster from inhibition, which increases the oscillation frequency, but does not strongly alter the delay between I and E cells.

E or I cells are depolarized by neuromodulators, such as, for instance, acetylcholine released by projections from the basal forebrain or dopamine. These projections affect E and I cells differently, which allows for a division of labor because the preceding results show that targeting E or I cells has different effects on spike rate, oscillation frequency and phase. Under the hypothesis that in visual cortex feedforward inputs drive the E cells and top-down inputs target I cells, the PING mechanism predicts that contrast increases gamma power and oscillation frequency and attention alters the relative phase between I and E cells.

# On the contribution of structural inhomogeneities to network burst initiation and propagation in dissociated cortical cultures

Sarah Jarvis[1], Stefan Rotter[1,3], Ulrich Egert[1,2]

[1]University of Freiburg, Bernstein Center Freiburg, Hansastr 9A, 79104 Freiburg, Germany

[2]Biomicrotechnology, Dept. of Microsystems Engineering, Faculty of Engineering, University of Freiburg, Germany

[3]Computational Neuroscience, Faculty of Biology, University of Freiburg, Germany

Dissociated cortical cultures grown on multielectrode arrays (MEA) have been established as a useful biological model in the analysis of network dynamics. Present in their dynamics are periods of strongly synchronized spiking by the network, termed `bursting', whose role is not understood but dominates network dynamics. It has been demonstrated that bursts have different motifs and contain structure, refuting the possibility that they are merely chaotic activity. Of particular interest are the conditions required for bursting to be initiated and propagated throughout the entire network. Within cultures, initiation sites can be well characterized in their location, even while varying different parameters such as cell density, while propagation waves display fairly regular patterns of neuron recruitment within the network burst. However, in order to minimize bursting and promote closed-loop communication with the disassociated culture, it is of interest to understand what conditions are necessary for bursting to arise.

Interestingly, the propagation of bursts has been observed to be faster than can be accounted for by only local connectivity. While paired intracellular recordings have revealed some clues as to the local structure and short range connectivity, they are unable to clarify the contribution of long-range connectivity of neurons to burst propagation. Additionally, pharmacological studies which result in freezing of synaptic plasticity and impaired cell migration have demonstrated that modifications to connectivity can greatly disrupt the pattern of burst propagation.

As network structure has been established to strongly affect dynamics, we identify network topologies that can account for observed burst initiation and propagation patterns by considering network models implementing several likely models of long-range connectivity. Specifically, we investigate the contribution of long-range connections within a 2D model network of spiking neurons representing a mature dissociated cortical culture. We previously demonstrated with networks of rate-based units that within clustered topologies, the relative number of long-range connection to cluster size greatly affects the robustness of the network to noise and ability to sustain activity. Here, we extend these networks to a population of spiking units and chart the effect of introducing inhomogeneities and a non-uniform distribution of connections, while also considering parameters such as the numbers and location of post-synaptic connections of each unit. By driving the network with low levels of background activity, we observe how different configurations change the burst initiation site and alter the propagation wave throughout the network. We establish the burst profiles for different network configurations and comment on their comparability to their biological equivalents.

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# Optimal Distribution of Spatial Periods for Grid Cells Ensembles on Finite Space

Alexander Mathis[1], Martin Stemmler[1,2], Andreas Herz[1,2]

[1] Division of Neurobiology, Ludwig-Maximilians-Universität München, 82152 Planegg-Martinsried, Germany
[2] Bernstein Center for Computational Neuroscience Munich, 82152 Planegg-Martinsried,Germany
[3] Graduate School for Systemic Neuroscience, LMU, 82152 Planegg-Martinsried, Germany

A grid cell in the medial entorhinal cortex or subiculum spikes at multiple locations in the environment of a rat. Remarkably, these locations are arranged in a hexagonal lattice [1, 2]. However striking such a pattern is, the presence of multiple firing fields for each grid cell introduces ambiguity in the coding of space at the single cell level. Hence, a population of grid cells is needed to represent the entire environment and encode space precisely.

We set out to compute the resolution of grid codes, defined as the mean square error of the maximum likelihood estimate, in a realistic parameter regime. For this purpose, we assumed a finite number of spiking grid cells that encode a limited region of space using different configurations of spatial periods. To achieve the best encoding of location, we predict that there should be far more small spatial periods present than large periods. This predicted distribution largely resembles empirical observations by Brun et al. [3]. Furthermore, we predict that the area of the firing fields should cover roughly one-third of the spatial period of the hexagonal grid.

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# Optimisation of Tonically Firing Neurones

Biswa Sengupta[1], Jeremy E. Niven[1], Simon B. Laughlin[1], Martin Stemmler[2]

[1]University of Cambridge, Neural Circuits Design Group, Downing St, Cambridge, CB2 3EJ Cambridge, United Kingdom
[2]BCCN Munich, LMU Munich, Großhaderner Str. 2, 82152 Martinsried, Germany

The design of engineering and biological systems is influenced by the balance between the costs incurred and the benefits realised. In stark contrast to man-made systems, it is only recently that we have been able to reverse engineer biological systems to analyse the trade-offs incurred during natural selection (Alexander, 1996; Sutherland, 2005). One such example is the nervous system, whose design is subject to metabolic energy constraints (Niven & Laughlin, 2008).

A large proportion of neurones in the nervous system display pulsatile spiking behaviour, represented mathematically by relaxation oscillations. Such oscillations emerge from opposing currents flowing on at least two different time scales, fast and slow. Previous work has quantified the energy cost of these currents in different models of neurones, concluding that experimentally measured biophysical parameters are locally optimal (Sengupta, Stemmler, Laughlin, & Niven, 2010). Here we address whether a globally optimal spike exists that uses the least possible energy. For this purpose, we devise a novel framework for studying optimality in general nonlinear, periodic dynamical systems by combining adjoint methods of sensitivity analysis and Poincare-Lindstedt perturbation theory. With this technique, we can optimise any functional, such as the energy, the voltage variance, or even the information rate carried in the spike times based on time-varying input stimuli, and handle thousands of model parameters.

Optimisation demonstrates five main innovations that are necessary for energy efficient action potentials (APs). First, increased $Na^+$ and $K^+$ channel densities enable currents that are intense, yet short-lived, causing spikes to have the sharp onsets and offsets predicted by bang-bang control theory. Second, $Na^+$ currents that inactivate with voltage, which allow the inward current to turn off prior to the downstroke of the AP. Third, $Na^+$ and $K^+$ currents whose kinetics have a bell-shaped voltage-dependence, which prevents premature activation or reactivation of currents. Fourth, the introduction of multiple gates that have to open in concert to allow current flow makes it possible for the onset of the outward $K^+$ current to be delayed relative to the inward $Na^+$ current. Fifth, passive $K^+$ channels that inwardly rectify thereby reducing the dramatic increase in leak current during the rising phase of the spike.

Our optimisation demonstrates that starting point of previous analyses of energy efficiency, the Hodgkin-Huxley formalism, already contains the essential ingredients for an energy efficient action potential. On this basis, we suggest that this form of action potential could have evolved to be energy efficient.

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# Optimizing charge-balanced bipolar rectangular current pulses for low-threshold neuronal stimulation

Thomas Schanze[1]

[1]University of Applied Sciences - FH Gießen-Friedberg, FB Krankenhaus- und Medizintechnik, Umwelt- und Biotechnologie, Wiesenstr. 14, 35390 Gießen, Germany

Knowledge of the mechanisms and dynamics of neural stimulation is very important for the development of electro-neural interfaces, especially for the design of electrodes and efficient stimulation current time-courses for neuroprosthetical applications. The basic time-course to activate neuronal tissue is a rectangular current pulse. However, such monopolar stimuli generally cause tissue injury or electrode damage [1]. In order to avoid such adverse effects charge-balanced bipolar rectangular current pulses have been used.

It is well known that extracellularly applied cathodic pulses have lower stimulation thresholds than anodic pulses, the opposite holds for intracellular stimuli. However, it is often assumed that charge-balanced, bipolar symmetrical rectangular stimulation current pulses are less efficient compared to monopolar current pulses. To disprove this assumption we performed computer simulations. We solved the Hodgkin-Huxley (HH) equations [2] with the implicit trapezoidal rule for different stimulation current time courses: monopolar rectangular and charge-balanced bipolar and symmetric rectangular pulses. We varied the duration of the extracellular pulses and the inter-pulse interval $t_i$ of two subsequent charge-balanced symmetric anodic-cathodic or cathodic-anodic current pulses systematically. Stimulation current thresholds were efficiently estimated by a modified bisection method. The thresholds were used to figure out the strength-duration relationships. These relationships were found to differ significantly from those for classical RC-membrane models. The ratio of the strength-duration curves of anodic and cathodic monopolar current pulses is not constant. For pulses with durations of less than 1 ms the ratio is about 3.07. The ratio decreases at approximately 2 ms and slows down to 1.24 for longer stimulation pulses. This indicates a change of the HH-stimulus-response dynamics. To test whether charge-balanced bipolar and symmetric rectangular pulses are less effective than cathodic pulses we set the duration of a single pulse to a fixed value, e.g. 50 µs, and used the stimulation threshold of a single cathodic pulse as reference. The resulting normalized stimulation current threshold curves show totally different behaviour (Fig. 1). The lowest threshold is obtained for anodic-cathodic stimulation and its normalized value is approximately 0.72. The optimal inter-pulse interval is about 5 ms, which is nearly a quarter of the eigen time of the HH-model for subthreshold stimulation. If we assume ohmic energy dissipation, then the energy supplied by the current source is nearly the same for this optimal anodic-cathodic pulse as for a single cathodic pulse.

Safety and efficacy of electrical stimuli is still a challenge in neuroprosthetical research. We have shown that a careful selection of the timing parameters of charge-balanced bipolar rectangular pulses is necessary to realize safe and low-threshold stimulation. Our results can be explained by the different dynamical properties of the sodium and potassium channels and, in particular, by the oscillating subthreshold pulse-response characteristic of the HH-model. If we interpret this oscillatory characteristic as a transient storage of energy or as a resonance phenomenon, it is evident that an appropriate timing of stimulation pulses is useful. These results provide a biophysical basis for understanding stimulation dynamics and some guidance to optimize the safety and efficacy of electrical neural tissue stimulation.

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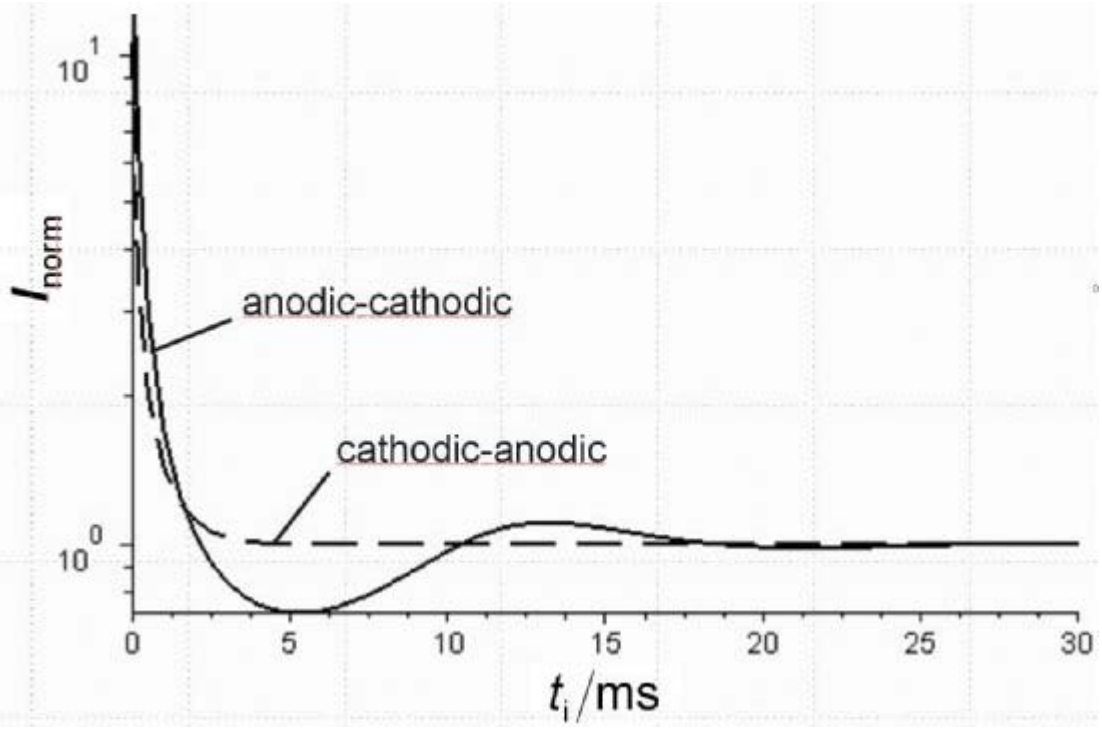

$10^1$
$I_{norm}$
anodic-cathodic
cathodic-anodic
$10^0$
0
5
10
15
20
25
30
$t_i$ /ms

# Properties of Statistical Tests for Spike Coincidences

Christian Braune[1], Sonja Grün[2,3], Christian Borgelt[1]

[1]European Centre for Soft Computing, c/ Gonzalo Gutierrez Quiros s/n, 33600 Mieres, Spain
[2]RIKEN Brain Science Institute, Wako-Shi, Japan
[3]Institute of Neuroscience and Medicine, Research Center Jülich, Germany

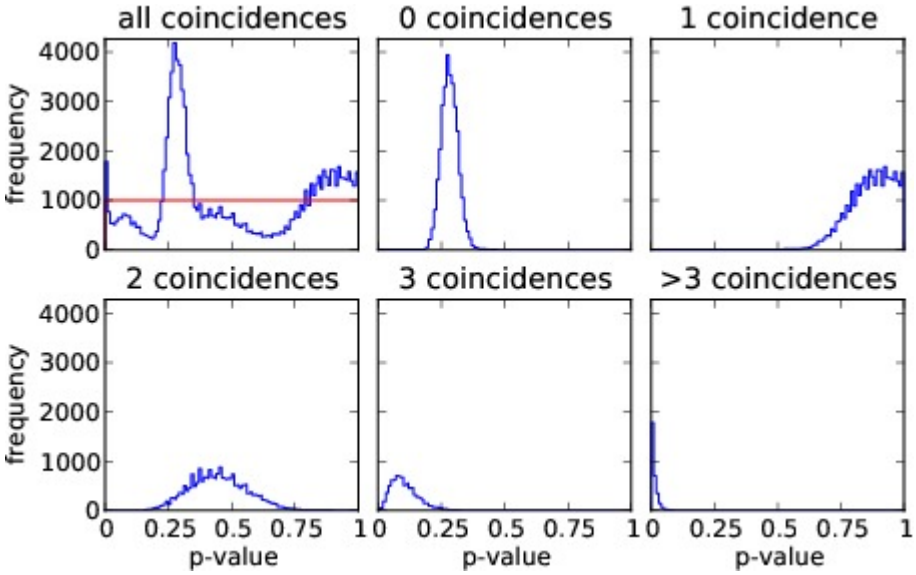
In an accretion-based approach [Gerstein et al. 1978, Berger et al. 2009] to detect synchronous spiking activity in parallel neuronal spike trains we observed an unexpected level of false positive outcomes. The objective to reduce or even eliminate them triggered us to investigate in more detail the properties of statistical tests for the dependence of neurons that are based on contingency tables of their spikes. To our surprise, the quality of these tests turned out to deviate from what we expected, and we have to conclude that p-values computed from them are biased. Our goal here is to understand the reasons for the bias and to develop methods to avoid them.

For simplicity, we confined ourselves to a simple Poisson model of neuronal activity or even a Bernoulli model as its discretized form. However, our negative results cannot be attributed to the choice of this model and can be transferred to other models (e.g. Gamma processes etc.), since the problematic aspects that revealed themselves are independent of the process model.

Based on a Bernoulli model, we simulated spike trains by discretizing them into a certain number of time steps of length dt, in each of which a neuron i fires with probability $p = l_i\ dt$, where $l_i$ is the firing rate. For efficiency we even sampled contingency tables directly from a multinomial distribution with parameters $p^2$, pq, pq and $q^2$ (where q = 1-p), having made sure that this yields the same results as generating (independent) parallel spike trains explicitly and computing contingency tables from them. For each contingency table we computed the p-value of a statistical independence test, for which we tried the $chi^2$ test, the G-test (based on mutual information) and Fisher's exact test. We then analyzed the distribution of these p-values over a large number of trials, checking whether it conforms to the expected uniform distribution: for a random variable X representing the p-value computed from a perfect independence test executed on data sampled from the null hypothesis (independent neurons) we should have $P(X <= x) = x$ for all x in [0,1]. However, for standard spike train parameters (recording time, firing rate), we observed grave deviations from this expectation, regardless of the statistical test.

Figure 1 shows the result of a particular example, in which we executed 100000 trials. In each trial two independent spike trains of 10s length were simulated with 1ms resolution (i.e. 10000 time steps) and a firing rate of 10.8Hz for both neurons (and thus 1.1664 expected coincidences). The p-values were computed from a standard $chi^2$ test. The top left diagram shows the distribution of all p-values (blue line) and the expected uniform distribution for an optimal test (red line). Clearly, there are significant deviations from the optimal behavior. In particular, the number of false positives on a 1% significance level is almost twice as large as expected. The other diagrams show the p-value distributions separated according to the number of observed coincidences, which explains the bumpy structure of the joint p-value distribution: the $p$-value is mainly determined by the number of coincidences, which lies in a very restricted range, since spikes are comparatively rare (and this is independent of the process model).

Note that the smaller ripples that are visible on the bumps are not accidental, but result in a similar way from the possible marginal spike frequencies. Note also that the p-value distributions for higher firing rates (not shown in the figure) are somewhat less extreme, but still strongly bumpy and not in line with the expected uniform distribution. Furthermore, we obtained similar diagrams for the G-test and Fisher's exact test, showing that it is not the $chi^2$ test that is at fault. It is rather the limited number of possible contingency tables that creates this behavior from which all tests then must suffer. As a consequence, testing for dependence based on coincident spikes has to be handled with care and may need specially adapted tests.


all coincidences
0 coincidences
1 coincidence
2 coincidences
3 coincidences
>3 coincidences
frequency
frequency
4000
3000
2000
1000
0
0
0.25
0.5
0.75
1
p-value

# Rate dynamics in highly structured population models of the rat amygdala

Oliver Schmitt[1], Eipert Peter[1], Andreas Wree[1], Klaus-Peter Schmitz[2]

[1]University of Rostock, Dep. of Anatomy, Gertrudenstr. 9, 18055 Rostock, Germany
[2]University of Rostock, Dep. of Biomedical Engineering, Friedrich-Barnewitzstr. 4, 18199 Rostock, Germany

The generic framework *neuroVIISAS* allows to assign symbolic information of a neuroontology to regions in atlases or serial histologic sections of complete central nervous systems. The connectivity information derived from tracing studies of the rat brain and spinal cord have been integrated into the neuroontoloy and atlas mappings to be used for simulations in the resultant highly structured neurobiological networks with the leaky integrate and fire neuron (LIAF) model of *NEST*. The rat project within *neuroVIISAS* consists of 32000 connectivities in 6300 CNS regions analyzed from 550 tracing studies. 91 subregions of the rat amygdala possess 254 real neurobiological afferents and efferents including connectivity weights. The amygdala network has been investigated with regard to graphtheoretical features. We found a scale free network with specific and highly significant distributions of certain 3 node and 4 node network motifs. This neuroanatomic connectivity structure of the rat amygdala have been used to model a network for simulations taking region volume dependent estimates of population sizes into account.
PyNEST scripts are automatically generated by defining LIAF, network parameters and neuroanatomical subregions of the intrinsic amygdala network. By applying a spike injection into the posteromedial cortical nucleus we found specific patterns such as oscillations, and correlations, spike coincidences and synchonicity at the neuron and population levels. Several regions of the intrinsic amygdala network show significant stronger correlations of their inter-spike intervals (ISI) than the average ISI distribution distances of population pairs. This finding points to a correlation structure of the intrinsic amygdala network and need to be investigated by analyzing the signal flow at different scales of the network.

# Refractoriness of individual neurons exposed in population spike trains

Moritz Deger[1], Moritz Helias[2], Clemens Boucsein[1], Stefan Rotter[1]

[1]Bernstein Center Freiburg & Faculty of Biology, Albert-Ludwig University, 79104 Freiburg, Germany

[2]RIKEN Brain Science Institute, Wako City, Saitama 351-0198, Japan

Poisson processes are often used to model neuronal population spike trains. It is known, though, that superpositions of realistic, non-Poissonian spike trains do not necessarily inherit the Poisson propery [1,2]. We indeed confirm that the population activity constructed from in vivo recordings in rat somatosensory cortex strongly deviates from the Poisson assumption. An improved minimal model of single neuron spike trains, the Poisson process with dead time (PPD; [3]), overcomes the associated difficulties by taking the effective refractoriness of neurons into account. In fact, many aspects of the statistics of superpositions constructed from in vivo spike trains agree with our analytical results for superpositions of the PPD matched to individual spike trains. In simulations, we observed that model neurons receiving superimposed spike trains as input are highly sensitive for the statistical differences induced by refractoriness of the component spike trains. We further present an efficient generator of superpositions of PPDs. It can be applied to produce more realistic background input for simulations of networks of spiking neurons.

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# Reliability and Information Transfer in Resonate-and-Fire models with hyperpolarizing resets.

