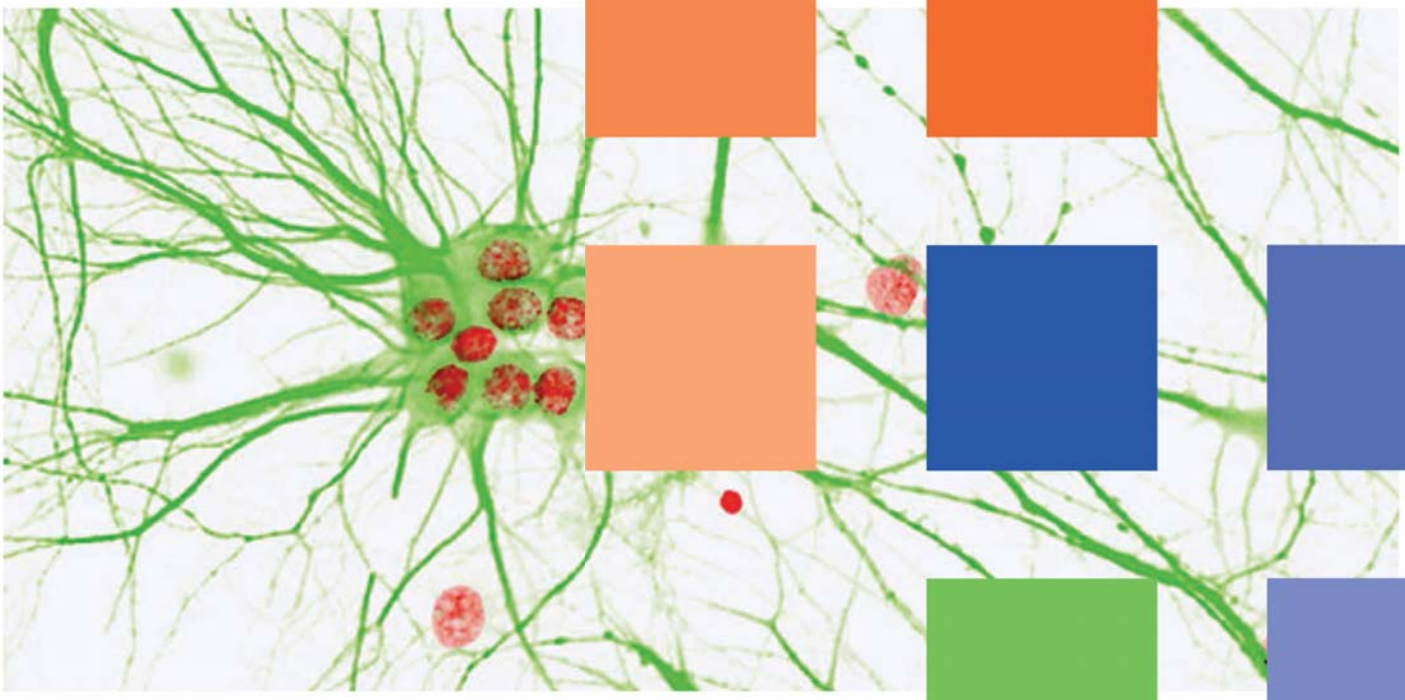

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Plenary Lectures

Genetic therapies – what does the future hold for neurological disorders?

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There are no effective disease-modifying therapies for neurodegenerative diseases such as Alzheimer's (AD), Parkinson's (PD), amyotrophic lateral sclerosis (ALS) or Huntington's disease (HD). Huntington's disease (HD) is a devastating autosomal dominantly inherited neurodegenerative disease and the genetic predictability of HD provides an opportunity for early therapeutic intervention many years before overt symptom onset and at a time when reversal or prevention of neural dysfunction may still be possible. As HD is monogenetic, fully penetrant, and characterised by a long premanifest phase, it is emerging as a potential model for studying therapeutic intervention in other neurodegenerative conditions such as Alzheimer's or Parkinson's disease where no preclinical diagnostic tests exist. In addition, HD manifests with a broad range of clinical symptoms and signs, many of them common to these other diseases, and involves widespread pathology throughout most of the brain involving similar protein misfolding. Understanding of HD pathogenesis is evolving, and I will present an overview of important approaches in development for targeting mutant HTT DNA and RNA, the cause of HD pathogenesis (Tabrizi Neuron 2019), and in particular I will present our recent successful phase 1b/2a clinical trial testing the effects of antisense oligonucleotide therapy (ASO) with Tominersen (formerly known as IONIS HTT Rx) in patients with early Huntington's Disease and present the results of the first successful HTT-lowering drug trial (Tabrizi et al New England Journal of Medicine 2019). This study is the first to demonstrate antisense-mediated protein suppression in patients with a neurodegenerative disease. While this ASO has potential for HD, our findings have broader implications. These data suggest that antisense technology has the potential to provide disease-modifying benefits in other neurodegenerative diseases associated with aberrant production of proteins, including ALS, Alzheimer's disease and many other diseases that currently lack adequate treatments (Tabrizi Science 2020). In my talk I will review ASO approaches in development for CNS diseases.