Wei Wu[1], Susanne Schreiber[1,2]

[1]Humboldt University of Berlin, Institute for Theoretical Biology, Invalidenstr. 43, 10115 Berlin, Germany
[2]Bernstein Center for Computational Neuroscience Berlin, Philippstr. 13, Haus 6, 10115 Berlin Germany

Subthreshold activation of ionic currents is known to contribute to neuronal firing. One example is the subthreshold frequency filtering in resonant cells, which leads to maximal firing rates for stimuli whose frequency content is in the range of the resonant frequency. In addition to subthreshold dynamics, however, also spike-induced currents have been found to shape the firing patterns. Here, we use simple resonate-and-fire models to explore how spike-induced effects, such as spike afterhyperpolarization, contribute to signal processing beyond the effects attributable to subthreshold dynamics. Employing mathematical modeling, we focus on the example of resonant cells, where an intricate interplay between subthreshold resonance -induced membrane-potential oscillations and spike-induced dynamics is to be expected. Prominent examples of such cells are the presumed grid cells in the entorhinal cortex - the layer II stellate cells - for which it has been argued that both the subthreshold membrane-potential oscillations (Giocomo et al., 2007) as well as spike-induced dynamics (Navratilova et al., 2008) may contribute to grid-cell firing. We find that the spike-induced dynamics influence the reliability of neural responses as well as the amount of information transmitted. Indeed, in resonate -and-fire neurons the characteristics of spike-afterhyperpolarization may determine to what extent reproducibility of responses to rhythmic stimuli is favored over information transfer for nonrhythmic stimuli.

Acknowledgments
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# Single drops decide about rise and fall of the diffusion approximation - Neuronal consequences of pulsed communication

Markus Diesmann[1], Moritz Helias[1], Moritz Deger[4], Stefan Rotter[4,5]

[1]RIKEN Brain Science Institute, Laboratory for Computational Neurophysics, Wako City, Japan
[2] Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany
[3] Brain and Neural Systems Team, RIKEN Computational Science Research Program, Wako City, Japan
[4] Bernstein Center for Computational Neuroscience, Freiburg, Germany
[5] Computational Neuroscience Lab, Faculty of Biology, Albert-Ludwig University, Freiburg, Germany

Email: diesmann@brain.riken.jp

A generic property of the communication between neurons are synaptic pulses exchanged at discrete points in time defined by the action potentials. However, the current theory of spiking neuronal networks of integrate-and-fire neurons invokes the diffusion limit [1,2] to approximate synaptic impulses by Gaussian noise. Associating each impulse with a rain drop, performing the diffusion limit corresponds to the change from torrent rain to dense fog. The shishi odoshi (A), a device found in traditional Japanese gardens, is in some respects a suitable analog of a neuron. As a single drop of rain ultimately causes the shishi odoshi to tilt (B), a single additional synaptic impulse will finally cause an action potential in a neuron. This explains why the membrane voltage density close to firing threshold sensitively depends on the size of the synaptic amplitude [5,6]. Here we combine pulsed synaptic interaction with the analytical ease of the diffusion limit by means of a novel boundary condition at threshold [5,6]. Our theory explains why neural transfer is instantaneous rather than exhibiting lowpass characteristics, depends nonlinearly on the amplitude of synaptic impulses, is asymmetric for excitation and inhibition and is promoted by a characteristic level of synaptic background noise. These findings finally resolve contradictions between the earlier theory [1,2] and experimental observations [3,4].

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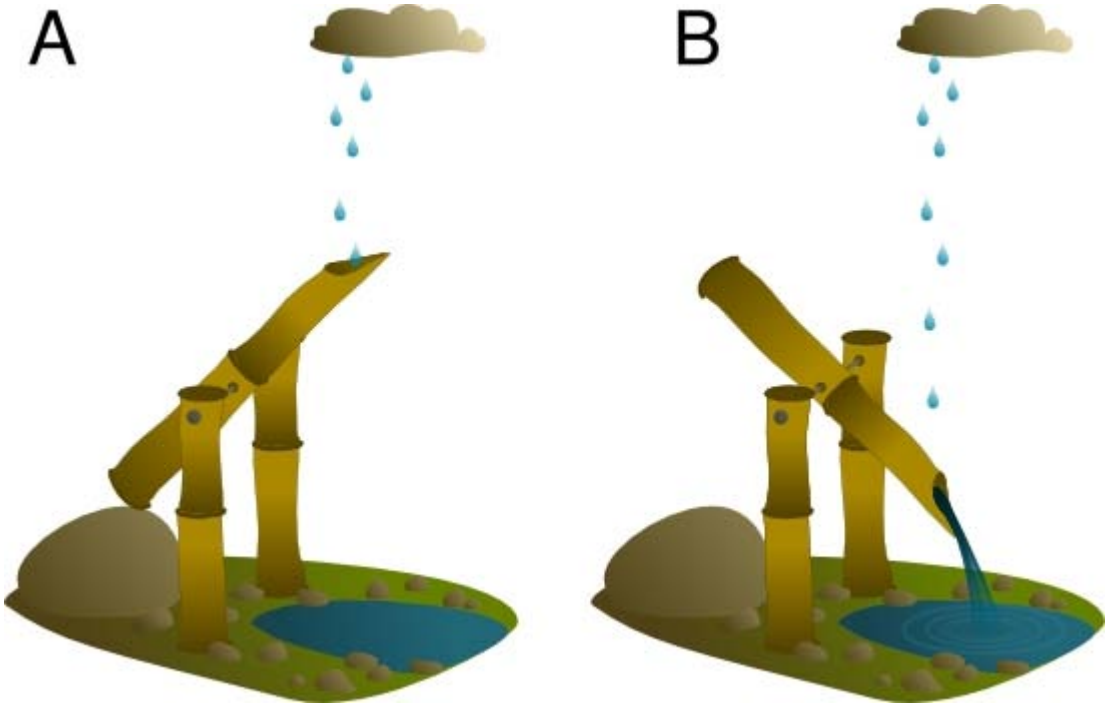

# Spike initiation and response dynamics of neuronal models with cooperatively gating $Na^+$ channels

Pinar Öz[1], Min Huang[1], Fred Wolf[1]

[1]MPI for Dynamics and Self-Organization, Theoretical Neurophysics, Bunsenstrasse 10 Haus 2 , 37073 Goettingen, Germany

Biophysical models of action potential generation are generally built upon two important assumptions: 1) Individual channels act statistically independently from their neighboring channels 2) The only coupling occurs through the membrane potential. However, a wide variety of ion channels for physiologically important ions are shown to undergo conformational changes also in a coordinated fashion, such that opening (and closing) of one channel increases the opening (and closing) probability of the neighboring channels (Saito et al. 1988; Undrovinas et al. 1992; Marx et al. 1998; Dekker et al 2006; Molina et al.2006). Recently, it was shown that the canonical gating kinetics,i.e. the Hodgkin-Huxley type gating, fail to reproduce the rapid onset and the threshold variability of the cortical action potentials that were recorded in vivo and in vitro (Naundorf et al. 2006). Furthermore,it was suggested in the same study that if the Hodgkin-Huxley type kinetics are replaced by cooperative channel gating, these characteristics of cortical action potentials can be reproduced.In this study, we explored this hypothesis by implementing cooperative channel activity at the axon initial segment (AIS), the site of action potential initiation (Palmer and Stuart, 2006; Kole et al. 2008). Our preliminary results on a spherical single compartment model indicated that replacing the canonical gating kinetics with cooperative gating can indeed produce sharper action potential onsets. The onset rapidness of the action potentials were closely related to the coupling strength of neighboring channels and the fraction of ionic channels that coupled in a chosen area. Next, we built a multi-compartmental model with a homogeneous cable-like dendrite, an octagon shaped soma and a myelinated cable-like axon. The conductances and gating kinetics were adapted from Wang-Buzsaki model (Wang and Buzsaki,1996) and cooperativity was implemented at AIS. The sodium channel densities in AIS were altered from 3- to 20- times somatic channel density and its relation with the initiation site and action potential onset was compared to the previous studies. Here, we present our recent results on the multi-compartmental cooperative AIS (MCCAIS) model.

# Spike Sorting by Stochastic Simulation

Di GE[1], Eric Le Carpentier[2], Jérôme Idier[2], Dario Farina[3]

[1]Glaizer Group, Applied Mathematic Dep, 32 rue Guy Moquet, 92240 Malakoff, France

[2] Institut de Recherche en Communications et Cybernétique de Nantes (IRCCyN UMR-CNRS 6597), École Centrale de Nantes, 1 rue de la Noë, 44321 Nantes Cedex 03, France

[3]Department of Neurorehabilitation Engineering, Bernstein Center for Computational Neuroscience, Georg-August University of Göttingen, Göttingen, Germany and the Center for Sensory-Motor Interaction (SMI), Department of Health Science and Technology, Aalborg University, Fredrik Bajers Vej 7D-3, DK-9220 Aalborg

The decomposition of multiunit signals consists of the restoration of spike trains and action potentials in neural or muscular recordings. Because of the complexity of automatic decomposition, semiautomatic procedures are sometimes adopted. The main difficulty in automatic decomposition is the resolution of temporally overlapped potentials.

We previously proposed [1] a Bayesian model coupled with a maximum a posteriori (MAP) estimator for fully automatic decomposition of multiunit recordings and we showed applications to intramuscular EMG signals. In particular, the inter-spike intervals (ISI) for each given neuron source were modeled by a Gaussian-like distributed variable due to the observed regularity property [2].

A more complex signal model including the variability in amplitude of each unit potential is proposed in this study. In order to derive an estimation from the the joint posterior law (of the signal model), the numerical method based on Markov Chain Monte Carlo (MCMC) simulation is adopted. The latter generates a population of samples that converges in distribution to the target joint posterior law after a number of iterations (burn-in period). The estimator is then calculated from valid samples (i.e., those after the burn-in period) and its quality improves as the number of samples increases according to the Monte Carlo principle. The Metropolis-Hastings (MH) and Gibbs algorithms are among the most classic algorithms in the MCMC family, while Reversible Jump MCMC (RJMCMC) [3] further extends the application field by including variable dimension problems. An example of the multiunit single-channel spike sorting with the proposed approach is shown in Fig. 1.

We prove the convergence property of this approach mathematically and we test the method representatively on intramuscular multiunit recordings. The results showed that the accuracy in spike identification of the proposed method is on average greater than 90% for intramuscular signals with up to 8 concurrently active units. In addition to intramuscular signals, the method can be applied for spike sorting of other types of multiunit recordings.

A further refinement in the model implies a more complex discrete random variable model of ISI for each unit, for example a negative binomial distribution:

P(X=k|r,p)=C_{k+r-1}^{r-1} (1-p)^r p^k, for k=0,1,2,⋯;

such that two degrees of freedom (both the mean and the variance) can be parameterized by (r, p). This extension is especially useful for discrete random variables over an unbounded positive range (k = 0) whose variance exceeds the mean, which is particularly true for ISI in EMG signals for example. If a Poisson distribution is used to model such data, its mean and variance are equal. In that case, the observations are over-dispersed w.r.t. the Poisson model, while in the negative binomial distribution model the second parameter can be used to adjust the variance independently of the mean. Its advantage w.r.t. the current model (Gaussian -like distribution) is the suppression of the model approximation.

Finally, we conjecture that an even more complex joint model might further exploit the inter-unit firing synchrony through a negative multinomial distribution, for which the variance matrix term takes into account correlations of ISI from concurrently active units.

Fig. 1.Example of multiunit decomposition (intramuscular EMG signals) using the proposed approach. A total of 7 sources are active. The numbers on top of the raw signal represent the source labels as identified by the automatic decomposition with the proposed method (top numbers) and the decomposition with interaction of an expert

operator using EMGLAB [4] (lower numbers). The labeling of sources in this example led to perfect matching with the reference result. The raw (solid line) and the reconstructed signal (dashed line) are shown in the upper panel whereas the residual error is plotted in the lower panel.

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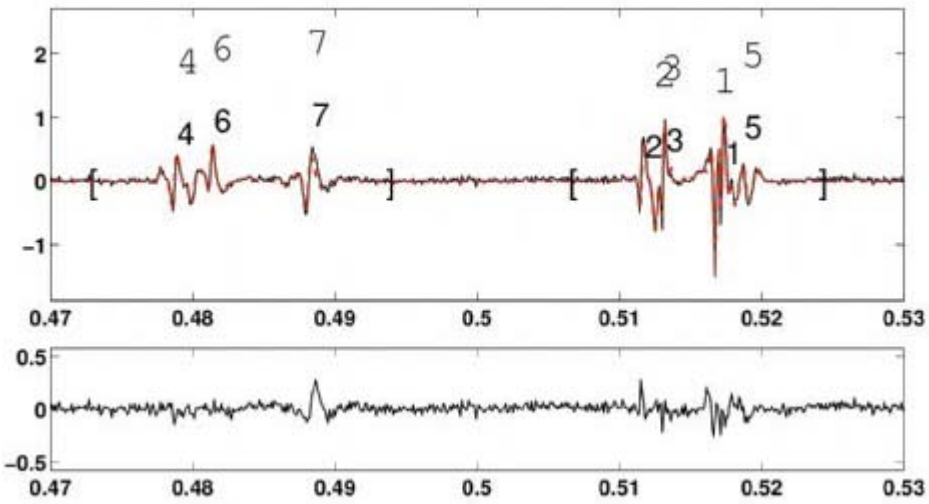

# Spike sorting of retinal ganglion cell responses effects stimulus reconstruction and change-point detection

Lena Sophie Köpcke[1], León M. Juárez Paz[1], Insa Winzenborg[2], Jutta Kretzberg[1]

[1]University of Oldenburg, Computational Neuroscience, Carl-von-Ossietzky-Str. 9-11, 26111 Oldenburg, Germany [2]University of Ulm, Institute of Number Theory and Probability Theory, Helmholtzstraße 18, 89081 Ulm, Germany

The central nervous system depends on spikes of retinal ganglion cells (RGC) as the only source of information about the visual environment. The exact time points of stimulus changes are generally not available to the nervous system and therefore cannot be used by the visual system as triggers to compute RGC response properties. In this contribution, we identified strong changes in population activity called population events and used them as reference points for stimulus reconstruction. We could show that spike sorting strategies significantly influence the performance of population events detection and event based stimulus reconstruction.

We have analyzed multi-electrode recordings of turtle RGC. The retina was stimulated with a moving pattern of dark dots on a bright background. The pattern moved with nine different velocities in one dimension along one axis of the electrode array. The velocity remained constant for 500ms and then changed abruptly to a different one.

We have used the Offline Sorter Version 2.8.8 (Plexon Inc) to classify spike waveforms. After automatic pre-sorting by E-M (Expectation Maximization) clustering selection, we manually classified the spike waveforms with a template matching algorithm. We had two different spike sorting strategies. The first strategy considered only the responses that showed the characteristic shape of a spike (noiseless sorted data). In contrast, in the second strategy all responses that did not show the characteristic shape of a spike were clustered into one additional unit per electrode (sorted data with noise).

The population events detection algorithm identifies strong changes in population activity by computing the moving average and the standard deviation of the smoothed population activity. A time point is defined as a population event if the population activity deviates from the moving average a pre-defined number of standard deviations. We have assessed the performance of the event detection algorithm by quantifying the number of correct stimulus change-points (correct events) and false positive change-points (false events).

For the stimulus reconstruction we applied Bayesian stimulus estimation. The main idea behind Bayesian reconstruction techniques is to determine the most probable stimulus given the observed response by using prior knowledge about the probability of stimulus occurrence combined with the statistics of response properties, conditioned on the stimulus.

Our main finding is that sorted data with noise allows better change-point detection performance than noiseless sorted data. Nevertheless, for sorted data with noise the proportion of false event detection is greater than the proportion of correct events detection. The correlation between the numbers of correct and false events is much greater in the case of data with noise than in the noiseless case.

If stimulus reconstruction uses knowledge about the timing of stimulus changes, no significant influence of the spike sorting has been observed. Nevertheless, sorting with noise has a positive effect on stimulus reconstruction based on the detected population events, leading to higher percentages of correctly reconstructed stimulus velocities.

In conclusion, even extracellular recorded activity that does not show the characteristic shape of a spike can improve the stimulus reconstruction.

# Spiking activity reflects structure in networks incorporating nonlinear dendrites

Sven Jahnke[1], David Breuer[1,2,3], Raoul-Martin Memmesheimer[4], Marc Timme[1,2,3]

[1]Network Dynamics Group, Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience (BCCN) Göttingen, Göttingen, Germany
[3]Georg August Universität, Göttingen, Germany
[4]Donders Institute, Department for Neuroinformatics, Radboud University, Nijmegen, Netherlands

So far, studies on the relation between network structure and network dynamics have focused on neurons with linear or sublinear input summations. However, single neuron experiments in different neuronal systems have shown supralinear dendritic interactions that are mediated by dendritic spikes. Here, we study structured networks that incorporate active dendrites, starting from feedforward chains. We develop analytical techniques to describe the propagation of synchronous activity in such networks. We find that structured networks with nonlinear dendritic interactions lead to precise spatio-temporally coordinated spiking activity, even if the networks are noisy and very sparse. Our findings suggest a mechanism underlying precisely timed patterns of spikes in cortical activity as well as replay of spike patterns as observed in hippocampal regions.

# State-dependent network reconstruction from calcium imaging signals

Olav Frank Stetter[1], Demian Battaglia[1,2], Jordi Soriano[3], Theo Geisel[1,2]

[1]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen, Germany
[3]Universitat de Barcelona, Spain

Calcium imaging has in recent years become a standard technique for the measurement of bulk activity of a population of cultured neurons. Typically these recordings are slow compared to the cell dynamics and display a low signal-to-noise ratio, but they allow for the simultaneous recording of hundreds of neurons.

We are interested in reconstructing an approximation of the structural connectivity of a culture of neurons. First, we study simulations of the fluorescence signal and examine established methods of inferring the topology such as Granger causality. It turns out that we can improve on these methods if we turn to measures from information theory, which do not rely on a linearity assumption.

Because we are interested in directed networks, our measure of choice in this case is Transfer Entropy, which is computationally very efficient and can also be used to reconstruct the topology in real time. It turns out that we can achieve a very reasonable quality of the reconstruction if we allow for novel extensions of this measure. Specifically, we need to take into account the ability of the network to display different dynamical states, where we need to focus on phases of activity where the dynamics of the system are dominated by pairwise synaptic interactions. Additionally, we need to include the fact that information is flowing faster between nodes as our recording is able to capture.

As a last step, we demonstrate post-processing improvements of the reconstruction using the Data Processing Inequality that are only possible in the case of information theoretical measures. These methods, already applied with success in the reconstruction of gene regulatory networks, take into account triples of neurons and help to discriminate indirect from direct interactions.

Finally, we illustrate our findings in real data of hippocampal neurons in vitro. For instance, we showing how the probability of connection between two nodes depends on the distance and how it compares to our simulations. We analyze as well more in detail the topological properties of the reconstructed network, finding a significant amount of clustering in the culture.

# Switching between Up and Down states in a conductance-based cortex model

Jens Christian Claussen[1], Arne Weigenand[1,2,3], Hong-Viet V. Ngo[1,2,3], Thomas Martinetz[1,2]

[1]University of Luebeck, Institut f. Neuro- und Bioinformatik, Ratzeburger Allee 160 , 23562 Luebeck, Germany
[2]SFB 654 Plasticity and Sleep
[3]Graduate School Computing in Medicine and Life Science, Luebeck

In a recent experiment [1] investigated the on and off switching of bursting activity in ferret brain slices. This experiment is seen as a paradigmatic system towards the understanding of the emergence of cortical slow waves. The basic dynamics can be modeled by a simplified discretized integrate-and-fire model having intrinsic inhibitory currents but lacking inhibitory connections [2]. Here we use a conductance-based model to reproduce the spike-burst dynamics and the triggering of Up–states as observed in [1].

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# The honeybee olfactory system as a template for better neuromorphic classifiers

Michael Schmuker[1], Chris Häusler[1,2], Martin P Nawrot[1,2]

[1]Neuroinformatics & Theoretical Neuroscience, Institute of Biology, Freie Universität Berlin, Berlin, Germany
[2]Bernstein Center for Computational Neuroscience Berlin, Berlin, Germany

Honeybees can identify odorant stimuli in a quick and reliable fashion, as is demonstrated by a large body of behavioral discrimination experiments [1]. The neuronal circuits involved in odor discrimination are well described at a structural level. We decompose the honeybee's olfactory pathway into local circuits that represent successive processing stages. Using spiking neuronal network models, we infer the specific functional role of these microcircuits in odor discrimination, and measure their contribution to the performance of a neuronal implementation of a probabilistic classifier, trained in a supervised manner [2,3].

In the insect olfactory system, primary receptor neurons project to the antennal lobe (AL). The AL is organized in compartments called glomeruli. Each glomerulus receives input only from one type of receptor neurons. Each odorant activates many different receptor types, inducing a spatial pattern across the AL. Strong lateral inhibitory interactions between glomeruli make an impact on information processing [4]. We illustrate how lateral inhibition enhances linear separability of stimulus patterns by increasing contrast between input dimensions.

From the glomeruli in the AL, uniglomerular projection neurons (PNs) send their axons to Kenyon cells (KCs) in the mushroom body, a central brain structure where different sensory modalities converge and stimulus associations are formed [5]. Connections between PNs and KCs are realized within small local microcircuits, where PNs and KCs interact with an inhibitory cell population [6]. We demonstrate how this microcircuit can create non-linear transformations of the input patterns. At this stage, about 950 PNs project onto a sizeable fraction of the total 160.000 KCs, providing a massive "fan-out" of connections. Taken together, this organization resembles the working principle of a support vector machine, transforming data which is not linearly separable into a higher-dimensional representation, in which linear separation is possible.

At each stage of the model, we use a two-dimensional toy data set to illustrate the classification problem and the processing principle.

Currently, we are testing implementations of our models on neuromorphic hardware, and we test classifier performance on benchmark data sets and a real-world odorant data set [7]. This is a first step towards implementations of fast and powerful neuromorphic classification devices, applicable to a wide range of parallel sensor data.

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# The Structure of Endogenous Activity in Spiking Cortical Networks with Randomly Coupled Synfire Chains

Chris Trengove[1], Cees van Leeuwen[2], Markus Diesmann[1,2,3]

[1]RIKEN, Brain and Neural Systems Team, 2-1 Hirosawa, 351-0198 Wako, Saitama, Japan
[2]RIKEN, Brain Science Institute, 2-1 Hirosawa, 351-0198 Wako, Saitama, Japan
[3]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Juelich, Juelich, Germany

Recent work demonstrated for the first time that a large number of synfire pools organized into synfire chains, with the number of pools being of the same order of magnitude as the number of neurons, can be embedded in a balanced random cortical network of spiking neurons on the scale of a cortical macro-column or greater (Trengove et al, 2010). Key elements of the model are balanced synfire chains containing both excitatory and inhibitory neurons and conductance -based synapses. Such a network supports simultaneous propagation of a number of synfire pulse packets (or waves). The number of waves is regulated by global feedback in the form of balanced noise, which acts to destabilize wave propagation.

We report on our current investigations into the extension of this model into a compositional system. We find that ongoing endogenous activity (that is, not dependent on external input) can result from synfire waves percolating through a system of randomly coupled (or composed) synfire chains, given a suitable composition topology. We find that such endogenously generated background activity in the network can exhibit firing rates ranging from 20 Hz down to 0.5 Hz or lower depending on the width of the chains and the size of the network.

We suggest a range of synfire chain composition topologies, focusing here on branching networks where a chain may have two or more successor chains according to some distribution. To make branching networks we consider two kind of pairwise composition: Type 1 (longitudinal or sequential linkage); and Type 2 (lateral via weak crosslinks, with an additional temporal offset) (see Figure).

We allow for a distribution of the strengths of the chains and the couplings (where strength involves both number of convergent inputs and synaptic strength). This constitutes a topography on the topology. We find that activity is regulated in the system so that the effective connectivity is poised at the critical state for percolation of wave activity, this being governed by the interaction between global feedback (balanced noise) and the topography.

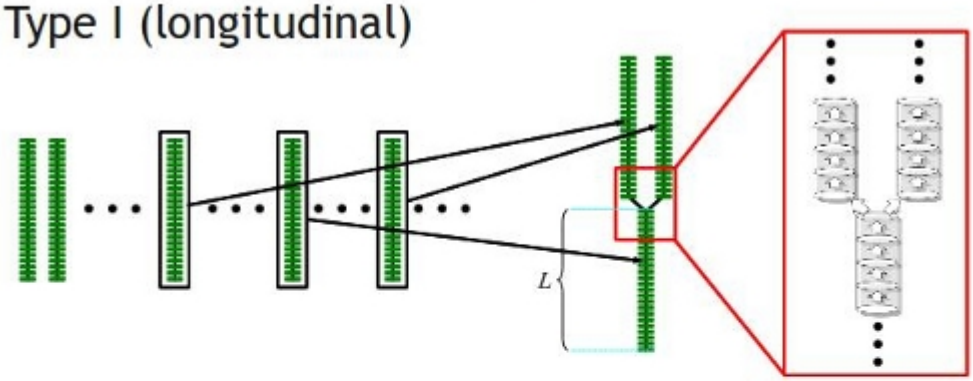
Within this space of compositional topologies, we investigate the bounds on the rate of branching and the distributions of chain and coupling strengths that are necessary in order to preserve the realistic regime of endogenous activity.

We characterize the nature of the endogenous state of activity; in particular we examine the fluctuations in activity to determine under what conditions, if any, these fluctuations obey a power law distribution. In addition, we characterize the structure of the evolving set of active synfire waves in terms of its youngest-common-ancestor age distribution and compare this with purely random model of percolation of a fixed number of waves on a branching network, in order to determine if avalanche phenomena are occurring.

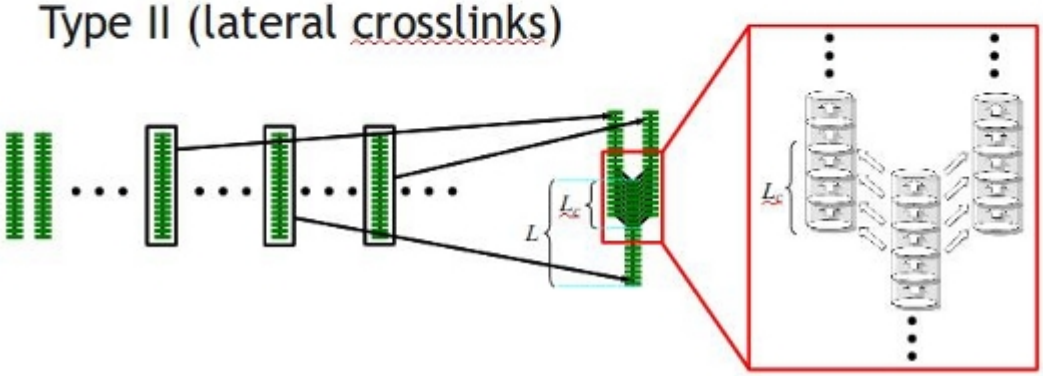
Reference:
Trengove C, van Leeuwen C, Diesmann M (2010) High storage capacity of synfire chains in large-scale cortical networks of conductance-based spiking neurons, CNS 2010, San Antonio.

Two schemes for branching chains:

Type I (longitudinal)




Type II (lateral crosslinks)



# Variability of Grid Cell Firing on a Trial-to-Trial Basis

Andreas VM Herz[1], Carleen Kluger[1,2,3], Alexander Mathis[1,2,4], Martin Stemmler[1,2]

[1] Division of Neurobiology, Ludwig-Maximilians-Universität München, Germany
[2] Bernstein Center for Computational Neuroscience Munich, Germany
[3] Department of Physics, Ludwig-Maximilians-Universität München, Germany
[4] Graduate School of Systemic Neurosciences, Munich, Germany

The spatially modulated firing of grid cells in the entorhinal cortex [Fyhn et al, Science, 2004] has radically changed our understanding of how the rodent brain represents space. Whereas the beauty of the hexagonal pattern, which is revealed by pooling the spikes of multiple runs for one grid cell over the environment, is striking, far less is known about the properties of single passages through a "firing field." Here we study the statistics of grid cell spiking, based on data recorded in the Moser lab, Trondheim.

Our results show that spike counts for a single run through the grid field are generally Poisson-distributed. This is particularly surprising, as place cells in the hippocampus exhibit spike counts with surplus variance and are therefore not Poisson-distributed [Fenton and Muller, PNAS, 1998]. Furthermore, we describe the spatial tuning of grid cells by tuning curves obtained by our non-parametric method [see the poster of Patirniche et al. at this conference] and show that the statistical properties of grid cell firing are well captured by a doubly stochastic process defined by these tuning curves [similar to work on place cells by Lansky and Vaillant, Biosystems, 2000]; such a statistical model can then be used to estimate the coding properties of grid cells more accurately than what is possible based on previous models.

# What correlation coefficients can and cannot tell.

Tatjana Tchumatchenko[1], Theo Geisel[1], Maxim Volgushev [2,3,4], Fred Wolf[1]

[1]Bernstein Center Göttingen, MPI Dynamics and Self-Organization, Theoretical Neurophysics, Bunsenstr. 10, 37073 Göttingen, Germany
[2]Dep. of Neurophysiology, Ruhr-University Bochum, Bochum, Germany, Germany
[3]Inst. of Higher Nervous Activity and Neurophysiology, RAS, Moscow, Russia
[4] Dep. of Psychology, University of Connecticut, Storrs, USA

Concerted neural activity can reflect specific features of sensory stimuli or behavioral tasks. Monitoring this activity offers a possibility to understand the neuronal mechanisms governing sensory perception and behavior. While recent experimental progress has enabled simultaneous recording of activity of a large number of neurons, it is still an open question which theoretical tools are appropriate to understand and quantify neuronal interactions. Correlation coefficients and count correlations are frequently used to measure correlations between neurons, the degree of synchrony in a network, to design synthetic spike trains and to build population models. But are correlation coefficients always a reliable measure of input correlations? Here, we consider a stochastic model for the generation of correlated spike sequences which replicates neuronal pairwise correlations in many important aspects [1,2]. We investigate under which conditions the correlation coefficients reflect the degree of input synchrony and when they can be used to build population models. We find that depending on the strength of input correlations, the firing rate and the bin size, correlation coefficients may reliably capture input correlation strengths or be an ambiguous measure of synchrony. Moreover, when computed for large time bins, the count correlations may vanish even in the presence of input correlations. In other words, if a pairwise correlation coefficient obtained in an experiment is indistinguishable from zero it does not allow concluding that the activity of the two neurons is unrelated to each other [2]. In Fig. 1 we show how a particular functional choice of input correlations can lead to vanishing correlation coefficients. Fig. 1 shows correlation coefficient $\rho_{ij}$ as a function of bin size for three different voltage correlation functions, one of which, $C_3(t)$, leads to vanishing correlation coefficients for bin sizes much larger than the intrinsic voltage correlation time $t_s$. These findings suggest that network models or potential coding schemes of neural population activity need to incorporate temporal properties of correlated inputs and take into consideration the regimes of firing rates and correlation strengths to ensure that their count correlations are unambiguous measures of synchrony.

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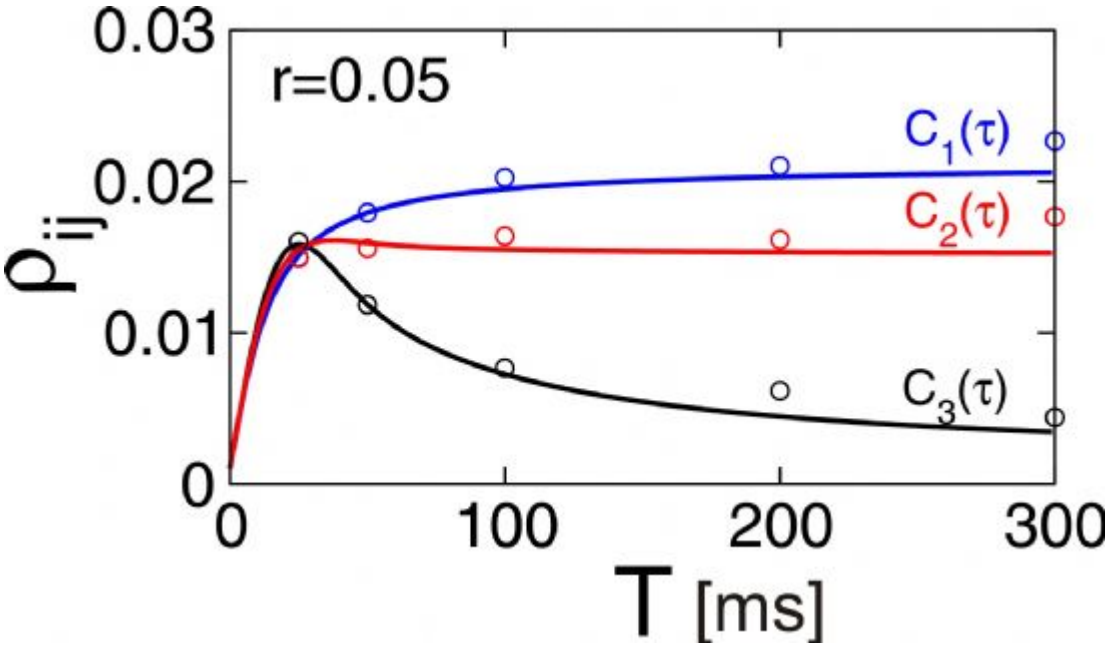
Caption to Figure 1: Influence of temporal structure on pairwise spike correlations. Spike correlation coefficient $\rho_{ij}$ vs. bin size T for voltage correlation functions $C_1(t)=s_V^2/\cosh(t/t_s)$, $C_2(t)= s_V^2/\cosh(t/(\mathrm{sqrt}(2)t_s))\cos(t/(\mathrm{sqrt}(2)t_s))$, $C_3(t)=s_V^2(\exp(-t^2/(6t_s^2))-t^2/(3t_s^2)\exp(-t^2/(6t_s^2)))$. Note, all voltage correlation functions share the same correlation time $t_s$ but have a different functional form. Here, the correlation time $t_s$ = 10 ms, voltage correlation strength r=0.05, voltage variance $s_V^2$, firing rate of both neurons is $\nu$=5Hz, circles denote the corresponding simulation points [2].


0.03
r=0.05
0.02
$\rho_{ij}$
0.01
0
0
100
200
300
T [ms]
$C_1(\tau)$
$C_2(\tau)$
$C_3(\tau)$

# Poster Topic

# T27: Techniques and Demonstrations

T27-1A A setup for automated experiments on visual behavior of fish under different temperature conditions
*Malte T. Ahlers, Josef Ammermüller*

T27-2A A spectral-selective stimulator for obtaining full-field ERGs in animal research
*Arne Liebau, Karl-Heinz Esser*

T27-3A Analysis of developmental transcriptional profiles of dopaminergic neurons in zebrafish by means of FACS and deep sequencing
*Martha Manoli, Sebastian Hobitz , Antonio Miguel Fernandes, Thorsten Kurz, Wolfgang Driever*

T27-4A Cluster analysis as a method to identify medullary nuclei of toothed whales by routine histology.
*Lyuba Zehl, Helmut H.A. Oelschläger, Wolfgang Walkowiak, Stefan Huggenberger*

T27-5A Development and testing of a wireless raw data acquisition system for neuronal activities from freely moving animals
*Lutz Rafflenbeul, Roland Werthschützky, Alexander Gail*

T27-6A Dynamic, semi-quantitative optical imaging of intracellular ROS levels and redox status in rat hippocampal neurons
*Frank Funke, Florian J. Gerich, Michael Müller*

T27-7A Enhancing neuronal cell proliferation using a 3D cell culture system.
*Dennis Jgamadze, Steffen Vogler, Sophie Pautot*

T27-8A Expression of recombinant protein in honeybee brains by *in vivo* electroporation
*Gerard Leboulle, Nora Gehne, Anja Froese, Randolf Menzel*

T27-9A Closed-loop electrophysiological experiments and automated metadata acquisition with RELACS
*Jan Benda, Jan Grewe*

T27-10A The Laboratory Logbook
A database-driven approach for project documentation
*Adrian Stoewer, Jan Benda, Jan Grewe*

T27-1B Extension of protease based cellular sensors to analyze complex signal processes in the brain
*Wilko Hinrichs, Moritz Rossner*

T27-2B Fuzzy classification and inference of interneuronal types
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T27-3B High-throughput, quantitative mass spectrometry of acutely stimulated synapses
*Sidney B. Cambridge, Marcus Krüger, Matthias Mann*

T27-4B How to deal with the heterogeneity of neural responses: A demixing method
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T27-5B In vivo SPECT-imaging of spatial patterns of neuronal activity in rodent brain using 99mTcHMPAO and 201TlDDC as tracers
*Jenni Neubert, Angela Kolodziej, Marc Zappe, Julia Georgi, Eike Budinger, Anton Ilango, Marie Woldeit, Frank Angenstein, Frank W. Ohl, Henning Scheich, Jürgen Goldschmidt*

T27-6B Light-inducible protein synthesis inhibition
*Kathrin Marter, Janina Schaal, Jenny Eichhorst, Julien Colomb, Burkhard Wiesner, Volker Hagen, Dorothea Eisenhardt*

T27-7B MAGNETIC RESONANCE IMAGING OF THE RHESUS MONKEY BRAIN
*Roland Tammer, Sabine Hofer, Klaus-Dietmar Merboldt, Jens Frahm*

T27-8B Microfluidics and insect cell culture
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T27-9B NEST: An efficient simulator for spiking neural network models
*Jochen Martin Eppler, Susanne Kunkel, Hans Ekkehard Plesser, Marc-Oliver Gewaltig, Abigail Morrison, Markus Diesmann*

T27-10B Neurophysiology data management for efficient analysis and collaborative work
*Andrey Sobolev, Philipp Rautenberg, Christian Kellner, Jan Benda, Jan Grewe, Martin P. Nawrot, Willi Schiegel, Tiziano Zito, Andreas V. Herz, Thomas Wachtler*

T27-11B New possibilities for advanced analysis methods in neuroscience through modern approaches to trivial parallel data processing
*Abigail Morrison, Michael Denker, Bernd Wiebelt, Denny Fliegner, Markus Diesmann*

T27-1C Novel approach for remote long-term recordings of sleep-wake rhythms, core body temperature and activity in single- and group-housed rats
*Kerstin Plaßmann, Eberhard Fuchs*

T27-2C Objective-coupled planar illumination microscopy – A novel technique for neuronal population imaging
*Oliver Braganza, Rolf Beck, Rainer Meyer, Heinz Beck*

T27-3C Organotypic brain slice co-cultures of the dopaminergic system- a versatile tool for the investigation of toxicological properties of novel substances
*Katja Sygnecka, Claudia Heine, Marcus Grohmann, Nico Scherf, Heike Franke*

T27-4C Protein Macroarray:
A new approach to indentify NCAM binding partners
*Hilke Wobst, Agathe Sekulla, Christine Laurini, Brigitte Schmitz, Simone Diestel*

T27-5C Reconstructing the *in vivo* brain: a CT/MRT aided stereotaxic atlas of the Mongolian gerbil brain (*Meriones unguiculatus*)
*Susanne Radtke-Schuller, Frank Angenstein, Jürgen Goldschmidt, Oliver S. Grosser, Gerd Schuller, Eike Budinger*

T27-6C Reconstruction and dissection of the entire human visual pathway using diffusion tensor MRI
*Sabine Hofer, Alexander Karaus, Jens Frahm*

T27-7C Recurrence-Based Estimation of Time-Distortion Functions for ERP Waveform Reconstruction
*Matthias Ihrke, Hecke Schrobsdorff, J. Michael Herrmann*

T27-8C The performance of an automatic algorithm in isolating single units in primate cortex
*Shubo Chakrabarti, Paul Hebert, Michael Wolf, Michael Campos, Alexander Gail, Joel Burdick*

T27-9C The use of the K+-probe thallium (Tl+) for imaging CNS potassium metabolism and neuronal activity - from microscopy to in vivo SPECT-imaging
*Jürgen Goldschmidt, Tim Wanger, Jenni Neubert, Marc Zappe, Ulrich H. Schröder, Frank Angenstein, Klaus G. Reymann, Henning Scheich*

T27-10C "Virtual Pre-embedding Labeling": A novel approach for correlative light and electron microscopy and double labeling in affinity cytochemistry
*Vince Istvan Madai, Rene Bernard, Wolfram Poller, Gregor Laube, Rüdiger W. Veh*

T27-11C Local noise amplification and synaptic depression control the spontaneous activity in neuronal cultures
*Javier G. Orlandi, Enric Alvarez-Lacalle, Sara Teller, Jordi Soriano, Jaume Casademunt*

T27-12C A microfluidic culturing platform for studying intra-dendritic signaling and dendrite to nucleus communication
*Carlos Bas Orth, Michael S Cohen, Hyung Joon Kim, Noo Li Jeon, Samie R Jaffrey*

# A setup for automated experiments on visual behavior of fish under different temperature conditions

Malte T. Ahlers[1], Josef Ammermüller[1]

[1]Carl von Ossietzky Universität Oldenburg, AG Neurobiologie, Carl-von-Ossietzky-Str. 9 - 11, 26129 Oldenburg, Germany

By extracellular recordings from fish ganglion cells (GC) with multi electrode arrays we were able to show strong temperature influences on GC light response properties. Response mean spike rate and latency are significantly altered by temperature changes. In addition, more complex response properties like temporal structure of burst patterns undergo significant changes with temperature, too. This leads to the question which consequences arise from these physiological effects on a behavioral level. To address this question we developed a setup to perform semi-automated and automated experiments on visual behavior of fish under different temperature conditions.

Our setup consists of the following parts: (1) an aquarium in which the experiments are conducted. The aquarium is placed in a housing made of Styropor shielding it from ambient temperature and light fluctuations. The fish is kept in the aquarium permanently. The aquarium is therefore equipped with a water filter which additionally serves to thermally homogenize the water. (2) The water inside the aquarium is tempered by Peltier elements affixed to one of the aquarium's walls made of aluminium. A purpose built closed loop control system adjusts the water temperature. It allows varying the temperature in a range of approx. 10 – 40°C with a precision of approx. +/- 0.1°C in steady state. The control algorithm is a PID type implemented on an Atmel AVR microcontroller. The PID parameters were fitted manually. The temperature control system is connected to a PC. (3) Light stimulation is performed by a purpose built light stimulation system. A horizontally oriented row of 6 "white-light" LEDs is mounted outside on one translucent aquarium wall. The light intensity of each LED can be varied with a resolution of 16 Bit by a digital-to-analog-converter (DAC). The DAC is connected to an Atmel AVR microcontroller which is interfaced to a PC. (4) For ambient lighting an array of 60 "white-light" LEDs is situated above the aquarium on the top cover of the Styropor housing. The light intensity of the LED array is controlled by an Atmel AVR microcontroller which is connected to a PC. (5) The same AVR also controls a purpose built feeding automat. (6) A camera is situated above the aquarium with a field of view directed to the area in front of the stimulation LEDs. The camera is connected to a PC via USB providing a real-time video stream of the animal's behavior. (7) A Windows PC running a purpose built Matlab program is used to centrally control all aspects of the experiments.