What the mouse's eye tells the mouse's brain: Visual feature extraction in the retina

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To provide a compact and efficient input to the brain, sensory systems separate the incoming information into parallel feature channels. In the visual system, parallel processing starts in the retina. Here, the image is decomposed into multiple retinal ganglion cell (RGC) types, each selective for a specific set of visual features like motion, contrast or edges. Recent work in mice provides a thorough classification of RGCs, revealing that the retina sends approx. 40 distinct information channels to the brain. In my talk, I will summarize our recent work on the neural mechanisms underlying this great diversity and on how the neural selectivity for distinct visual features arises across the retinal network. With this, we hope to increase our understanding of how the mammalian retina processes the stream of incoming visual information to extract relevant features from the environment.

Clinical and experimental studies of autoantibodies to CNS membrane receptors and associated proteins: many questions still unanswered

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Over the last 20 years it has become clear that autoantibodies to neuronal or glial surface membrane proteins can cause central nervous system (CNS) disease. The patients usually present with seizures, cognitive and psychiatric disturbance and movement disorders, which are collectively termed “autoimmune encephalitis” to distinguish from those patients who have an infectious form of encephalitis. The autoimmune disorders present over days or weeks and progress rapidly, often requiring treatment in intensive care facilities. Importantly, these patients can make substantial recoveries when treated with immunotherapies that reduce antibody levels and immune cell activation. The main targets for the autoantibodies are membrane receptors and ion-channel related proteins. (a) Autoantibodies to the NMDA receptor (NMDAR) are found (in serum and CSF) in children and younger adults with seizures, behavioural and cognitive disturbance; the patients can progress to develop complex movement disorders, autonomic instability and loss of consciousness. Ovarian tumours that express the NMDAR are found in about 30% of females. Treatment with combinations of steroids, plasmapheresis, intravenous immunoglobulins are typical; anti-CD20 therapeutic antibodies or cyclophosphamide are used in severe cases. Recovery is often slow but can be impressive with return to normal life. (b) A distinct form of “limbic encephalitis” is associated with antibodies to LGI1. This protein regulates the activity of voltage gated potassium channels and AMPA receptors at CNS synapses, particularly in the limbic system. Anterograde memory loss is severe and seizures can be resistant to anti-epileptic medication. The cognitive problems can be preceded by a distinct seizure type called facio-brachial dystonic seizures and immunotherapies at this stage may prevent development of the full limbic encephalitis. CASPR2 is another protein that associates with potassium channels both in the CNS and at the axonal juxtaparanodes. CASPR2 antibodies are also found in limbic encephalitis but more specifically in patients with insomnia and peripheral nerve hyperexcitability, known as Morvan’s syndrome. Lung cancers and thymomas are found in a minority of these patients. (c) Antibodies to inhibitory GABA receptors are, not surprisingly, often associated with seizures and cognitive problems. Glycine receptor antibodies are rare but can cause not only muscle rigidity and exaggerated reflexes but also life-threatening respiratory failure due to involvement of the brainstem. The clinical syndromes associated with these and other antibodies are now widely recognised and immunotherapies used with successful outcomes. In vitro studies have demonstrated some of the mechanisms by which the antibodies interfere with function, and the diseases can be partially reproduced in mice by injection of patients’ purified or monoclonal antibodies. The lecture will show videos of typical patients and describe briefly some of the experiments performed by ourselves and others, drawing attention to the range of activities in this exciting field and to the many challenges and unanswered questions.