In semi-automatic mode of operation all experimental parameters are loaded from a predefined protocol file and the devices are set accordingly. This simplification of the experimental procedure makes it less error prone and more convenient for the experimenter. The behavior of the fish is observed on the computer screen, circumventing any disturbances of the animal that may be caused by direct observation e.g. movements of the experimenter. Experiments can be conducted double blinded.

Establishment of the automatic mode of operation is ongoing work. Regions of interests (ROI) are defined by the experimenter, typically one in front of each stimulation LED. An algorithm based on frame differencing detects motion in the ROIs. Thereby it's objectively identified if the fish prefers to stay in a specific ROI. This can be used to automatically trigger feeding during training and as an objective criterion for the detection of a specific trained stimulus.

# A spectral-selective stimulator for obtaining full-field ERGs in animal research

Arne Liebau[1,2], Karl-Heinz Esser[1,2]

[1]Institute of Zoology, Auditory Neuroethology and Neurobiology, University of Veterinary Medicine Hannover, Bünteweg 17, 30559 Hannover, Germany

[2]Center for Systems Neuroscience Hannover

The electroretinogram (ERG) is commonly accepted as the only measurement that allows an objective evaluation of retinal function. The most widely used ERG method is the full-field (Ganzfeld) ERG. For obtaining full-field ERGs it is mandatory that the retina is homogeneously stimulated. This is typically achieved by illuminating the respective eye with indirect light from the interior of a reflective hollow sphere or dome.
The arrangement of the light sources for illuminating the interior of the sphere in commercial full-field stimulators leads to some difficulties in animal research. As the head of the tested animal has to be placed inside the sphere, the retina receives direct light from the light sources which makes a homogeneous retinal stimulation impossible. Here, we describe a custom-made full-field stimulator for use with the most common laboratory animal species (e.g. mice, rats and rabbits), which provides strictly indirect stimulation of the eyes. For this, the hollow sphere is uniformly illuminated by up to 37 individual fibre optics and a corresponding number of directly attached diffusing disks. All fibre optics terminate equidistantly spaced on a ring in front of the opening (diameter = 25 cm) of the above sphere. To guarantee the functionality of the system in the UV-range, all optical components are made of quartz glass. Further, for the same reason, the inner surface of the hollow sphere has a barium sulphate-based coating.
Since the visual spectral ranges of animals can differ from the human situation it is suitable to have a broad range of spectral selective-stimulation i.e. from the UV to the NIR band (300 nm – 1100 nm; UV = ultraviolet, NIR = near infrared).
The full-field stimulator presented here allows for a narrow-band spectral-selective stimulation (10 - 25 nm) throughout its whole working range. The light is delivered by a triggerable xenon-light source (TYP SP-20-X6, Rapp OptoElectronic, Hamburg, Germany) that allows for pulsed stimulation according to the customer's needs. Spectral composition and intensity of light stimuli can be adjusted by an optical filter system which combines band-pass and neutral-density filters. In order to be able to record ERGs during different light-adaptation states of the animal under consideration, an adjustable (re intensity) background illumination (29 white LEDs) is integrated in the above mentioned front ring.
Different animal holders considering the respective body proportions allow for the use of animals varying greatly in body size and weight. The subject holders can be easily changed between recording sessions/ experiments and the access to the animal's head and eyes, and hence to the recording electrodes, is granted by a sliding mechanism under the sphere.

# Analysis of developmental transcriptional profiles of dopaminergic neurons in zebrafish by means of FACS and deep sequencing

Martha Manoli[1], Sebastian Hobitz [2], Antonio Miguel Fernandes[1], Thorsten Kurz[3], Wolfgang Driever[1,3,4]

[1]University of Freiburg, Biology 1, Hauptstraße 1, 79104 Freiburg, Germany
[2]Max-Planck-Institute of Immunobiology, Stübeweg 51, 79108 Freiburg, Germany
[3]Zentrum für Biosystemanalyse ZBSA, University of Freiburg, Habsburgerstr. 49, 79104 Freiburg, Germany
[4]FRIAS University of Freiburg, Freiburg, Germany

The dopaminergic (DA) systems represent some of the key neuromodulatory systems. DA neurons control initiation of movement, postural reflexes, reward association behavior and hormonal homeostasis. Deficits in DA neuron activity contribute to a wide range of diseases, including Parkinson's disease, schizophrenia, and addiction. While research has focused on the development of mammalian mesencephalic substantia nigra neurons, little is known about the developmental control of diencephalic DA neurons. For example, A11-type Otp-dependent DA neurons connect to the spinal cord, the telencephalon, and the hypothalamus, and are involved in restless legs syndrome.

We utilize the zebrafish as a model to gain more knowledge of mechanisms implicated in the differentiation of diencephalic DA neurons. For this purpose we established a protocol for the purification and the enrichment of embryonic and early larval DA neurons by fluorescent activated cell sorting (FACS). We use transgenic zebrafish lines that label DA neurons, including the ETvmat2:GFP line by Wen et al. (Dev.Biol. 2007). First, we have compared and optimized cell dissociation procedures to obtain an optimal yield of viable GFP positive cells. Depending on the line and developmental stage the number of GFP positive cells isolated from one fish ranges from 6 to 60 cells. Starting with 500- 1000 embryos or early larvae, up to 50000 cells have been collected. The sorted cells are directly lysed to isolate total RNA. The amount and length profile of the total RNA are inspected with a Bioanalyzer 2100 (Agilent), which revealed high quality of isolated RNA. The amount of total RNA varies between 5 ng and 50 ng per sort. Since this amount is insufficient for direct library construction in the Illumina/Solexa Genome Analyzer workflow, extraction and amplification of mRNAs from the total RNA was performed (MessageAmp II Kit; Ambion). From 10 ng of total RNA about 1 µg of aRNA have been synthesized. Amplification efficiency was controlled by qPCR for several transcripts. These qPCR data indicate that amplification introduces only minor transcript specific amplification bias. Thus, it can be used to semi-quantitatively establish transcriptomes with the Illumina/Solexa technology. We plan to combine the data we attain from different fish lines and at different developmental stages to establish transcript profiles characteristic for early and late stages of DA differentiation, as well as for different DA neuronal subtypes.

# Cluster analysis as a method to identify medullary nuclei of toothed whales by routine histology.

Lyuba Zehl[1], Helmut H.A. Oelschläger[2], Wolfgang Walkowiak[1], Stefan Huggenberger[1]

[1]Cologne Biocenter, University of Cologne, Germany
[2]Department of Anatomy III (Dr. Senckenbergische Anatomie), University of Frankfurt am Main, Germany

Toothed whales (odontocetes) are the only aquatic animals known to use echolocation. Considering that sound travels much faster in water than in air the question arises how these animals specifically adapted to acoustic perception and processing. In this context the comparatively large superior olivary complex (SOC) is of special interest, being the first auditory centre showing binaural integration of acoustic information and largely responsible for the localization of sound sources in mammals. Accordingly, we started to analyze the histology of the SOC in coronal microslide series of the common dolphin (*Delphinus delphis; D.d.*), a typical off-shore species with world-wide distribution using broad-banded low-frequency clicks. In addition, we examined a rather different odontocete, the La Plata dolphin (*Pontoporia blainvillei; P.b.*), a pristine dolphin -like species from coastal and estuarine habitats of Uruguay which echolocates with narrow -banded high -frequency clicks. The available microslide series of *D.d.* and *P.b.* consist of brain sections of 30µm and 20µm, respectively. Every 20th section of both series was mounted and Nissl stained. This led to around 25 sections comprising the SOC in each brain stem. The analysis of the SOC in these animals is challenging because its neuron populations are scattered diffusely between a few more prominent surrounding structures like the facial nucleus, pons, and the inferior olive. Moreover, only routine histological cell- or fibre-stained sections of our odontocete brains were available. This is due to the fact that, in these animals, tissue can only be taken from stranded dead specimens because of ethical reasons and strong protection. Likewise, immunohistochemistry could not be used for the characterization of neurons within the SOC. In the literature of odontocetes, only four subnuclei of the SOC have been reported so far, in contrast to up to 14 in other mammals. Our method combines digital data acquisition with cluster analysis, a common pattern recognition technique, providing a largely "observer-independent" opportunity to identify and categorize effectively large numbers of neurons. Therefore, we made a topographical analysis using morphological criteria and the quantitative distribution of neuron populations. Prior to a complete analysis of the SOC, we reduced the data set in this preliminary study to around 2,000 neurons obtained from one microslide of each brain, representing the estimated total of 50,000 neurons. A visual control of the method was possible in these cross sections, because the location of the SOC was defined by the sagittal plane medially, the brainstem contour ventrally and the facial nucleus laterally. Our method allowed us to distinguish up to six subnuclei of the SOC and discriminate them against the surrounding structures in both species. Our method is, therefore, an effective tool for future multivariate analysis of brainstem nuclei in routine-stain microslide series concerning specific neurobiological adaptations of odontocetes to their aquatic habitat.

# Development and testing of a wireless raw data acquisition system for neuronal activities from freely moving animals

Lutz Rafflenbeul[1], Roland Werthschützky[1], Alexander Gail[2]

[1]Institute of Electromechanical Design, Technische Universität Darmstadt, D-64283 Darmstadt
[2]Sensorimotor Group, German Primate Center, BCCN Göttingen, D-37077 Göttingen, Germany

Neurophysiological research aims at understanding the brain activity underlying behaviour. Many forms of complex behaviour in primates can only be investigated in natural environments. Recording neuronal activities in freely moving or socially interacting monkeys is still challenging due to the required bandwidth for signal transmission and recording stability, while at the same time energy supply, construction space, and acceptable weight for telemetric devices are very limited.

We designed a telemetric device to record neuronal activity without a need for constraining the animal's movements. We aim to amplify and transmit the recorded raw data from currently four microelectrodes or a tetrode to a Lab PC for further analysis. We use digital wireless transmission, which is very robust to electromagnetic noise sources. Also, in contrast to widespread analogue systems, our digital device is capable of signal pre-processing and event triggered actions, like a wake-up in response to a change in signal intensity.

Importantly, we implemented a bi-directional wireless communication protocol. The communication channel from the host PC to the remote system serves two purposes. First, a cascade of two programmable amplifiers fits the different amplitudes independently for each channel to allow the highest possible resolution. In combination with the fixed pre-amplification a gain between 20 dB and 80 dB can be programmed remotely. Second, we implemented the possibility to control a micromanipulator wirelessly. Different from other available systems, this allows to remotely re-adjust the microelectrode positions without the need to handle the animal. This new technology ensures a precise tracing of signals from individual neurons during free movement.

With a footprint of 2 by 3 cm² and a weight of less than 20 grams our current test system is small enough to be mounted on a rhesus monkey's head. It contains the microcontroller, the pre- and main amplifier and the motor driver. The system is powered by a LiPo-battery with a capacity of 380 mAh allowing a continuous operation for more than two hours.

First tests were conducted in awake monkeys, using an existing stationary recording system as reference for the neuronal data recordings, and a modified conventional head-mounted micro-drive for testing remote controllability of the micro-electrodes. Wireless recording of neuronal activity was possible over a distance of approx. 8 meters with our device.

Restrictions in usable bandwidth of the currently used Bluetooth module limited the sampling rate of our analog-digital-converter to 10 kSamples/s. The signal quality allowed reliable detection of single spiking events, while off-line spike-sorting via waveform clustering suffered from the reduced sampling rate. Bandwidth limitations are expected to be overcome by the next generation prototype using an alternative wireless transmission module.

Summary

A stand-alone digital wireless system for acquisition of neuronal activity on primates is described. It is capable of recording, processing and transmitting four independent neuronal signals. Bi-directional communication allows the adjustment of signal amplifications and the integration of micro-drives for electrode repositioning.

# Dynamic, semi-quantitative optical imaging of intracellular ROS levels and redox status in rat hippocampal neurons

Frank Funke[1], Florian J. Gerich[1], Michael Müller[1]

[1]DFG Research Center Molecular Physiology of the Brain (CMPB), Neuro- und Sinnesphysiologie, Humboldtallee 23, 37073 Göttingen, Germany

The cellular redox status is determined by various extra- and intracellular factors, and contributes to cytosolic signaling as well as oxidative stress. Especially mitochondria modulate the cytosolic redox balance by oxidizing NADH and $FADH_2$ and generating reactive oxygen species (ROS). Excessive ROS production may cause oxidative damage and contribute to neurodegenerative processes. Whereas cellular NADH and FAD levels can be monitored as autofluorescence, quantifying cellular ROS production is more demanding as the various redox-sensitive dyes share major disadvantages such as irreversible oxidation, autooxidation and photosensitivity. As an alternative, we therefore took advantage of a genetically engineered redox -sensitive green fluorescent protein (roGFP1) and carefully evaluated its response properties. The roGFP1 responds reversibly to oxidation/reduction and is ratiometric by excitation (395 nm/470 nm), thereby enabling semi-quantitative analyses of cytosolic ROS levels and redox status. So far, roGFP1 has mostly been used in various cell lines but only rarely in primary cultured neuronal preparations. Therefore, we now elucidated its response properties in rat hippocampal neurons, performing ratiometric CCD-camera imaging as well as 2-photon microscopy. We confirmed that cytosolically expressed roGFP1 readily and reversibly responds to hydrogen peroxide, superoxide as well as hydroxyl radicals. Calibration of its response range allows for the direct comparison of different preparations and various experimental conditions. We found that roGFP1 is only negligibly affected by changes in intracellular pH or $Cl^-$ content and well suited also for 2-photon excitation. Modulating the cellular scavenging systems also evokes roGFP1-responses, confirming its functional integration into the cytosolic redox balance. Furthermore, roGFP1 is sufficiently sensitive to detect changes in endogenous ROS production and to visualize the formation of perimitochondrial ROS microdomains. The ability to correlate dynamic changes in cellular ROS levels with mitochondrial metabolism and neuronal network activity is a promising step towards a detailed mechanistic understanding of redox- and ROS-mediated signaling in normal and diseased brain function.

# Enhancing neuronal cell proliferation using a 3D cell culture system.

Dennis Jgamadze[1], Steffen Vogler[1], Sophie Pautot[1]

[1]Center for Regenerative Therapies Dresden, Pautot AG, Tatzberg 47/49, 01307 Dresden, Germany

Over the years primary neuronal cell culture methods have undergone significant changes to recapitulate the features necessary for neuronal cell development and to obtain cells suitable for transplantation. In the absence of 3D stimuli and specific growth factors, traditional monolayer neuronal cell culture systems lead to post-mitotic neurons. Alternatively neuronal stem cells can be isolated and expanded. However, thus far, only a fraction of these cells differentiate into the desired neuronal cell types *in vitro* or *in vivo*. To improve cell replacement therapy it is necessary to develop a cell culture system which provides expandable, homogenous neuronal lineage committed cells.

Here we describe a cell culturing method that enables us to tune the *in vitro* culture environment to resemble the *in vivo* cell niche. We used colloid silica beads coated with Poly-L-Lysine to culture primary hippocampal cells. In low growth factor condition these cells showed enhanced survival and proliferation. They exhibit progenitor like characteristics. Once attached, neuronal cells continue to grow and by the third day in culture neurospheres sprout from the bead surface, and at the bead-bead contact interstices. Such a culture method prolongs the proliferation ability of a subpopulation of primary hippocampal cells. Moreover, these proliferating cells yield to neurons when replated on conventional 2D culture surfaces, as well as when they were transplanted into adult rat brain. Hence, 3D colloidal support provide a culturing environment capable of fostering cell survival and proliferation while preserving neuronal commitment highly desirable for cell replacement therapy.

# Expression of recombinant protein in honeybee brains by *in vivo* electroporation

Gerard Leboulle[1], Nora Gehne[1], Anja Froese[1], Randolf Menzel[1]

[1]Freie Universität Berlin, Neurobiologie, Königin-Luise-Str. 28/30, 14195 Berlin, Germany

The honeybee *Apis mellifera* is a valuable model system in neuroscience. It has a rich behavioural repertoire and high learning capabilities, yet its nervous system is relatively simple. For a long time, there was no alternative to pharmacological strategies to perform functional analysis. Recently, molecular techniques (e.g. antisense oligos and RNA interference) allowing specific inhibition of gene expression were developed. However, these techniques allow only the inhibition of gene expression. In addition, the effects are often of low amplitude and limited in time and in space. For this reason, it would be of great interest to develop tools that allow better control of gene expression. This could be achieved with *in vivo* electroporation, a technique that allows gene transfer *in vivo* into many cell types. This technique was already successfully used in the honeybee.

In this study, we evaluated if we could induce recombinant protein expression in the brain of adult honeybees by using this technique. We were able to induce the expression of enhanced green fluorescent protein (eGFP) in the brain. The expression of eGFP was demonstrated by Western blot and by immunohistochemistry. The expression was induced in almost every electroporated animal and lasted several days. Histological analysis showed that sparse clusters of cells were efficiently transfected in different regions of the brain. Several cell types were identified in the mushroom body, among them type I and type II Kenyon cells. Other cells, probably glial cells, were also efficiently transfected. However these could not be precisely identified.

In its present state, the technique can be used to evaluate if particular promoters are functional in honeybee neurons. This constitutes an important preliminary step towards the development of more sophisticated gene transfer techniques (e.g. viral expression system). In the next future, we want to express other recombinant proteins in the brain. We intend to induce the expression of proteins that diffuse more efficiently in neurons, like CD8-GFP. This will improve the characterisation of the transfected cells. We also think that the technique can be used to express calcium sensor genes to monitor *in vivo* neuronal activity.

# Closed-loop electrophysiological experiments and automated metadata acquisition with RELACS

Jan Benda[1], Jan Grewe[1]

[1]Department Biology II, Ludwig-Maximilian Universität München, Großhaderner Str. 2, 82152 Planegg-Martinsried, Germany

Relacs ("Relaxed ELectrophysiological data Acquisition, Control, and Stimulation", www. relacs.net) is a fully customizable software platform for data acquisition, online analysis, and stimulus generation specifically designed for electrophysiological recordings. Filters and spike detectors can be applied instantly on the recorded potentials. Freely programmable, hardware independent C++ plugins can access the preprocessed data for further online analysis and visualization. Therefore the experimental protocols can automatically adapt a stimulus (e.g. offset, variance, etc.) in a closed loop fashion and thus completely control the running experiment.

For offline data-analysis, data management, and data sharing the annotation of the raw data with metadata that specify the stimuli, the recorded neuron, the animal, as well as context of the experiment is necessary. Traditionally, such data have been written into lab books. File formats for metadata are often tailored to a specific database schema and thus assume a very detailed and specific structure and context.
Within a lab this specificity is usually not a problem as long as the same database schema is used. However, these file formats for metadata are very difficult to handle, when it comes to sharing data within a larger community, in particular via the upcoming initiatives of public databases. In addition, in most cases, such metadata files do not even exist.

We here want to stress that the recording software used for acquiring the raw data knows already many of the metadata. In particular, these are properties of the generated stimuli and settings of the controlled hardware. Therefore metadata in principle can and should be immediately acquired during the acquisition of the raw data in order to minimize manual efforts in providing this important data. As a closed-loop software RELACS has all this information available and saves it to disk.

We also developed a file format for hierarchically organized key-value pairs that is independent of any specific database-schema and thus can be used as a general file format for exchanging metadata. Through customized terminologies the metadata can be structured and standardized for ensuring interoperability while at the same time not restricting the content, thus ensuring immediate flexibility.
This way, metadata can be submitted to a local as well as public data-bases, like for example to the German neuroinformatics node at www.g-node.org, without any manual interference (see talk by Jan Grewe at symposium 10).

In our live demonstration we exemplify this approach by acquiring simulated data with RELACS and diretly importing the metadata to LabLog - the laboratory logbook, a datamanagement program for any neuroscientific lab (http://lablog.sourceforge.net/, see poster by Adrian Stoewer and Jan Grewe).

# The Laboratory Logbook
# A database-driven approach for project documentation

Adrian Stoewer[1], Jan Benda[2,3], Jan Grewe[2,3]

[1]German Neuroinformatics Node, Biozentrum Martinsried, Ludwig-Maximilians Universität München, Germany
[2]Department Biology II, Ludwig-Maximilians-Universität München, 82152 Martinsried, Germany
[3]Bernstein Center for Computational Neuroscience Munich, BCCN Munich, Germany

Documentation of scientific projects and storing metadata of recorded data is essential for data analysis and interpretation of the results. Ideally, all information is recorded and kept available.
Usually such information is stored in handwritten laboratory journals and/or protocol sheets which comes along with some drawbacks:

- Laboratory journals are (or are considered) personal property and often vanish from the lab once the respective author leaves the lab.

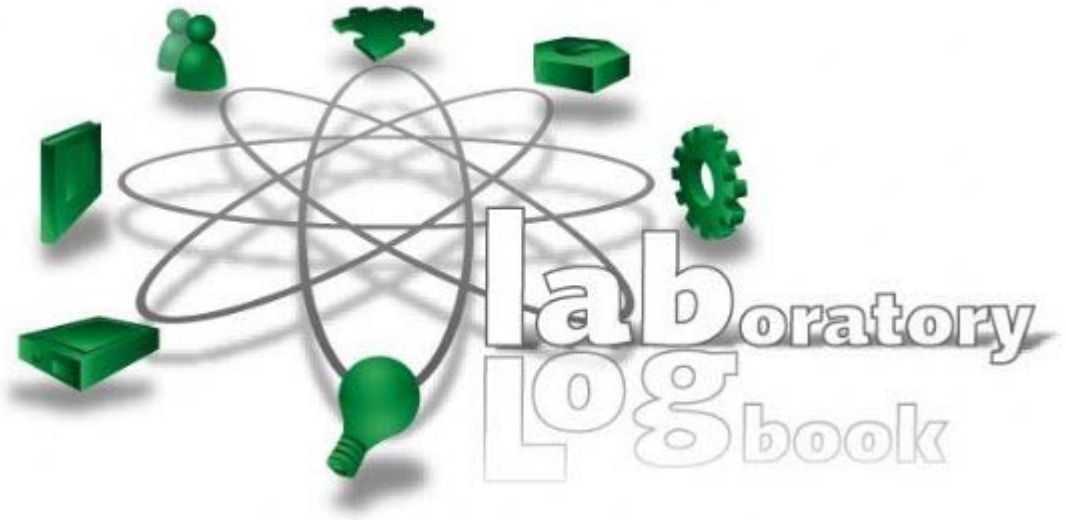
- Due to the fact, that every scientist has his or her own way to name and sort things, information is hard to share and compare among other scientists.

- It is extremely uncomfortable to search for a specific piece of information in a handwritten journal.

- Manual data annotation is time consuming and extraction of these tedious.

The Laboratory Logbook (LabLog) tries to complement the conventional laboratory journals. LabLog is basically a graphical front end to a relational database that stores project related information as well as metadata. On the one, hand it offers modules to accomplish tasks like (i) Management of persons and subgroups of the lab (ii) Description of projects, experiments, setups, stimuli and hardware (iii) Browsing and retreiving datasets and dataset related information. On the other hand, LabLog can be integrated into the laboratory workflow of data acquisition and analysis tools by (semi-)automatic import and export of metadata via odML (www.g-node.org/odml), a rather generic, easy to use, format for metadata exchange.

LabLog is primarily designed to store and manage metadata and not the actual data itself. However, it is possible to attach arbitrary files to projects, experiments or datasets and therefore associate data and metadata, if needed.
LabLog is open source (http://lablog.sourceforge.net) and written in Java. Thus, it runs on many operating systems and a wide variety of hardware.


laboratory
Log book

# Extension of protease based cellular sensors to analyze complex signal processes in the brain

Wilko Hinrichs[1], Moritz Rossner [2]

[1]MPI of experimental medicine, Neurogenetic, Hermann-Rein Str. 3, 37075 Göttingen, Germany

Inter- and intracellular signalling events are pivotal to the development of the nervous system. Here, cell-cell contacts and soluble ligands of cell surface receptors mediate cellular behaviors such as cell migration, differentiation and apoptosis. Prime examples for intricate cell-cell interaction and trophic dependence are the interactions between myelinating glia, oligodendrocytes and Schwann cells, with neurons of the central and peripheral nervous system.
Transmembrane receptor tyrosine kinases (RTKs) of the erbB family and their ligands of the neuregulin-1 family have been shown to be essential for mediating intercellular signals that regulate cell survival and myelination. ErbB receptors are expressed by glial cells, where they form upon neuregulin-1 binding signaling-competent homo- or heterodimers.
The hetero- or homodimerization of ErbB RTKs initiate autophosphorylations in trans, which triggers the recruitment of phospho-adapter proteins such as Grb2, SHC or PIK3a to further induce intracellular signaling cascades.
Thus far, we were able to assess RTK dimerization, or the phosphorylation-dependent recruitment of adaptor proteins to erbB receptors through the use of a protein complementation assay termed SPLIT-TEV (Wehr et al., 2006 and 2008). Split-TEV is based on the functional complementation of inactive fragments of the NIa protease TEV (from the tobacco etch virus) fused to interacting proteins. However, Split-TEV is limited to binary protein-protein interactions and it would therefore be desirable to expand the protease repertoire to be able to quantitatively measure the formation of ternary protein complexes simultaneously, such as ErbB dimerization and the recruitment of a phospho-adapter proteins.

Citations

Monitoring regulatet protein-protein interactions using split TEV
Wehr et al. Nat Methods. 2006 Dec;3(12):985-93. Epub 2006 Oct 29.

Analysis of transient phosphorylation-dependent protein-protein interactions in living mammalian cells using split-TEV.
Wehr et al. BMC Biotechnol. 2008 Jul 13;8:55.

# Fuzzy classification and inference of interneuronal types

Harold Gutch[1], Demian Battaglia[1,3], Anastassios Karagiannis[4], Thierry Gallopin[5], Bruno Cauli[4]

[1]Max Planck Institute for Dynamics and Self-Organization, Department of Nonlinear Dynamics, Bunsenstr. 10, 37073 Göttingen, Germany
[2]Technical University of Munich, Munich, Germany
[3]Bernstein Center for Computational Neuroscience, Goettingen, Germany
[4]University Pierre et Marie Curie, CNRS UMR 7102, Paris, France
[5]ESPCI, CNRS UMR 7637, Paris, France

Cortical interneurons display an impressive diversity of morphological, electrophysiological and molecular properties. Various authors have proposed classification schemes that rely on the concomitant observation of several multimodal features. However, despite attempts to establish a common nomenclature, an agreement on the definition of standard types is still lacking. In addition, a broad variability is observed even among cells grouped into a same class, suggesting a potential continuum of anatomical and physiological subtypes. As a further contribution to this classes-versus-continuum debate, we approach here the problem of unsupervised classification of interneurons introducing a novel protocol, grounded on a well-established fuzzy clustering algorithm. We propose to associate to each interneuron in a dataset a multi-component vector of memberships in distinct reference fuzzy classes (without defuzzification). Our method quantifies the relationship of a given cell with multiple template types simultaneously, thus supplying an improved categorization of atypical interneurons whose phenotypes do not fit properly into a rigid classification. Outlier or misclassified cells are detected by inspection of the memberships evolution in proximity of phase transitions of the algorithm, associated to switchings between classifications with a progressively smaller number of reference types. Furthermore, we design a Fuzzy Inference System, trained on a subset of our data. Human-readable inference rules, akin to a set of guidelines for decision-making, are extracted in an automated way and used to predict the expression of specific proteins from the measurement of electrophysiological parameters. Cells unlikely to express a target protein are filtered out with reduced false negatives scores, limiting eventual biases induced by our guided sampling procedure. Large fractions of poorly characterized interneurons expressing uncommon enzymes like the Nitric Oxide Synthase NOS1 can then be efficiently separated by our tool and made available for further targeted analyses.

# High-throughput, quantitative mass spectrometry of acutely stimulated synapses

Sidney B. Cambridge[1], Marcus Krüger[2], Matthias Mann[2]

[1]University of Heidelberg, Heidelberg, Germany [2]Max-Planck-Institute of Biochemistry, Munich-Martinsried, Germany

To discover the molecular changes that accompany the eletrophysiological changes after acute synaptic stimulation, we established a high throughput mass spectrometry method for quantitative comparison of synaptic proteomes. Using standard hippocampal slices of wild-type and metabolically labeled SILAC mice, the method was applied to characterize the effects of short term ethanol exposure at physiological concentrations. Of the 2084 identified pre- and postsynaptic proteins, 72 showed highly significant stimulus-dependent changes in synaptic abundance. Despite the moderate ethanol stimulus, several proteins decreased or increased about two-fold and more compared to controls including proteins known to be affected by ethanol. Overall, our results link short-term ethanol exposure to synaptic plasticity, mood, apoptosis, and (neurodegenerative) diseases. Thus, this method offers the unprecedented possibility to identify acute molecular changes of dynamic synaptic proteomes at a systemwide level.

# How to deal with the heterogeneity of neural responses: A demixing method

Wieland Brendel[1], Christian Machens[1]

[1]Group for Neural Theory, INSERM Unité 960, Département d'Etudes Cognitives, École Normale Supérieure, Rue d'Ulm 29, 75005 Paris, France

Neural responses in higher cortical areas often display a baffling complexity. In animals performing behavioral tasks, single neurons will typically encode several parameters simultaneously, such as stimuli, rewards, decisions, etc. We present a method that can be used to demix these heterogeneous neural responses.

Recordings from many different brain areas show two salient features. First, responses of single neurons in a given area are often quite diverse and heterogeneous, being influenced e.g. by the sensory stimuli and/or the subject's decision. Second, responses of single neurons across areas show a remarkable overlap in activity patterns. These two observations make it quite difficult to summarize or visualize the data, which in turn impedes our ability to interpret what is going on.

To make sense of these data, researchers typically seek to relate the firing rate of a neuron to one of various controlled task parameters, e.g. sensory stimulus, reward or decision of the subject. To this end, a number of statistical tools are exploited such as regression, signal detection theory or discriminant analysis. The population response is then characterized by quantifying how each neuron in the population responds to a particular task parameter. Subsequently, neurons can be attributed to different (possibly overlapping) response categories, and population responses can be constructed by averaging the time-varying firing rates within such a category.

This classical, single-cell based approach to electrophysiological population data has been quite successful in clarifying what information neurons in higher-order cortical areas represent. However, the approach rarely succeeds in giving a complete account of the recorded activity on the population level. Furthermore, the strongly distributed nature of the population response, in which individual neurons can be responsive to several task parameters at once, is often left in the shadows.

Principal component analysis (PCA) and other dimensionality reduction techniques seek to alleviate these problems by providing methods that summarize neural activity at the population level. However, such "unsupervised" techniques will usually neglect information about the relevant task variables. While the methods do provide a succinct and complete description of the population response, the description may yield only limited insights into how different task parameters are represented in the population of neurons.

Therefore, we propose an exploratory data analysis method that seeks to maintain the major benefits of PCA while also extracting the relevant task variables from the data. Our basic insight is that variance in neural firing rates can have different origins (such as changes in a stimulus, a reward, or the passage of time), and that, instead of lumping them together, as PCA does, we need to treat these sources separately. We present a method that seeks an orthogonal coordinate transformation such that the variance captured from different sources falls into orthogonal subspaces and is maximized within these subspaces. Using simulated examples, we show how this approach can be used to demix heterogeneous neural responses. Our method may help to lift the fog of response heterogeneity in higher cortical areas.

# In vivo SPECT-imaging of spatial patterns of neuronal activity in rodent brain using 99mTcHMPAO and 201TlDDC as tracers

Jenni Neubert[1], Angela Kolodziej[1, 2], Marc Zappe[1], Julia Georgi[1], Eike Budinger[1, 2], Anton Ilango[1, 2], Marie Woldeit[1, 2, 3], Frank Angenstein[1], Frank W. Ohl[1, 2], Henning Scheich[1, 2], Jürgen Goldschmidt[1, 2]

[1]Leibniz Institute for Neurobiology, Brenneckestr.6, 39118 Magdeburg, Germany
[2]Otto-von-Guericke University Magdeburg, 39106 Magdeburg, Germany
[3]Institute of Biology, Otto-von-Guericke University Magdeburg, Leipziger Str. 44, 39120 Magdeburg Germany

During the past years significant progress has been made in small animal single-photon emission computed tomography (SPECT). Dedicated small-animal SPECT scanners have been developed and are now commercially available. These scanners provide spatial resolutions in the submillimeter range up to ca. 500 micrometers.
We here explored the use of SPECT for in vivo imaging of spatial patterns of neuronal activity in the rodent brain. We present results from studies in Mongolian gerbils and in mice using two tracers, 99m-Technetium-hexamethylpropyleneamine oxime (99mTcHMPAO) and 201-Thallium-Diethyldithiocarbamate (201TlDDC).
99mTcHMPAO is a well-established tracer for imaging regional cerebral blood flow. 201TlDDC can be used for imaging cerebral potassium metabolism. Both tracers accumulate in the brain in an activity-dependent manner since blood flow and potassium uptake are tightly coupled to neuronal activity.
Both tracers are lipophilic complexes that are readily cleared from the plasma. Animals have to be injected with the tracers during – and not before – they are stimulated or exposed to a certain experimental setting. We therefore chronically implanted the animals with jugular vein catheters. The tracers were intravenously injected over time spans of five minutes during ongoing behavior. We studied the spatial patterns of neuronal activity during intracranial self-stimulation and during auditory learning in the shuttle-box.
We show that the spatial resolution of small-animal SPECT is sufficient to reveal activations of small brain regions such as the accumbens nucleus upon medial forebrain bundle stimulation in mice. Small-animal SPECT also reveals complex spatial patterns of neuronal activity in trained animals solving tasks. The technique is a promising new approach for in vivo imaging of spatial patterns of neuronal activity in unrestrained behaving animals.

# Light-inducible protein synthesis inhibition

Kathrin Marter[1], Janina Schaal[2], Jenny Eichhorst[2], Julien Colomb[1], Burkhard Wiesner[2], Volker Hagen[2], Dorothea Eisenhardt[1]

[1]Freie Universität Berlin, Neurobiologie, Königin-Luise-Str. 28/30, 14195 Berlin, Germany
[2]Leibniz-Institut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin, Germany

Photolabile protecting groups can be bound to functional groups of biomolecules to block their chemical and biological activity. The inactivated biomolecule ("caged compound") can be released by irradiation with short wavelength light, removing the protecting groups and restoring the molecule's bioactivity. This enables temporal precision and accurate delivery to a specific tissue of interest (spatial control) and minimizes side effects in other surrounding tissues.
We characterized new caged compounds that are important to study translation, namely two inhibitors of protein synthesis (PSI), emetine and anisomycin. Both are frequently used in neuroscience to interfere with protein synthesis-dependent processes like long-term synaptic plasticity and memory consolidation.
The biological activity of the PSIs was masked with different protecting groups. The resulting caged compounds were photochemically characterized and their uncaging kinetic was analyzed in detail in an in vitro translation system. All caged compounds have high long-wavelength absorptions, good photorelease rates, are resistant to spontaneous hydrolysis in the dark and perform excellent in the in vitro translation assay. Our caged anisomycin has, compared to previous reports (Goard et al., Chem. & Biol. 2005 and Sadovski et al., Bioorg. Med. Chem. 2010), a higher solubility in aqueous buffers and can be used in lower concentrations.
Additionally, we tested our caged compounds applicability in transfected HELA cells and in vivo in Drosophila transgenic flies expressing GFP after being heat-shocked.
We conclude that our new caged PSIs are efficient and reliable tools to probe the role of translation in a variety of neuroscientific applications and biological systems.

# MAGNETIC RESONANCE IMAGING OF THE RHESUS MONKEY BRAIN

Roland Tammer[1], Sabine Hofer[1,3], Klaus-Dietmar Merboldt[1], Jens Frahm[1]

[1]Max-Planck-Gesellschaft, Biomedizinische NMR Forschungs GmbH, Am Fassberg 11, 37070 Göttingen, Germany, [2]DFG Center of Molecular Physiology of the Brain (CMPB), 37073 Göttingen, [3]Bernstein Center for Computational Neuroscience, 37073 Göttingen

In the past two decades there have been tremendous advances in noninvasive imaging methods. In particular, magnetic resonance imaging (MRI) has developed into the most important modality for diagnostic imaging with about 100 million examinations per year. Practical applications are not limited to the clinic but extend to animal research with a special emphasis on the neurosciences. The ability to provide detailed insights into the intact living brain (from a mouse to a human) underlines the specific role of MRI in the growing field of translational research. With respect to animal experimentation, MRI has shown to be particularly attractive as it reduces the number of animals in follow -up studies that monitor developmental changes, aging, disease progression, or therapeutic efficacy.

For example, studies dealt with psychosocial stress in tree shrews (Tupaia belangeri), models of multiple sclerosis in common marmosets (Callithrix jacchus), and structural assessments of individual anatomy in squirrel monkeys (Saimiri sciureus) and rhesus monkeys (Macaca mulatta). Nonhuman primates are indispensable because of their immunological, physiological, and behavioral similarities to humans.

During the course of these studies it became clear that there is a lack of adequate anatomical MRI data of the rhesus monkey brain which was a strong motivating factor for the preparation of this atlas. MRI allows to visualize anatomical structures at high spatial resolution in all three dimensions. The technique yields excellent soft-tissue contrasts even without the application of an exogenous contrast agent and is characterized by a marked sensitivity to pathological tissue alterations. This MRI atlas is expected to serve the community of neuroscientists and primatologists as a reference source for easy identification of anatomical structures in the rhesus monkey brain. All cross-sectional images are presented in a stereotaxic coordinate system that is defined in accordance with internal brain structures rather than outer landmarks of the head or skull.

# Microfluidics and insect cell culture

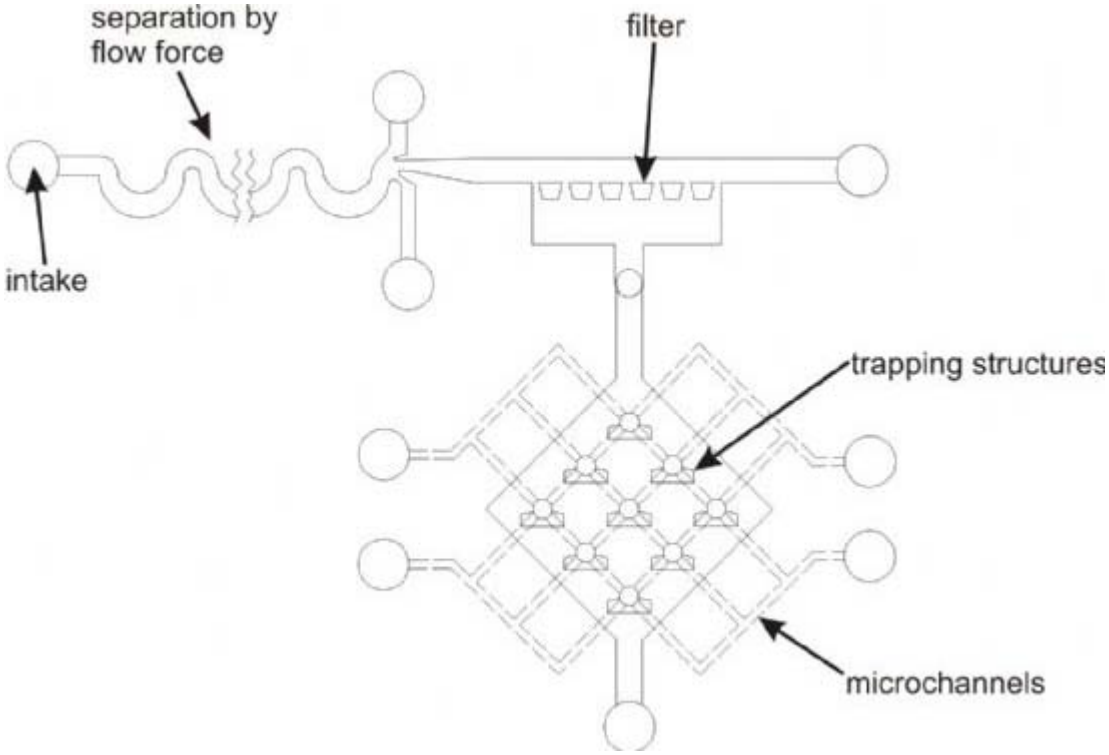
Katrin Göbbels[1], Anja Lena Thiebes[1], Andreas Buchenauer[2], Akram El Hasni[2], Uwe Schnakenberg[2], Peter Bräunig[1]

[1]RWTH Aachen University, Institute of Biology II, Lukasstr. 1, 52074 Aachen, Germany
[2]RWTH Aachen University, Institute of Materials in Electrical Engineering (IWE 1), Sommerfeldstr. 24, 52074 Aachen, Germany

The investigation of neuronal networks *in vitro* provides insight into principles of their development, structure and function such as neuritic outgrowth, development of synapses, or synaptic plasticity in a much less complex situation than *in vivo*. Multielectrode arrays (MEAs) offer the ability to investigate the activity of such neuronal networks non-invasively over an extended time period. A detailed understanding of neuronal networks necessitates the analyses of the activity of individual neurons in low-density networks as well as their synaptic connections. In this context, the major difficulty is a directed positioning of the neurons on electrode surfaces. Usually the positioning is either a random or a manually-guided mechanical process.

A new approach to overcome the problem of positioning is the development of neuronal networks in closed-channel microfluidic devices. Our approach of combining microfluidics and insect cells in culture as biological test system is aimed at solving the challenge of cell separation as well as directed positioning of neurons. Furthermore, the combination of microfluidic devices with MEAs in the future could lead to an automated setup usable, for example, in basic research or as screening tool.

Our microfluidic device is separated in two components, a hydrodynamic filter for separation of neurons up to a certain diameter and a trapping structure for storage of neurons (Fig. 1). The trapping structure offers the possibility to retain neurons on certain positions, e.g. certain electrodes on a MEA. Microchannels in a second layer of the microfluidic device allow for directed guiding of neurites to help them to establish synaptic contacts with adjacent neurons.



# NEST: An efficient simulator for spiking neural network models

Jochen Martin Eppler[1], Susanne Kunkel[2], Hans Ekkehard Plesser[3], Marc-Oliver Gewaltig[4], Abigail Morrison[2], Markus Diesmann[1,5]

[1]Research Center Jülich GmbH, Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Leo-Brandt-Straße, 52428 Jülich, Germany

[2]Albert-Ludwig University of Freiburg, Faculty of Biology, Functional Neural Circuits, Hansastr. 9a, 79104 Freiburg, Germany

[3]Norwegian University of Life Sciences, Deptartment of Mathematical Sciences and Technology, PO Box 5003, 1432 Aas, Norway

[4]Honda Research Institute Europe GmbH, Carl-Legien-Str. 30, 63073 Offenbach, Germany

[5]RIKEN CSRP, Brain and Neural Systems Team, 2-1 Hirosawa, Wako City, Saitama 351-0198, Japan

NEST is a simulator for networks of point neurons or neurons with a few electrical compartments [1]. It is suited for a broad range of spiking neural network models and runs on standard desktop computers, computer clusters, or HPC facilities such as the IBM BlueGene. Distributed simulations show excellent scaling up to the order of one thousand processors, and current research extends the scalability into the range of ten thousand processors and beyond [2,3]. Recent additions to NEST include the incorporation of new neuron models such as the MAT(2) model [4] and spike-timing and neuromodulation dependent plasticity [5,6]. To increase its usability and follow software trends in the neuroscience community, NEST provides a convenient user interface based on the Python programming language [7]. NEST also supports the MUSIC interface to communicate with other simulators [8] and provides visualization tools [9] and a topology module that allows the specification of spatially structured networks [10]. The developers continually improve the algorithms in NEST, e.g. for the calculation of 'off-grid' spike times and the integration of non-linear neuron models such as the AdEx model [11,12,13]. Release stability is guaranteed by an automated test suite [14].