The elusive mechanisms of CSF secretion - we know so much, but understand so little

Nanna MacAulay[1], Annette B. Steffensen[1], Eva K. Oernbo[1], Dagne Barbuskaite[1], Niklas Gerkau[2], Katerina Tritsaris[3], Christine R. Rose[2]

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The mammalian brain is bathed in the cerebrospinal fluid (CSF), which is continuously secreted by the choroid plexus located in each of the four ventricles. The CSF production has generally been assumed to take place by transepithelial transport of ions followed by osmotically obliged, passive movement of water, partly via the water channel aquaporin 1 (AQP1) expressed at the luminal membrane of the choroid plexus. The limitations of such a conventional osmotic model for CSF production are apparent from 1) the lack of osmotic driving force across the choroid plexus epithelium, 2) the minimal effect of genetic deletion of AQP1, and 3) the ability of the choroid plexus epithelium to transport water uphill against a transepithelial osmotic gradient. A number of cotransporter proteins have the inherent ability to cotransport water along with the ions/solutes in the translocation mechanism in a manner that permits water to be transported independently of an osmotic gradient. This talk will introduce the water-translocating Na^+,K^+,$2Cl^-$ cotransporter, NKCC1, as a key contributor to CSF production in the murine choroid plexus. The NKCC1 cotransport protein is located in the luminal membrane of the choroid plexus and is poised for ion and water transport from the choroid plexus epithelial cell to the ventricle. With its inherent ability to transport water along with the ion translocation, NKCC1 is able to move water independently of the osmotic gradient and in this manner contribute approximately half of the CSF secretion across the luminal membrane.

How the fly brain encodes time

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Circadian physiology provides a useful context in which to study the neural control of behavior. When applied to *Drosophila*, the studies take further advantage of the powerful genetics and cellular resolution available in this model system. Rhythm-generating neurons can be identified and manipulated, and the neural circuits analyzed in-depth. We are interested to learn how the brain uses circadian timing information to organize daily rhythmic locomotion. This talk will review our recent in vivo imaging measurements of the pacemaker circuit throughout its daily cycle of activity. We propose a model whereby the intrinsic clocks of pacemaker neurons and their cellular interactions together create proper timing cues (outputs) by which rhythmic behaviors such as sleep & locomotion may be properly aligned across the solar day. Finally, we will discuss on-going studies of the neuromodulatory peptidergic cell-signaling that is critical to the functions of this rhythm-generating circuit.

Emergence of memory traces in the dentate gyrus

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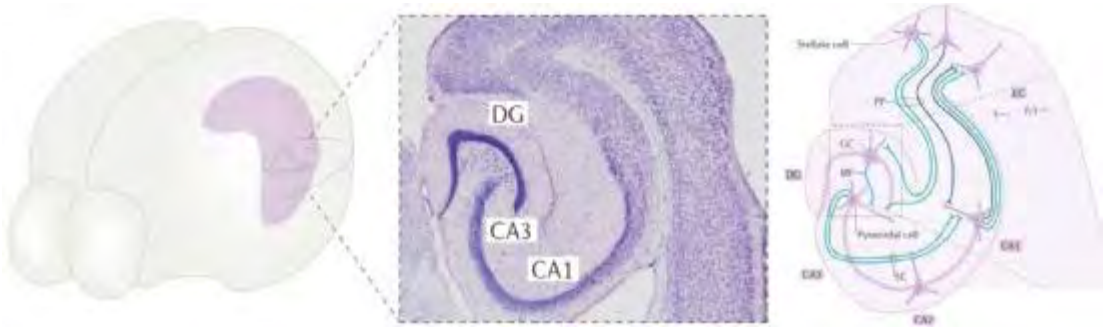
The dentate gyrus (DG) is the entrance gate of the hippocampus and translates the rich input stream from the entorhinal cortex into sparse non-overlapping memories. The network mechanisms underlying sparse coding are however largely unknown. In this talk, I will highlight new insights on the role of the various cellular components of the DG network, glutamatergic granule cells (GCs) and GABAergic inhibitory interneuron types in the sparse coding of information and the spatio-temporal emergence of DG population activity during learning. I will provide new insights on the relationship between the rich input stream provided by the medial entorhinal cortex to the DG and the hippocampal areas CA1 and CA3 and the spatial code generated by the principal cell output, which is forwarded to downstream brain areas for mnemonic associations. I will present our recently published and unpublished data obtained with state-of-the-art techniques including single unit recordings and 2-photon population imaging of neuron types in behaving rodents, to test their role in cell assembly formation for the representation of space and context during learning.