NEST can be extended by the user through dynamically linked modules that contain new neuron and synapse models, stimulus and recording devices, or functionality for the analysis of the resulting data.

NEST is developed by the NEST Initiative, an international collaboration between academic and industrial research institutes. The NEST Initiative provides regular public releases of NEST to give users access to the newest technology. The releases together with documentation on the usage of NEST, and a list of neuroscientific publications that use NEST are available on the homepage of the NEST Initiative at www.nest-initative.org.

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# Neurophysiology data management for efficient analysis and collaborative work

Andrey Sobolev[1], Philipp Rautenberg[1], Christian Kellner[1], Jan Benda[1], Jan Grewe[1], Martin P. Nawrot[2], Willi Schiegel[3], Tiziano Zito[4], Andreas V. Herz[1], Thomas Wachtler[1]

[1]Ludwig-Maximilians-Universität München, Department Biology II, Großhaderner Straße 2, Planegg-Martinsried, Germany
[2]Freie Universität Berlin, Berlin, Germany
[3]Humboldt-Universität zu Berlin and technologit, Berlin, Germany
[4]Technische Universität Berlin, Berlin, Germany

Scientific progress depends increasingly on collaborative efforts that involve exchange of data and re-analysis of previously recorded data. A major obstacle to fully exploit the scientific potential of experimental data is the effort it takes to access both data and metadata for application of specific analysis methods, for exchange with collaborators, or for further analysis some time after the initial study was completed. To cope with these challenges and to make data analysis, re-analysis, and data sharing efficient, data together with metadata should be managed and accessed in a unified and reproducible way, so that the researcher can concentrate on the scientific questions rather than on problems of data management. At the German Neuroinformatics Node (www.g -node.org), an infrastructure for cellular and systems neuroscience is being developed to improve key ingredients of neuroscientific research: data access, data storage and exchange, and data analysis. Central component is a data management platform where neuroscientists can store and organize their data for sharing and analysis (portal.g-node.org/data). To facilitate collecting of metadata in machine readable form, a flexible XML-based format is proposed, together with recommended terminologies for neurophysiology (www.g-node.org/odml). Furthermore, tools and interfaces for data access through a variety of applications are being developed. This approach will enable researchers to seamlessly integrate data access into the laboratory workflow and efficiently perform management and selection of data in a systematic and largely automatized fashion for data sharing and analysis.

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# New possibilities for advanced analysis methods in neuroscience through modern approaches to trivial parallel data processing

Abigail Morrison[1], Michael Denker[3], Bernd Wiebelt[2], Denny Fliegner[4], Markus Diesmann[3,5,2]

[1]Functional Neural Circuits Group, Faculty of Biology, Albert-Ludwigs University, Freiburg, Germany
[2]Bernstein Center Freiburg, Albert-Ludwigs University, Freiburg, Germany
[3]RIKEN Brain Science Institute, Wako-shi, Japan
[4]Max-Planck Institute for Dynamics and Self-Organization, Göttingen, Germany
[5]Institute of Neuroscience and Medicine, Computational and Systems Neuroscience (INM-6), Research Center Jülich, Germany

In addition to the increasing amounts of data gushing out from neuroscientific experiments, the complexity of modern data analysis techniques places new demands on the computing infrastructure required for data processing. In particular, the observation that neuronal data typically exhibit non-stationary statistics complicates the task of finding the correct null-hypothesis to assess the significance of a variety of test parameters. Modern computer resources enable a data-based approach to tackle significance estimation: surrogate techniques. In this framework the original data is modified in a specific way so as to keep some aspects of the data (e.g., the non-stationary nature of the data), while deliberately destroying others (i.e., those described by the test parameter). Repeating this procedure many times estimates the distribution of the test parameter under the null hypothesis.

However, the required resources exceed the speed and memory constraints of a classical serial program design and require scientists to parallelize their analysis processes on distributed computer systems. Here, we explore step-by-step how to transform on-the-fly a typical data analysis program into a parallelized application. This approach is facilitated by the observation that a typical task in neuronal data analysis constitutes an embarrassingly parallel problem: the analysis can be divided up into independent parts that can be computed in parallel without communication. In particular for surrogate-based analysis programs, finding the decomposition of the analysis program into independent components is often trivial due to the inherent repetition of analysis steps. On the conceptual level, we demonstrate how in general to identify those parts of a serial program best suited for parallel execution. On the level of the practical implementation, we introduce four methods that assist in managing and distributing the parallelized code. By combining readily available high-level scientific programming languages and techniques for job control with metaprogramming no knowledge of system-level parallelization and the hardware architecture is required. We describe the solutions in a general fashion to facilitate the transfer of insights to the specific software and operating system environment of a particular laboratory.

The details of our technique accompanied by concrete examples form a chapter of the new book “Analysis of parallel spike trains” edited by Sonja Grün and Stefan Rotter and published at Springer 2010.

# Novel approach for remote long-term recordings of sleep-wake rhythms, core body temperature and activity in single- and group-housed rats

Kerstin Plaßmann[1], Eberhard Fuchs[1]

[1]German Primate Center, Clinical Neurobiology Laboratory, Kellnerweg 4, 37077 Göttingen, Germany

Sleep is an extremely dynamic and complex process and, despite intense research, its function is still not fully understood. Physiologic sleep is characterized by the occurrence of two distinct sleep stages – NREM (non rapid eye movement) and REM (rapid eye movement) – that alternate in ultradian rhythms. The regulation of the sleep-wake-cycle includes homeostatic and circadian processes. This cycle is, however, very vulnerable to a number of internal and external factors and sleep disturbances are among the most reported health problems. Sleep disorders also typically occur in a variety of mental and neurological disorders, such as depression, anxiety disorders and dementia. The commonly used animal model in sleep research is the laboratory rat. In order to categorize different sleep stages, cable-restricted, polysomnographic recording systems are widely used. These systems require conditions of individual housing and often restrict the animals in their ability to move. In contrast to this, telemetric systems enable the recording of freely moving animals. However, these systems are restricted by a low number of recording channels or a limited operating time of the included batteries.

To overcome these limitations, we tested two recently developed recording devices in order to establish a system for long-term recordings of sleep-wake rhythms and the closely related rhythms of core body temperature and motor activity in freely moving single-and group-housed rats.

For registration of core body temperature we used the implantable Remo 200 transmitter (Remo Technologies, Salisbury, UK). This system is suitable for long term use and allows for simultaneous monitoring of a group of subjects in their home cage. For wireless EEG and EMG registrations and actimetry we adapted the NeuroLogger® (NewBehavior, Zürich, Switzerland). A stepwise optimization of NeuroLogger® connection on the animals´ heads was performed, as well as the development of protection casing. Implantation of the systems was followed by long -term recordings during which the animals were single-housed. After that, animals were group-housed and telemetric recordings were continued for another 2-3 weeks. Additional NeuroLogger® recordings were performed on 3 rats after 16 weeks of group housing.

Parameters measured were compared between different time points and housing conditions. Results show that modifications of the NeuroLogger® system allowed for the use of this system in single- and group-housed rats. Parallel recordings of EEG, EMG and activity enable the separation of sleep phases to wake, NREM and REM. The Remo 200 transmitter enabled parallel recordings from multiple animals in social context. Recordings of activity patterns were possible to perform with the accelerometer integrated in the NeuroLogger® system. Comparison of recording periods showed no evidence of an impairment of the animals and their physiological parameters by an experimental manipulation to replace the NeuroLoggers®. In group -housed animals, a consolidation of sleep parameters was observed which points to improved environmental conditions.

In conclusion, the combination of two innovative technical approaches allows measuring of circadian rhythms of sleep-wake cycle, core body temperature and activity in multiple subjects simultaneously, improving the quality of results with significant improvements in animal welfare.

# Objective-coupled planar illumination microscopy – A novel technique for neuronal population imaging

Oliver Braganza[1], Rolf Beck[2], Rainer Meyer[2], Heinz Beck[1]

[1]Department of Epileptology, University of Bonn Medical Center, D-53105 Bonn, Germany
[2]Institute of Physiology II, University of Bonn Medical Center, D-53105 Bonn, Germany

To truly arrive at an understanding of normal and pathological behavior of complex neuronal networks, it would be desirable to achieve sampling of the activity of a large number of anatomically identified cellular elements in a network at high temporal resolution. Current electrophysiological approaches are limited in either cell number or availability of exact anatomical information. Rapid functional multineuron $Ca^{2+}$ imaging in combination with advanced imaging approaches, however, offer an alternative. Chemical or genetically encoded fluorescent $Ca^{2+}$-indicators are utilized to monitor action potential generation in several hundreds of cells simultaneously. However, laser scanning approaches, such as two- photon or confocal microscopy are subject to a fundamental tradeoff between the acquired field size, signal-to-noise ratio, photo damage and temporal resolution. Further, widespread use in the field is limited by their high cost.
A novel imaging technique, objective-coupled planar illumination microscopy (OCPI), first described by Holekamp et al., 2008, ingeniously circumvents parts of this tradeoff and potentially offers an affordable alternative. In it, the tissue sample is illuminated by a few micron thick planar light-sheet which is mechanically coupled to the objective and aligned with its focal plane, thereby achieving low photobleaching and a high signal-to-noise ratio. As a consequence, hundreds of neurons within an individual frame can be acquired with a single exposure. In combination with a rapid, low light, charge coupled device (CCD) camera, pixel rates at least 100-fold higher than in laser scanning microscopy can be achieved (Holekamp et al., 2008).
We describe here the construction and characterization of an OCPI-microscope prototype highlighting technical challenges and potential applications.

*Terrence F Holekamp, Diwakar Turaga, and Timothy E Holy. Fast three-dimensional fluorescence imaging of activity in neural populations by objective-coupled planar illumination microscopy. Neuron, 57(5):661–672, Mar 2008.*

# Organotypic brain slice co-cultures of the dopaminergic system- a versatile tool for the investigation of toxicological properties of novel substances

Katja Sygnecka[1], Claudia Heine[1,2], Marcus Grohmann[2], Nico Scherf[1], Heike Franke[2]

[1]Translational Centre for Regenerative Medicine Leipzig, University of Leipzig,
[2]Rudolf Boehm Institute of Pharmacology and Toxicology, University of Leipzig

Organotypic brain slice cultures are a useful tool to test novel substances *ex vivo*. Here we present an organotypic co-culture model which allows us to reconstruct the dopaminergic projection system and to investigate the toxicological properties of compounds under *ex vivo* conditions. Therefore brain slices of the ventral tegmental area/substantia nigra (VTA/SN) and the prefrontal cortex (PFC) or the striatum (STR) were used.
This co-culture model has already been characterised immunohistochemically in our lab. The quantification and analysis of fibre outgrowth into the target region of the projection system has been well established. The aim of the present study is to supplement the model with qualitative and quantitative toxicological methods. Moreover the effect of the applied substances on the expression/modification of certain proteins (neuronal and glial markers, members of signalling pathways) should be investigated.
For the determination of toxicity after substance application in comparison to untreated control co-cultures the following methods were established: (1) Lactate dehydrogenase activity measurement in the culture medium, (2) Propidium iodide staining followed by image processing and subsequent densitometric quantification, (3) Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)-assay allowing the visualisation of apoptotic cell nuclei, (4) Active caspase 3 staining, (5) Celestine Blue for the labelling of damaged cells and (6) Hoechst staining to show nuclear shrinkage in apoptotic cells. As reference substances glutamate (excitotoxic concentrations) and the well known apoptosis inducing compound staurosporine were chosen.
The data presented on the poster show the feasibility of the above mentioned techniques to investigate the properties of unknown compounds in comparison to well known controls under *ex vivo* conditions.

# Protein Macroarray: A new approach to indentify NCAM binding partners

Hilke Wobst[1], Agathe Sekulla[1], Christine Laurini[1], Brigitte Schmitz[1], Simone Diestel[1]

1 Institute of Animal Sciences, Department of Biochemistry, University of Bonn, Katzenburgweg 9a, 53115 Bonn, Germany

The neural cell adhesion molecule (NCAM) has been implicated in neural development and in the adult brain. NCAM interacts with several proteins which initiates intracellular signal transduction pathways ultimately causing cellular proliferation, differentiation, migration and survival. To identify further potential intracellular interaction partners the cytosolic domains of human NCAM (hNCAM180 -CT or hNCAM140 -CT, respectively) were expressed in bacteria. The purified domains were applied onto a protein macroarray containing 38016 protein-expression-clones of human fetal brain. Using this approach we identified several novel potential cytosolic interaction partners of NCAM. The binding of interesting partners will be verified by other approaches. The results may give an advanced understanding of the cellular mechanisms of NCAM action during neural development and in the adult brain.

# Reconstructing the *in vivo* brain: a CT/MRT aided stereotaxic atlas of the Mongolian gerbil brain (*Meriones unguiculatus*)

Susanne Radtke-Schuller[1], Frank Angenstein[2], Jürgen Goldschmidt[4], Oliver S. Grosser[3], Gerd Schuller[1], Eike Budinger[4]

[1]LMU Munich, Department Biology II , Division of Neurobiology, Grosshadernerstrasse 2, 82152 Planegg-Martinsried, Germany
[2]Leibniz Institute for Neurobiology Magdeburg, Special Lab Non-Invasive Brain Imaging; Magdeburg, Germany.
[3]University Hospital Magdeburg, Clinic for Radiology and Nuclear Medicine, Magdeburg, Germany.
[4]Leibniz Institute for Neurobiology Magdeburg, Department of Auditory Learning and Speech; Magdeburg, Germany.

The Mongolian gerbil (*Meriones unguiculatus*) is an important animal model in neurosciences, especially for the study of the auditory and other sensory systems, their anatomy, physiology, development and plasticity as well as for the investigation of related behavioral mechanisms, learning and memory. A large body of literature is related to these topics, however, the only published brain atlas for the gerbil (Loskota et al., 1974) is of limited use for stereotaxic procedures.
Thus, the need for a common brain anatomy data base that could be used as a reference frame for the identification and localization of neuroanatomical structures in a standardized way, is evident.
Traditional stereotaxic atlas techniques are based on brain sections which are inevitably subject to distortions during histological procedures (fixation, embedding, sectioning, staining and section mounting). In order to improve the accuracy of the new stereotaxic system, the skull-related *in vivo* brain position of the gerbil was recorded with computerized tomography (CT imaging) for the skull and magnetic resonance imaging (MRI) for the brain.
The atlas coordinate system was chosen in a way that an easily reproducible embedding protocol of the brain allows the sectioning of experimental brains in the standard frontal plane of the atlas. Key structural landmarks of the skull like lambda, bregma, ear canals, occipital crest or skull contours have well defined coordinates within the atlas reference system and thus can be used to line up the skull and the brain in standard coordinates in acute experiments.
A series of frontal sections was cut according to the standardized embedding procedure (using an adjustable perspex chamber). The sections were alternately stained for cell bodies and fibers and compared to the MRI sections relying on recognizable anatomical structures. This procedure yielded an atlas series in close correspondence to the MRI sectioning planes and to the skull reference marks. Distortions due to the histological processing were equalized by morphing the sections, so that the congruency of anatomical structures was optimal between sections and MRI.
The identification of anatomical structures was further backed up by the use of additional histochemical and immunohistochemical stains like cytochromoxidase, acetylcholine esterase, calcium-binding proteins, and the comparison with other species.

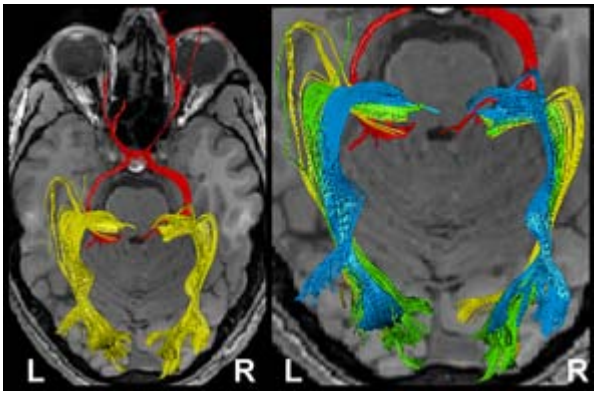
# Reconstruction and dissection of the entire human visual pathway using diffusion tensor MRI

Sabine Hofer[1], Alexander Karaus[1], Jens Frahm[1]

[1]Max-Planck-Institut fuer biophysikalische Chemie, Biomedizinische NMR Forschungs GmbH, Am Fassberg 11, 37070 Goettingen, Germany[1]

In humans the visual pathway extends from the eyes to the calcarine fissure and thereby passes through the entire brain in a roughly transverse orientation. The visual system represent a serious challenge for diffusion tensor imaging (DTI) and fiber tractography: while tracking of frontal fiber bundles may be compromised by the nearby presence of air-filled cavities, nerves, and eye muscles, the anatomic courses of the three main fiber bundles of the optic radiation are subject to pronounced inter-subject variability. Here, tractography of the entire visual pathway was achieved in 6 healthy subjects at high spatial accuracy, that is at 1.8 mm isotropic spatial resolution, without susceptibility-induced distortions, and in direct correspondence to anatomic MRI structures. Using a newly developed diffusion -weighted single -shot STEAM MRI sequence, we were able to track the thin optic nerve comprising the nasal optic nerve fibers, which cross the optic chiasm, and to dissect the optic radiation into the anterior ventral bundle (Meyerâ€™s loop), the central bundle, and the dorsal bundle. Apart from scientific applications these results in single subjects promise advances in the planning of neurosurgical procedures to avoid unnecessary damage to the visual fiber system.

(Left) Bi-hemispheric reconstructions of the optic nerve and tract (red) as well as of the optic radiation (yellow). (Right) Dissection of the optic radiation into Meyer´s loop (yellow), central bundle (green), and dorsal bundle (blue). R = right, L = left.



# Recurrence-Based Estimation of Time-Distortion Functions for ERP Waveform Reconstruction

Matthias Ihrke[1], Hecke Schrobsdorff[1,2], J. Michael Herrmann[2,3]

[1]Max-Planck-Institute for Dynamics and Self-Organization, Bunsenstrasse 10, 37073 Göttingen, Germany
[2]Bernstein Center for Computational Neuroscience Göttingen
[3]Institute for Perception, Action and Behaviour, University of Edinburgh, Informatics Forum, 10 Crichton Street, Edinburgh, EH8 9AB, U.K.

We introduce an approach to compensate for temporal distortions of repeated measurements in event-related potential research. The algorithm uses a combination of methods from nonlinear time-series analysis and is based on the construction of pairwise registration functions from cross-recurrence plots of the phase-space representations of ERP signals. The globally optimal multiple-alignment path is approximated by hierarchical cluster analysis, i.e. by iteratively combining pairs of trials according to similarity. By the inclusion of context information in form of externally acquired time markers (e.g. reaction time) into a regularization scheme, the extracted warping functions can be guided near paths that are implied by the experimental procedure. All parameters occurring in the algorithm can be optimized based on the properties of the data and there is a broad regime of parameter configurations where the algorithm produces good results. Simulations on artificial data and the analysis of ERPs from a psychophysical study demonstrate the robustness and applicability of the algorithm.

# The performance of an automatic algorithm in isolating single units in primate cortex

Shubo Chakrabarti[1], Paul Hebert[2], Michael Wolf[3], Michael Campos[4], Alexander Gail[1,5], Joel Burdick[2]

[1]Deutsches Primatenzentrum , Sensorimotor group, Kellnerweg 4, 37077 Goettingen, Germany[2]Mechanical Engin., Caltech, Pasadena, CA[3]Advanced Robotics Controls, Jet Propulsion Lab., Pasadena, CA[4]Dept. of Neurosurg., Massachusetts Gen. Hospital, Harvard Univ., Boston, MA[5]Bernstein Ctr. for Computat. Neuroscience

The need for reliable and stable electrophysiological recordings is a common problem in neurophysiology. In acute recordings considerable effort is required by the experimenter to continually analyze signal quality and manually adjust microelectrode positions for optimum results. Furthermore, it becomes increasingly difficult to control recordings from a multi-channel device. To address these problems, we present an improved version of our autonomous electrode control algorithm, SpikeTrack 2, which allows for minimal user interaction and is equipped to handle a variety of commercial multi-channel drives. Innovations include the incorporation of recent advances in spike sorting and an improved neuron tracker. SpikeTrack operates on a closed loop control cycle. In short intervals action potentials are autonomously detected via a wavelet-based method. These are then analyzed to determine the appropriate electrode movement to keep a neuron isolated. The unsupervised spike sorting method, based on an extension of the traditional mixture model EM optimization, incorporates clustering results from preceding intervals in a Bayesian manner. In addition to the tracking provided by linking current and prior neurons from the clustering method, a multiple hypothesis tracker for clusters (MHTC) is employed to provide a delayed-decision framework for robust multi-target tracking (MTT) of neurons. Both the improved clustering and tracking provide SpikeTrack 2 the ability to accurately build up a history of the signal and isolation quality of the tracked neurons. A supervisory finite state machine (SFSM) is utilized to transition between states of the algorithm and to predict the next optimal movement of the electrode in order to isolate a neuron and optimize its signal.
We compared SpikeTrack 2 with the performance of human experts during multichannel recordings from the dorsal premotor cortex of awake behaving rhesus monkeys. In each session not more than 3-5 electrodes were used simultaneously. Electrodes were positioned such that high levels of neural activity were encountered. After allowing the electrodes to settle, either SpikeTrack 2 was turned on, or neurons were tracked manually. The final test and control sets consisted of 64 functional electrode penetrations and a total of approximately 30 hours of recording each. Following equivalent off-line cluster sorting in both sets, we obtained a total of 37 well defined single units for the manually recorded files and 35 for those in which SpikeTrack 2 was used. The average time the neurons were kept isolated by SpikeTrack 2 was found to be at least as long as by the human experts.