Magnetoreception in Pigeons

David Anthony Keays[1]

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Using maps, compasses, and sextants, mariners in the early 1500's developed the first methods to navigate the open sea; heralding an age of exploration as humanity set sail for the horizon. Yet long before this time evolution had equipped life on the planet with a biological global positioning system that was far superior to those early navigational tools – the Magnetic Sense. While there is unequivocal behavioural evidence demonstrating that this faculty exists, it is the least understood of all senses. The location of the primary sensors, the underlying biophysical mechanisms, and the neurological basis of the sense are unknown. Currently, there are three ideas that aim to explain how magnetosensation might work: (1) a light sensitive radical pair based model; (2) magnetite based magnetoreception; and (3) electromagnetic induction. In this lecture I will present our work that has tested the magnetite theory of magnetosensation, a concept that argues that small crystals of the iron oxide magnetite act as an intracellular compass transducing magnetic information into a neuronal impulse. I will present our work that challenges the existence of a magnetic sense system in the beak of birds. Moving forward I will introduce our in vivo assay that assesses magnetically induced neuronal activation in the pigeon brain, which has implicated the vestibular system in magnetic sensation. Finally, I will present a model that predicts a magnetoreceptive system that is based on electromagnetic induction within the semicircular canals, and apically located electroreceptors (CaV1.3) in vestibular hair cells.

Symposia

S19 Same, same but different – Emergence of individuality in the nervous system

S20 Store-operated calcium entry in neurons and glia

S21 The impact of the immune system on psychiatric disorders (DGPPN Symposium)

S22 MultiSenses – MultiScales: Deciphering neural processing in multisensory integration

S23 Principles of decision-making across species

S24 Hypothalamic neuron-glial network in obesity and type 2 diabetes

S25 Optical imaging to assess the plasticity function of sleep

S26 Regulation of synaptic vesicle recycling: from physiology to disease

S27 Sound processing, adaptation, and perception in the auditory system - From midbrain to cortical networks

S28 FAIR data management and data sharing in neuroscience

S29 Odors and Metabolism - neuromodulation in sensory processing

S30 Structure and dynamics of inhibitory synapses in health and disease

S31 Odor spaces: from odor molecules to behavior

S32 Translational Aspects in Neurological Diseases: from pathophysiology to new therapeutic approache

S33 Genetic and environmental factors shaping neuronal network defects and cognitive impairment

Symposium

S1: Tools for the future of synaptic neuroscience: superresolution imaging meets artificial intelligence

S1-1 Molecular resolution imaging by post-labeling expansion dSTORM (Ex-dSTORM)
Markus Sauer, Christian Werner, Janna Eilts, Fabian Zwettler

S1-2 Faster and better: high speed localisation precision without artifacts
Susan Cox

S1-3 Nanoscale organization of glutamate receptors and synaptic function
Daniel Choquet

S1-4 Development of machine learning approaches for quantitative super-resolution microscopy of molecular interactions in neurons
Flavie Lavoie-Cardinal

S1-5 Protein mobility in the synaptic bouton
Sofiia Reshetniak, Jan-Eike Ußling, Eleonora Perego, Burkhard Rammner, Thomas Schikorski, Eugenio F. Fornasiero, Sven Truckenbrodt, Sarah Köster, Silvio O. Rizzoli

S1-6 Novel correlative imaging approaches for cellular structures
Felix Lange, Paola Agüí González, Nhu T. N. Phan, Stefan Jakobs, Silvio O. Rizzoli

Molecular resolution imaging by post-labeling expansion dSTORM (Ex-dSTORM)

Markus Sauer[1], Christian Werner[1], Janna Eilts[1], Fabian Zwettler[1]

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Expansion microscopy (ExM) enables super-resolution fluorescence imaging of physically expanded biological samples with conventional microscopes. By combining ExM with single-molecule localization microscopy (SMLM) it is potentially possible to approach the resolution of electron microscopy. However, current attempts to combine both methods remained challenging because of protein and fluorophore loss during digestion or denaturation, gelation, and the incompatibility of expanded polyelectrolyte hydrogels with photoswitching buffers. Here we show that re-embedding of expanded hydrogels enables dSTORM imaging of expanded samples and demonstrate that post-labeling ExM resolves the current limitations of super-resolution microscopy. Using reference structures, neurons and brain slices, we demonstrate that post-labeling Ex-SMLM can be used advantageously for super-resolution imaging. It preserves ultrastructural details, improves the labeling efficiency and reduces the positional error arising from linking fluorophores into the gel thus paving the way for super-resolution imaging of immunolabeled endogenous proteins with true molecular resolution.