# The use of the K+-probe thallium (Tl+) for imaging CNS potassium metabolism and neuronal activity - from microscopy to in vivo SPECT-imaging

Jürgen Goldschmidt[1], Tim Wanger[1], Jenni Neubert[1], Marc Zappe[1], Ulrich H. Schröder[1], Frank Angenstein[1], Klaus G. Reymann[1], Henning Scheich[1]

[1]Leibniz-Institute for Neurobiology, Brenneckestr. 6, 39118 Magdeburg

The monovalent thallium ion Tl+ is a well-established potassium (K+) probe. The gamma-emitting isotope 201-Tl has been used for several decades for single-photon emission computed tomography (SPECT) imaging of alterations in K+-metabolism in tumors and myocardial infarction in humans.
Tl+ or 201-Tl+ respectively have been of little use thus far for studying CNS K+-metabolism. Tl+-uptake in the CNS after systemic applications of Tl+-salts is very low because of the poor blood-brain barrier (BBB) K+-permeability.
We have recently shown that the BBB-related limitations in the use of Tl+ are no longer present when animals are intravenously injected with the lipophilic chelate complex thallium diethyldithiocarbamate (TlDDC). TlDDC crosses the BBB. Importantly, Tl+ is released from TlDDC after crossing the BBB but prior to neuronal or glial uptake. After intravenous TlDDC injections neurons and glial cells take up Tl+ and not TlDDC. Tl+ -uptake in neurons increases with increasing activity. TlDDC can thus be used for imaging cerebral K+-metabolism and neuronal activity.
Tl+ can be detected using a variety of different methods. Histochemically the ion can be detected at the light and electron microscopical level using a modified Timm-technique or autometallographic method for the detection of heavy metals. In vivo the distribution of the isotope 201-Tl in the brain can be imaged using SPECT. SPECT can also be used for ex-vivo imaging of the Tl+-distribution in excised brains.
We here demonstrate, focusing on cerebral ischemia, how TlDDC can be used to study CNS potassium metabolism from microscopical levels to in vivo SPECT-imaging.

# "Virtual Pre-embedding Labeling": A novel approach for correlative light and electron microscopy and double labeling in affinity cytochemistry

Vince Istvan Madai[1], Rene Bernard[2], Wolfram Poller[2], Gregor Laube[2], Rüdiger W. Veh[2]

[1]Charité Universitätsmedizin Berlin, Department of Neuroanatomy and Center for Stroke Research Berlin (CSB), Chariteplatz 1, 10117 Berlin, Germany

[2]Charité Universitätsmedizin Berlin, Department of Neuroanatomy, Chariteplatz 1, 10117 Berlin, Germany

Haptens are small and chemically inert molecules, which cannot elicit an immunological response on their own. Here we present the use of haptens for immunocytochemical labeling in a combination of the two, in principle different, pre- and post- embedding labeling techniques for electron microscopy. This approach, termed virtual pre-embedding (ViP), is a new immunocytochemical alternative for correlative light and electron microscopy and double labeling in affinity cytochemistry.

For this purpose, antigens in rat brain vibratome-sections were indirectly labeled with haptens prior to resin-embedding. Biotin, digoxigenin, tetramethylrhodamine and fluoresceine served as haptens. For double-labeling a combination of ViP with silver-enhanced immunogold detection was used. For correlative microscopy with fluorescent haptens sections were pre-examined by fluorescent microscopy. Subsequently, sections were embedded in epoxy-resin and tissue-bound haptens were visualized using standard post-embedding protocols and 10 nm colloidal gold particles.

After embedding all haptens used in this study could be clearly visualized in semithin sections by diaminobenzidine and in ultrathin sections by 10 nm colloidal gold particles. In double -labeling studies co-localization of antigens could be readily detected by the distinct appearance of DAB/colloidal gold vs. silver-enhanced particles. Fluorescent haptens were used in correlative light and electron microscopy for antigens with a highly restricted distribution as in tracer-labeled axon terminals. Detecting the antigens by their fluorescent signal prior to embedding greatly facilitated their identification by colloidal gold particles in ultrathin sections.

In conclusion, haptens are well suited for stable immunocytochemical pre -embedding labeling and post-embedding visualization of neuronal antigens in central nervous tissue (CNS) sections. This approach was successfully used for correlative light and electron microscopy and for double-labeling with immunogold-silver-enhancement, suggesting that ViP will serve as a useful tool in immunocytochemical studies of CNS structure and function.

# Local noise amplification and synaptic depression control the spontaneous activity in neuronal cultures

Javier G. Orlandi[1], Enric Alvarez-Lacalle[2], Sara Teller[1], Jordi Soriano[1], Jaume Casademunt[1]

[1]Universitat de Barcelona, Departament d'Estructura i Constituents de la Materia, Av. Diagonal 647, e-08028 Barcelona, Spain
[2] Universitat Politecnica de Catalunya, Departament de Fisica Aplicada, Av. Dr. Gregorio Marañon 50, e-08028 Barcelona, Spain

Spontaneous activity (SA) in cultures has been reported and studied for many years, with the observation of a rich repertoire of activity patterns[1]. However, little is known on the detailed mechanisms that control the generation and maintenance of these episodes of global activity. Experiments show that these episodes are a collective effect that strongly depends on the properties of the culture and the connectivity between neurons.

Here we present a numerical model combined with experimental measurements to understand the origins of SA. In our approach we consider the SA as a self-organized process, i.e. that neurons form connections and modify their strength until they are able to sustain episodes of SA with a given rate. We simulate realistically the way in which neurons grow and form connections to one another. This provides the connectivity matrix that defines the network and that mimics the one of the culture. Based on previous studies [2], we simulate the activity of each neuron by combining the behavior of its membrane potential with a dynamical coupling between neurons. Our model is able to reproduce the rhythmic SA episodes observed experimentally and the role of neuronal connectivity. It shows that global bursts of activity start through a local noise amplification process that depends on the connectivity map of the network. Also, it shows that burst termination and the posterior silent phase are controlled by short-term synaptic depression. The model also provides a basis for understanding many recent observations, such as the presence of leader neurons or burst initiation zones.

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# A microfluidic culturing platform for studying intra-dendritic signaling and dendrite to nucleus communication

Carlos Bas Orth[1], Michael S Cohen[1,5], Hyung Joon Kim[3], Noo Li Jeon[4], Samie R Jaffrey[1]

[1]Department of Pharmacology, Weill Medical College Cornell University, NY 10065, USA.

[2]Present address: Excellence Cluster CellNetworks at University of Heidelberg, and Department of Neurobiology, Interdisciplinary Center for Neurosciences (IZN), University of Heidelberg, 69120 Heidelberg, Germany.

[3]Department of Biomedical Engineering, University of California, Irvine, CA 92697, USA.

[4]School of Mechanical and Aerospace Engineering, Seoul National University, Seoul 151-742, Korea.

[5]These authors contributed equally to this work.

Dendrites are a major site of signaling in neurons. Nevertheless, few experimental techniques exist that allow signaling to be selectively manipulated in dendrites. Here we describe a microfluidic culture platform that allows dendrites to grow into a fluidically isolated environment. Using these microfluidic devices, we first studied intra-dendritic signaling. By applying the protein synthesis inhibitor, cycloheximide, selectively to dendrites for seven days we demonstrate that intra-dendritic protein synthesis regulates dendrite morphogenesis. Next, we studied dendrite to nucleus communication. We demonstrate that BDNF can act directly on dendrites to elicit an anterograde signal that induces transcription of the immediate early genes Arc and c-Fos. Intriguingly, the activity of TrkB, the BDNF receptor, is required in the cell body for the induction of Arc and c-Fos mediated by dendritically-applied BDNF. These results are consistent with the involvement of a signaling endosome-like pathway that conveys BDNF signals from the dendrite to the nucleus.
The results presented here demonstrate that microfluidic compartmentalization of dendrites allows dendritic signaling pathways to be selectively targeted and provides a strategy to identify signaling pathways that regulate dendrite to nucleus signaling.

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# Late Posters

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LP-T1-2B Determining the role of Sonic Hedgehog signaling in establishing midbrain dopaminergic neuron subclasses
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LP-T2-1B The role of Satb2/Ctip2 in cortical connectivity and the elucidation of their downstream pathways
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LP-T5-1B Phosphatidylinositol 4, 5- bisphosphate ($PIP_2$)-dependent modulation of TASK and HCN channels in the thalamocortical relay neurons
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*Tom Goeckel, Gerhard Lakemeyer, Hermann Wagner*

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*Julien Colomb, Ben Beuster, Marc-Nikolas Rentinck, Björn Brembs*

LP-T25-1B The *Drosophila* FoxP gene is necessary for operant self-learning: Implications for the evolutionary origins of language
*Björn Brembs, Diana Pauly, Rüdiger Schade, Ezequiel Mendoza, Hans-Joachim Pflüger, Jürgen Rybak, Constance Scharff, Troy Zars*

LP-T25-2B Retracted

# Defects in the neurovasular development of the forebrain in FoxG1cre/TßRII$^{flox/flox}$ mutants

Nicole Büttner[1], Tanja Vogel[1]

[1]Department of Neuroanatomy, Centre for Anatomy, University Medical Center Göttingen, Germany

Transforming Growth Factor ß (Tgfß) influences various processes in developing and adult organisms through cytostatic signals. Especially the role in neuronal and vascular development depend upon proper Tgfß-signaling. To investigate in vivo the influence of Tgfß in the developing forebrain, we mated floxed Tgfß-receptor 2 (TßRII$^{flox/flox}$) mice with FoxG1cre expressing mice and gained conditional knock-out FoxG1$^{cre}$/TßRII$^{flox/flox}$ mutants. FoxG1 is expressed in the developing telencephalon and is a known antagonist of Tgfß-signaling pathway in neuroepithelium. In this case Tgfß activates Smad proteins through phosphorylation. The Smad-complex translocates into the nucleus and binds there to unphosphorylated FoxO. This formed Smad/FoxO-complex promotes the transcription of p21Cip1. FoxG1 inhibits this binding and therefore blocks the transcription of p21Cip1. FoxG1cre/TßRII $^{flox/flox}$ mice might reveal inside information on the role of Smad and FoxG1 antagonism in neurogenesis, proliferation and angiogenesis.

Conditional knock-out FoxG1$^{cre}$/TßRII$^{flox/flox}$ mutants display detectable haemorrhages beginning around E13.5. These bleedings infiltrate further into brain parenchyme with increasing developmental stages and are mostly localized in telencephalon and diencephalon. Also an enlargment of the ventricles is detectable. The vascular phenotype of mutants varies compared to controls, e.g. vessels of mutants show an atypical appearence. The embryos die between E16.5 and E17.5.

To analyse possible molecular causes of mutants we use datasets which were gained from two different microarray analyses. Both microarrays are based upon primary cultured cortical cells from E13.5 embryos to investigate the gene regulation on a homogene background of cells. One dataset is gained from the comparision of Tgfß and ALK4,5,7-inhibtor SB431542 treated cells to analyse gene regulations induced by Tgfß at E13.5. In this case we cannot detect a change in the amounts of neurons in the neuronal cell-pool after Tgfß-treatment. The other array shows gene regulations from Tgfß-treated FoxG1-deficient cells and controls. The second array reveals gene regulations due to loss of FoxG1, but with consistent Tgfß -level. In FoxG1 -deficient cells, Tgfß can induce an increase of neurons. Both datasets were examined for interesting candidate genes with influence on angiogenesis, extracellular matrix and cytoskeleton. Expression of these genes are analysed in mutants as well as controls with Real-Time PCRs. Regulated candidate genes might play an important role in Tgfß-signaling in neurovascular development.

# Determining the role of Sonic Hedgehog signaling in establishing midbrain dopaminergic neuron subclasses

Anna Kabanova[1], Sandra Blaess[1]

[1]Neurodevelopmental Genetics, Institute of Reconstructive Neurobiology, Life and Brain Center, University of Bonn, Sigmund-Freud-Str. 25, D-53127 Bonn

The midbrain dopaminergic (mDA) neurons of the substantia nigra (SN) and the ventral tegmental area (VTA) play critical roles in the control of voluntary movement, and reward behavior, respectively. These subpopulations of mDA differ in gene expression, axonal projections and their aberrant function underlies a wide spectrum of disorders, such as Parkinson's disease and schizophrenia. Recent studies have identified several signaling molecules, including Sonic Hedgehog (Shh), Wnt1 and Fibroblast Growth Factor 8 (Fgf8) that influence the development of mDA neurons.

Conditional mutant analysis and fate mapping of Shh-responding cells showed that Shh signaling is necessary for mDA precursor induction between embryonic day (E) 7.0 and E10.5. Furthermore, the fate mapping data suggest that Shh initially acts on precursors that give rise to SN mDA neurons while after E9.5 Shh signals preferentially to medial VTA progenitors. However, it remains unclear whether Shh signaling plays a direct role in the specification of mDA neuron subsets.

To investigate whether the timing of Shh signaling is involved in regulating the specification of ventral midbrain (vMb) precursors into specific mDA neuron subsets, we are using conditional gene inactivation. We generated mutants in which *Gli2*, the main activator downstream of Shh signaling, was removed in the vMb between E8.5-E9.0. In these mutant mice, we observe a reduction of mDA neurons in the adult midbrain, which appears to be particularly severe in the medial VTA and the SN.

Analysis of embryonic stages demonstrates that an abnormal mDA neuron distribution is already apparent in E12.5 mutant brains. Analysis of the expression of mDA subset markers shows that the majority of the remaining mDA neurons in the mutants adopt the fate of neurons in the SN, whereas only a small number of neurons are specified to the mDA neurons of the VTA. Finally, to investigate whether Shh plays a direct role in mDA specification, we are activating or inactivating Shh signaling in a temporally controlled and mosaic manner and are following the fate of the genetically mutated and permanently marked cells throughout development of the midbrain.

# The role of Satb2/Ctip2 in cortical connectivity and the elucidation of their downstream pathways

Paraskevi Sgourdou[1], Olga Britanova[1,3], Srinivas Parthasarathy[1,4], Victor Tarabykin[1,4]

[1]Max Planck for experimental Medicine, Göttingen, Herman Rein str. 3, 37075, Germany
[2]DFG Research Center for the Molecular Physiology of the Brain (CMPB)
[3]Shemiakin and Ovchinnikov Institute of Bioorganic Chemistry RAS, Miklukho-Maklaya 16 / 10, 117871 Moscow, Russia
[4]Institute of Cell Biology and Neurobiology, Charité medical school Hindenburgdamm 30, 12203 Berlin

Satb2, similarly to Satb1, belongs to a family of transcription regulators that control neuronal differentiation of specific cell types, by influencing the expression of multiple loci over long distances (Britanova et al., 2005). In particular, Satb2 has been shown to control the post mitotic fate of cortical neurons that reside on the upper layers (II-IV) of the developing neocortex, in part by downregulating the expression of Ctip2. Ctip2 is a transcription factor that is primarily expressed in layer V neurons, which are destined to form corticospinal connections (Britanova et al., 2008). In Satb2 null mice, which suffer multiple craniofacial abnormalities and ultimately exhibit perinatal lethality, the cortico -cortical connections fail to form and instead there is an ectopic induction of corticospinal connectivity. On the contrary, Ctip2 mutants loose their normal corticospinal connections of layer V neurons which are instead misrouted into forming callosal projections, similar to those seen in neuronal layers II to IV. Additionally there is a lack of fasciculated bundles that normally perforate the striatum to form the internal capsule and a complete absence of CSMN (corticospinal motor neurons) axons extending pas the pons ( Arlotta et al., 2005). These data along with the lack of Ctip2 expression in the ventricular and subventricular zones suggest a role of this gene in controlling the postmitotic differentiation of CSMN neurons. In order to investigate the genetic interactions between Satb2, Ctip2 and to identify the downstream targets of the above transcription factors we generated compound *Satb2-/-;Ctip2 -/-* mutants and analyzed the resulting phenotype by comparing it to the phenotypes of Satb2 and Ctip2 single mutants. The resulting connectivity phenotype and the downstream pathways will be discussed in detail.

# Phosphatidylinositol 4, 5- bisphosphate ($PIP_2$)-dependent modulation of TASK and HCN channels in the thalamocortical relay neurons

Pawan Bista[1], Matthias Pawlowski[1], Tatyana Kanyshkova[1], Hans Christian Pape[1], Sven G. Meuth[2], Thomas Budde[1]

[1]Institut für Physiologie I, Westfälische Wilhelms-Universität, Münster, Germany
[2]Department of Neuropathophysiologie, Westfälische Wilhelms-Universität, Münster, Germany

**Introduction:** PIP2 is a minority phospholipid of the inner surface of the plasma membrane. Many plasma membrane ion channels including TWIK-related acid-sensitive potassium (TASK) channels and hyperpolarization activated cyclic nucleotide gated (HCN) channels are modulated by $PIP_2$ and can be turned off by signaling pathways that deplete PIP2. The aim of this study was to analyze the role of PIP2 in the modulation of TASK and HCN channels in thalamocortical relay (TC) neurons of the rat.

**Methods:** In the present study we combined electrophysiology with immunohistochemistry in brain slices. Long Evans rats postnatal age of 12- 23 days were taken as the experimental animals. 250µM thick coronal thalamic slices were prepared on a vibratome. Whole cell voltage clamp recordings were performed on TC neurons in the dorsal lateral geniculate nucleus (dLGN) of the rat thalamus. TC neurons were held at a potential of -20mV and ISO amplitude was analyzed. Current through HCN channels ($I_h$) was analyzed by using hyperpolarizing voltage steps.

**Results:** A dose dependent decrease in the Oxotrimorine (OxoM)-sensitive current was observed with increasing concentration of $PIP_2$ added to the internal solution. Neomycin (Neo), a polycationic compound which binds to intracellular $PIP_2$, showed a significant increase in the OxoM-sensitive current at a concentration of 10µM. In contrast 5mM Neo completely bound all $PIP_2$ and inhibited TASK channels, thereby decreasing ISO and the OxoM-sensitive current. While intracellular addition of $PIP_2$ had no effect on the activation curve of $I_h$, increasing concentrations of Neo induced a dose-dependent modulation of HCN channels. Neo shifted the voltage-dependent activation of $I_h$ toward hyperpolarizing potentials.

**Conclusion:** Both TASK and HCN channels are modulated by $PIP_2$. However the sensitivity towards this phospholipid seems to be different.

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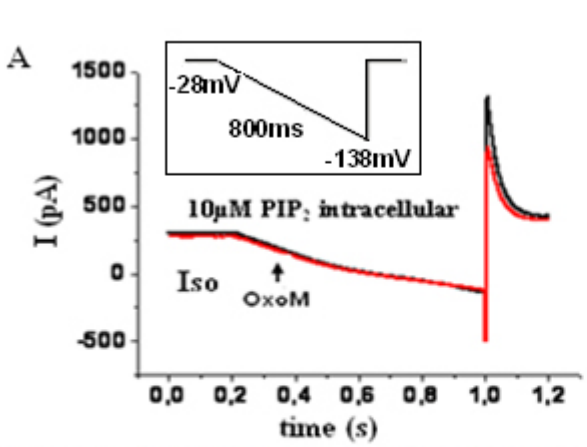
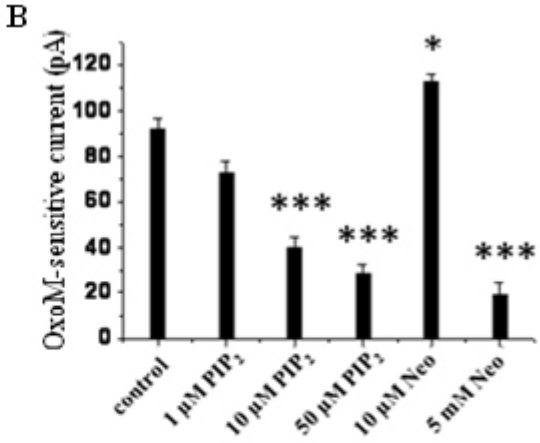



**A. Currents evoked by ramping the membrane potential from -28 to -138mV over 800ms with 10μM $PIP_2$ in internal solution (black trace) and during external application of 10 μM OxoM (red trace).**




**B. Mean bar graph representation of OxoM-sensitive current at -20mV under control conditions and with 1μM PIP2, 10μM $PIP_2$, 50μM $PIP_2$, 10μM Neo, and 5mM Neo added to the internal solution.**

# Quantification and Visualization of Serotonin Release in Stem Cell-derived Serotonergic Neurons

Thorsten Lau[1], Sarah Jacobs[1], Christoph Spieler[1], Patrick Schloss[1]

[1]Zentralinstitut für Seelische Gesundheit, 68159 Mannheim, Deutschland

Communication between neurons is commonly considered a straight forward pathway. Following stimulation of a neuron the resulting summation of excitatory postsynaptic potentials exceeds a certain threshold at the axon hillock and thereby triggers the generation of an action potential. This action potential runs in an unidirectional way down the axon towards the presynaptic nerve ending. Here, this signal induces exocytotic processes that result in neurotransmitter release into the synaptic cleft, the defined area of communication with the next neuron in line.
Serotonergic neurons are located in the raphe nuclei and project their axonal fibres into different areas of the brain. Along their extensive outgrowths, serotonergic neurons form axonal varicosities. These as well as somatodendritic regions can be stained for serotonin (5-hydroxytryptamine, 5HT) containing vesicles and the serotonin transporter (Sur et al., 1996). This provided first evidence for 5HT release and re-uptake at other cellular structures than synaptic boutons. These distribution patterns go along with the finding that in vivo serotonergic neurons communicate in addition to synaptic neurotransmission also via non-synaptic axonal and importantly somatodendritic release. As shown in recent in vivo microdialysis studies, the serotonergic neurotransmission system, much like the dopaminergic one, mainly functions via volume transmission (De-Miguel and Trueta, 2005).
Since serotonergic neurons cannot be isolated from rodent brains, murine embryonic and neuronal stem cell-derived serotonergic neurons provide a supreme tool to investigate (a) the organization of serotonin release sites and (b) serotonergic neurotransmitter release in living neurons.

# Association of Membrane Rafts and Postsynaptic Density: Proteomics, Biochemical, and Ultrastructural Analyses

Tatsuo Suzuki[1], Jingping Zhang[2], Shoko Miyazawa[1], Qian Liu[1], Michael R. Farzan[3], Wei-Dong Yao[2]

[1]Dept. Neuroplasticity, Shinshu Univ. Grad. Sch. Med., Matsumoto, Japan, [2]Psychiatry, [3]Microbiol & Mol Genet., Harvard Med. Sch. & New England Primate Res. Ctr., Southborough., MA, USA

Postsynaptic membrane rafts are believed to play an important role in synaptic signaling, plasticity, and maintenance. However, their molecular identities remain elusive. Further, how they interact with the well-established signaling specialization, the postsynaptic density (PSD), to regulate signaling in postsynaptic neurons is poorly understood. We previously detected a number of conventional PSD proteins in detergent-resistant membranes (DRMs) prepared from synaptic plasma membranes (SPMs) known to be enriched with membrane rafts. This suggests potential associations between the two postsynaptic structures. Here, we have performed LC-MS/MS (liquid chromatography coupled with tandem mass spectrometry) analyses on postsynaptic membrane rafts (SPM-DRMs) and PSDs. Our comparative analysis identified 370 proteins in SPM-DRMs, in which 214 proteins (57.8%) were also present in the PSDs, confirming an extensive overlap of protein components in the two structures. This overlapping distribution could be explained, at least partly, by a physical association of PSDs with membrane rafts. A significant number of proteins displayed biased distributions to either rafts or PSDs, suggesting distinct roles for the two postsynaptic specializations. Using biochemical and electron microscopic methods, we directly detected membrane raft-PSD complexes. *In vitro* reconstitution experiments indicated that the formation of raft-PSD complexes was not due to the artificial reconstruction of once-solubilized membrane components and PSD structures, supporting that these complexes occurred *in vivo*. Taking together, our results provide evidence that postsynaptic membrane rafts and PSDs may be physically associated. Such association could be important in postsynaptic signal integration, synaptic function, and maintenance.

# Synaptic organization of the anterior optic tubercles and the lateral triangles in the brain of *Drosophila melanogaster*.

Kouji Yasuyama[1]

[1]Department of Natural Sciences, Kawasaki Medical School, 577 Kurashiki, Okayama, 701-0192, Japan.

The lateral triangles (LTr) are small neuropil areas closely associated with the central complex in *Drosophila* brain. The two bilateral LTrs abut the fan-shaped body rostro-laterally and also lateral to the upper half of the ellipsoid body. The LTrs house the terminals of the neurons, termed small-field elements, which ascend from the anterior optic tubercle (AOTu) in the protocerebrum (Hanesch et al., 1989). In locust, both of the AOTu and the LTrs function as relay sites for the polarization-vision pathway (e.g., Homberg, 2003).

The recent ultrastractural study on the LTrs in locust revealed a novel type of microgromerular synaptic complex, in which large cup-shaped presynaptic terminals enclose many small ?-aminobutyric acid (GABA) immunoreactive profiles (Träger et al., 2008). In *Drosophila*, the LTrs as well as the AOTus are easily recognized by their prominent immunoreactivity against the anti-choline acetyltansferase antibody (ChAT-ir). In this study, in order to compare the synaptic organization of the *Drosophila* LTr with that of the locust LTr, we examined the fine structures of the LTr by the preembedding immuno-electron microscopy (immuno-EM) using ChAT immunolabeling as a marker for the LTrs and the AOTus.

In the AOTu, small globular structures (diameters of 5-10 ?m) are strongly labeled in a cluster. Immuno-EM revealed that mass of ChAT immunoreactive profies were loosely compartmentalized by the immunonegative thick fibers, so that the ChAT-ir are detected as the globular masses under light microscope. The AOTu receives projection of LC10 neurons carrying information from the lobula (Otuna and Ito, 2006). The anterior optic tract, through which the LC10 neurons reach to the AOTu, showed ChAT-ir, implying the LC10 neurons are cholinergic.

In the LTr, about forty of ChAT immunoreactive tiny granules were detected into two separate (dorsal and ventral) clusters. Immuno-EM demonstrated that these ChAT immunoreactive granules correspond to large immunopositive synaptic boutons bearing tiny spine-like processes. The ChAT immunoreactive boutons formed the core at each microglomerulous, and were presynaptic to the immunonegative thin fibers surrounding them. The fiber tract connecting the AOTu with the LTri, through which the small-field elements ascend, showed ChAT-ir, suggesting that the cholinergic terminal boutons in the LTr are attributed to the small-field elements.

In the locust LTri, no presynaptic large boutons were observed, whereas large cup-shaped terminals enclosing the postsynaptic fiber masses were found as presynaptic elements (Träger et al., 2008). In contrast, in the *Drosophila* LTri, the microglomeruli were exclusively detected, each of which comprises a large cholinergic presynaptic bouton at its core, encircled by a number of tiny immunonegative fibers. This micorglomerular organization in the LTr is similar to that found in the *Drosophila* calyx of mushroom body. The LTri boutons with ChAT-ir are characterized by having the spine-like processes, in contrast with the ChAT immunoreactive boutons in the calyx.

# Synaptic marker proteins as useful tools in the study of neural control on peptidergic neurons

Gergely Karsai[1], Christian Wegener[2], László Molnár[1], Edit Pollák[1]

[1]Department of General Zoology, University of Pécs, Pécs, Hungary
[2]Department of Biology - Neurobiology/Ethology, Philipps-University, Marburg, Germany

Some peptidergic neurons and neural networks, e.g. crustacean cardioactive peptide (CCAP)-expressing ones, have recently been identified in *Drosophila* larvae and imago. Based on three-dimensional visualisation of certain peptidergic neurons putative dendritic and axonal parts of their arborisation have been identified, but their synaptic inputs regulating their secretion activity remained mainly unknown.
Using GAL4/UAS-directed expression of certain neuropeptides in central nervous system of 3rd larval stage and adult *Drosophila*, we made an effort to refine available data on spatial characteristics of CCAP-containing neuronal structures as well as c929-carrying cells. Most of c929-positive neurons contain the marker enzyme PHM (peptidylglycine-a-hydroxylating monooxygenase), a peptide biosynthetic enzyme typical of majority of peptidergic cells. We also applied new clones transformed with gene constructions expressing different green fluorescent protein (GFP)-connected proteins known as synaptic marker molecules of pre- or postsynaptic regions.
Our experiments resulted in large amount of new information on the arborisation of the two selected peptidergic neuron populations. Further, applying confocal laser scanning microscopy, we improved success of documentation presenting much more complete and detailed maps on labelled structures than those have been published before.
Our results partially support earlier observations on neuronal in- and output compartmentalization. However, direct proof of synaptic activity could only be gained with the aid of fine structural analysis of identified neural elements. In order to get the answer to the newly emerging questions, immune electron microscopic experiments have been started.
Considering that peptides could act both synaptically and via volume transmission also extrasynaptically, exploration of peptidergic receptor distribution is essential to learn exact space of peptide transmitter liberation and action. Clarification of role and rate of classical synaptic activity and extrasynaptical modulation on regulation of secretion activity in peptidergic neurons needs further investigation.

# Novel Cell- and Tissue-Based Assays for Detecting Misfolded and Aggregated Protein Accumulation; Relevance to Neurological Disease Models

Walter Dittrich[1], Dee Shen[1], Wayne F. Patton[1]

[1]Enzo Life Sciences, R+D, Marie-Curie-Str. 8, 79539 Lörrach

Aggresomes are inclusion bodies that form when the ubiquitin–proteasome machinery is overwhelmed with aggregated proteins. Aggresomes and related inclusion bodies appear to serve as storage depots for misfolded and aggregated proteins within cells, which can potentially be degraded by the autophagy pathway. The deposition of protein aggregates is a pathological feature of a large number of diseases targeting neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease and related polyglutamine disorders. A homogenous fluorescence-based assay was devised to detect aggregated proteins within aggresomes in a cellular context, employing a novel red fluorescent molecular rotor dye. The assay has been validated using a range of conditions known to modulate proteosome pathways and has been optimized for co-localization studies with fluorescently-labeled antibodies, highlighting interactions between aggregated protein cargo and proteins implicated in aggresome formation, such as p62 and LC3. The assay is compatible with flow cytometry, allowing, for the first time, easy quantitation of denatured protein cargo. Furthermore, amyloid beta peptide 1–42 was shown to induce aggresome formation in the SK-N-SH human neuroblastoma cell line. SMER28, a small molecule modulator of autophagy acting via an mTOR-independent mechanism, blocked accumulation of amyloid beta peptide within the cells. The described assay allows assessment of the effects of protein aggregation directly in cells, without resorting to the use of non-physiological protein mutations or genetically engineered cell lines. With minor modification the assay was also adapted to the analysis of frozen or formalin-fixed, paraffin-embedded tissue sections, with demonstration of co-localization of aggregated cargo with ß-amyloid and tau proteins in brain tissue sections from Alzheimer's disease patients.

# Neural organisation and Visual Processing in the Anterior Optic Tubercle of the Honeybee Brain

THEO MOTA[1], Nobuhiro Yamagata[1], Martin Giurfa[1], Wulfila Gronenberg[2], Jean-Christophe Sandoz[1,3]

[1]Centre de Recherches sur la Cognition Animale; Université de Toulouse; UPS; 118 route de Narbonne, F-31062 Toulouse Cedex 9, France
[2]Arizona Research Laboratories; Division of Neurobiology; University of Arizona, Tucson, Arizona 85721
[3]Laboratoire Evolution, Génomes et Spéciation -CNRS, 1 avenue de la Terrasse, 91198 Gif-sur-Yvette, France

Vision in honeybees has been extensively studied at the behavioural level and with a reduced number of intracellular electrophysiological recordings allowing the study of one particular neuron at a time. However, our knowledge of visual processing in bees is still limited because no technique has yet allowed the functional study of visual processing at the circuit level. Here we present such an endeavour into a practically unstudied visual area of the bee brain, the anterior optic tubercle (AOTu). We characterised the neural connectivity and physiological properties of the AOTu using two main approaches: 1) neuro-anatomical characterisation of visual pathways to and from the AOTu, and 2) calcium imaging of visual stimulus-induced signals in the AOTu. We show that the AOTu is compartmentalised in different subunits. It receives visual input from different regions of the optic lobes (medulla and lobula) and this input is dorso-ventrally segregated into the distinct AOTu subunits. The AOTu also receives input from a specific extrinsic neuron of the mushroom body. Different categories of output neurons connect the AOTu to the lateral accessory lobe. Furthermore, two parallel inter-tubercle tracts passing through distinct protocerebral regions connect the AOTus of both brain hemispheres. For calcium imaging, the AOTu was retrogradely stained with a dextran-conjugated calcium indicator. Our experiments based on stimulation with LED arrays show that: 1) Lights presented in different visual fields induce distinct patterns of neuronal activity, especially for dorsal and ventral eye regions. Stimulations of dorsal or ventral eye induced activation of different AOTu subunits. These and the anatomical results suggest that some level of retinotopic organisation is retained in the AOTu; 2) Monochromatic lights matching to the bees' photoreceptors (UV, blue and green) induced different signal intensities, time-course dynamics and activity maps, thus showing a spatiotemporal coding of chromatic stimuli in the AOTu ; 3) Activation by blue-green polychromatic stimuli was always lower than to the strongest component (green light), revealing an inhibition phenomena ('suppression'). This non-additive occurrence concurs with colour-opponency theories. Our results strongly suggest the involvement of the AOTu in spatial and chromatic information processing by the bee brain.

# Modeling the precedence effect with an efficient and robust algorithm

Tom Goeckel[1], Gerhard Lakemeyer[2], Hermann Wagner[1]

RWTH Aachen University, Department of Zoology and Animal Physiology, Mies-van-der-Rohe Straße 15, 52056 Aachen
[2]RWTH Aachen, Knowledge-based Systems Group, Chair for Computer Science 5, Ahornstraße 55, 52056 Aachen

In reverberant environments not only the direct sound, but also sounds reflected from walls arrive at the sound source localization system. It is a challenge to discriminate between the direct sound (corresponding to the actual source) and the reflected sound (corresponding to virtual sound sources).
Barn owls (Tyto alba), similar to mammals, possess several mechanisms, subsumed under the term of the precedence effect, to suppress the localization information from virtual sound sources. Although the neuronal structures involved in the precedence effect are not yet fully understood, several models have been developed to simulate the physiological findings. Our goal was to design a system based on the Jeffress Model that suppresses localization information caused by echoes from virtual sources.
Faller and Merimaa (J Acoust Soc am 116: 3075 (2004)) introduced the normalized cross-correlation value (also known as Interaural Coherence (IC)), as a measured of the reliability of a sound localization cue. The IC can take values between +/-1. The IC value is determined for each sample of the recorded digital signal. The IC value describes how well the processed ITD value can specify a (single) free-field source. In our approach, the IC value serves as a weight for each ITD value. The recorded signal was subdivided into time frames of 4096 samples (at 44.1 kHz sample rate). The IC values of each time frame were accumulated in a histogram that spanned the physiological ITD range of the system. After normalizing the histogram the algorithm looked for local maximums that surpassed a predefined threshold value (0.25) and returned them as possible sound source directions. Additional time integration (time constant: 0.2 time frames) reduced noisy peaks to a minimum.
The system was tested using noise signals that were convoluted with the impulse response of several virtual rooms that had different echoic conditions and for a certain number of signal-to-noise ratios (SNRs). In addition, it was implemented on a mobile robot and tested in an office environment with a noise source at a distance of 3.5m. To simulate different source directions, the pan-tilt unit with the mounted microphones was rotated. We measured the mean of the localization error after the evaluation of 100 time frames.
The algorithm was able to detect the correct sound source with a small localization error for SNRs as low as -15dB, even in a simulated room with unpainted concrete walls. The errors of less than 3 degrees for lateral positions and less than 1.5 degrees for medial positions could mainly be accounted for by the restricted time resolution of the discrete input signal. While the algorithm did not mislocalize sources in anechoic or 50% echoic conditions, in two cases in 100% echoic conditions a reflection site was wrongly assumed as the original source. This also happened for an azimuthal source position in the real environment tests.

# Drosophila courtship: Male hybrid vigor after crossing isogenic lines?

Madeleine-Marie Gilles[1], Yasmine Graf[1], Saskia Rughöft[1], Kirstin Leinhoß[1], Björn Brembs[1]

[1]Institut für Biologie - Neurobiologie, Freie Universität Berlin

In many models of sexual selection it is assumed that attractive traits are passed on from father to son and thus also help the mother attain fitness benefits, since her sons would be more likely to father even more offspring. In *Drosophila simulans*, male attractiveness is heritable through the patriline: attractive fathers sire attractive sons, while unattractive fathers sire unattractive sons (Taylor et al., 2007). However, Pischedda et al. had previously concluded that "male fitness was not inherited by sons" in *Drosophila melanogaster*. In an attempt to further elucidate the evolutionary and neurobiological underpinnings of sexual signaling during courtship, we set out to test the attractiveness of recently published isogenic fly lines. It has been reported that these 40 lines exhibit considerable variability in copulation latencies and frequencies (Ayroles et al., 2009). We started by measuring copulation laten-cies and frequencies of a selected subset of these lines in standard courtship wheels against a Canton S tester female. The selected lines were the five lines with the highest (lowest, re-spectively) copulation latencies from experiments using large, food-containing vials with Oregon/Samarkand tester females. To identify the lines best suited for crossing, the copula-tion latencies were plotted against the copulation frequencies and four lines which were clearly separable on both variables were chosen for crossbreeding. In all cases, the male offspring from the reciprocal crosses were more attractive than the less attractive parent. Some offspring exceeded even the more attractive parent in attractiveness. This evidence demonstrates that the fathers from an unattractive *D. melanogaster* population can sire attractive sons if they mate with females from a population that produces attractive males.
These results also suggest a form of hybrid vigor which can render offspring more attractive than same-sex members from both their patri- and their matriline.

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# Hide or fly: phototaxis in flightless *Drosophila*

Julien Colomb[1], Ben Beuster[1], Marc-Nikolas Rentinck[1], Björn Brembs[1]

[1]Institut für Biologie - Neurobiologie, Freie Universität Berlin

About one hundred years ago, T.H. Morgan suggested to his student to clip the wings of flies and test their response to light. They discovered that flies with clipped wings did not respond to the light. Some years later, Robert McEwen continued to work on the subject (McEwen, Journal of experimental Zoology, 1918). In this study, McEwen showed that the effect was specific to wing clipping. Antennae clipping and even leg clipping had smaller effects than wing clipping. He also tested mutants with non-functional wings and reported low phototaxis scores and that cutting the wings of these flightless flies had no further effect. In 1963, Chiang correlated phototaxis behavior with flying abilities, by looking at the development of both traits in juveniles and compared them with adults: young flies with still un-expanded wings prefer shaded areas over lit ones, with a reversal of the preference at about 7h after emergence. Finally, in 1967, Benzer presented his counter-current apparatus which mass-assay for phototaxis behavior. He was also able to confirm that flightless flies show reduced phototaxis scores. What is the neurobiological mechanism by which flies modify their response to light when there are unable to fly?

We first used Benzer's countercurrent apparatus in different modes in order to reproduce the results of McEwen. We found that flightless flies are less attracted to the light even when tested together with flies that can fly. Flies with glued wings return to their normal phototaxis response when their wings are unglued, demonstrating the reversibility of the effect. We performed different control experiments to show that this change in behavior was not due to a locomotion deficit. For instance, we tested the response of flies when the light was coming from other directions or in complete darkness. We also found that flies without wings are more active in a modified Buridan walking paradigm and are heading for the targets more consistently. Flightless mutants with or without apparent wing defect do not show a further reduction in phototaxis. These results are consistent with the hypothesis that flies continuously monitor their flight ability and change the way they respond to sensory cues according to their flight ability at the time the sensory stimulus is perceived.

We are now screening for flying mutant flies which do not show any decrement in phototaxis after wing clipping, in order to decipher the neuronal mechanisms involved in this behavioral modification.

# The *Drosophila* FoxP gene is necessary for operant self-learning: Implications for the evolutionary origins of language

Björn Brembs[1], Diana Pauly[2], Rüdiger Schade[3], Ezequiel Mendoza[1], Hans-Joachim Pflüger[1], Jürgen Rybak[4], Constance Scharff[1], Troy Zars[5]

[1]Institut für Biologie - Neurobiologie, Freie Universität Berlin
[2]Robert Koch-Institut, Berlin
[3] Institut für Pharmakologie, Charité, Berlin
4[*/sup]Max Planck Institute for Chemical Ecology, Jena, Germany
[*sup]5 Division of Biological Sciences, University of Missouri, Columbia, Mo, USA

In humans, mutations of the transcription factor Forkhead box protein P2 (FoxP2) cause a severe speech and language disorder. Downregulating the Zebrafinch FoxP2 orthologue in development results in incomplete and inaccurate song imitation. These forms of vocal learning exhibit two common characteristics: 1. Spontaneous initiation of behavior ('trying out'); 2. Evaluation of sensory feedback shaping behavior. Using a torque learning essay in which both characteristics have been realized, we investigated the involvement of the fly orthologue, FoxP, in operant self-learning in the fruit fly *Drosophila*. The experiments were performed using stationary flying *Drosophila* at the torque compensator with heat as punishment. Both a P-Element insertion and RNAi-mediated knockdown of the isoform B of the *Drosophila* FoxP gene did not lead to alterations of the gross brain anatomy, nor to an impairment in operant world-learning, i.e., color-learning, compared to control flies. However, both fly strains were impaired in operant self-learning, i.e., yaw-torque learning without any environmental predictors. Neither the FoxP intron retention isoform nor isoform A appear to be involved in this form of learning. These results suggest a specific involvement of isoform B of the *Drosophila* FoxP gene in the neural plasticity underlying operant self-learning but not in other forms of learning. To investigate the effects of RNAi knockdown and P-Element insertion on FoxP abundance and localization in the fly central nervous system, we have generated polyclonal chicken antibodies against four different regions of the putative FoxP protein.
Perhaps not surprisingly, these results are consistent with the hypothesis that one of the evolutionary roots of language is the ability to directly modify the neural circuits controlling behavior. It is noteworthy that these roots can apparently be traced back to the Ur-bilaterian, the last common ancestor of vertebrates and invertebrates.

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