Faster and better: high speed localisation precision without artifacts

Susan Cox[1]

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Super-resolution microscopy is a powerful tool for imaging structures at a lengthscale of tens of nm, but its utility for live cell imaging is limited by the time it takes to acquire the data needed for an image. For localization microscopy the speed at which an image of a given structure can be acquired is directly linked to the structure being imaged, leading to a factor of more than 1000 difference in how fast a particular resolution can be achieved in different types of structure, even given identical performance of dyes and optics. Another result of this is that in almost all localization microscopy datasets there are images where fluorophores overlap. As an initial approach to this problem, we combined principle component analysis and a random forest classifier to allow data from overlapping fluorophores to be removed.
However, this approach can only deal with a small degree of overlap. For localisation microscopy the acquisition time can be cut by more than two orders of magnitude by using advanced algorithms which can analyse dense data, trading off acquisition and processing time. Information can be traded for resolution: for example, the whole dataset can by modelled as arising from blinking and bleaching fluorophores (Bayesian analysis of Blinking and Bleaching), although at a high computational cost. However, all these approaches will come with a risk of artefacts, which can mean that the image does not resemble the underlying sample. We have recently developed Harr Wavelet Kernel (HAWK) analysis, a multi-timescale prefiltering technique which enables high density imaging without artefacts. The results of benchmarking with other techniques reveal that at high activation densities many analysis approaches may achieve high apparent precision, but poor accuracy. However, HAWK analysis produces images free from sharpening artefacts allowing accurate images to be rapidly taken. Furthermore, this property of HAWK can be used to identify artificial sharpening artefacts and assess the quality of localisation microscopy images.

Nanoscale organization of glutamate receptors and synaptic function

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The spatio-temporal organization of neurotransmitter receptors in the postsynaptic membrane is a fundamental determinant of synaptic transmission and thus information processing by the brain. Ionotropic AMPA glutamate receptors (AMPAR) mediate fast excitatory synaptic transmission in the central nervous system. Using a combination of high resolution single molecule superresolution imaging and tracking techniques, we have established that AMPARs are not all stable in the synapse as thought initially, but in large part undergo continuous entry and exit to and from the post-synaptic density through lateral diffusion. The other fraction of AMPAR are highly concentrated inside synapses into a few clusters of around seventy nanometers. These results have opened the new possibility that glutamatergic synaptic transmission is controlled by the regulation at the nanometer scale of the position and composition of these highly concentrated nanodomains. The dynamic exchange of AMPAR within the PSD and between synaptic and extrasynaptic sites is highly regulated by neuronal activity. We have demonstrated that AMPAR conformation strongly impacts their mobility, desensitized receptors being more mobile than naïve ones. This property likely explains how post-synaptic AMPAR receptor mobility can regulate short term synaptic plasticity, a feature previously ascribed to pre-synaptic mechanisms. Recently, using new methods to exogenously control AMPAR surface diffusion, we have demonstrated that AMPAR surface diffusion directly controls the establishment of long term synaptic plasticity. We will now present a series of new results that 1) establish a link between regulation of AMPAR surface diffusion and changes in short term plasticity during Long Term Depression, 2) expand the role of AMPAR surface diffusion to synaptic plasticity in vivo and 3) present how controlling AMPAR surface trafficking can provide insight into the implication of synaptic plasticity in various learning paradigms.

Development of machine learning approaches for quantitative super-resolution microscopy of molecular interactions in neurons

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[2]CERVO Brain Research Center, Québec, Canada
[3]Institute for Intelligence and Data, Psychiatry and Neuroscience

Understanding the molecular mechanisms underlying synaptic transmission is challenging in part because synapses are tiny (less than a micron), exhibit a wide range of shapes and internal structures, undergo activity-dependent plasticity, and their molecular components are dynamic. We must be able to observe the molecular dynamics and interactions of synaptic proteins at their scale: the nanoscale. Super-resolution microscopy (or optical nanoscopy) techniques allow characterizing molecular interactions inside living cells with unprecedented spatiotemporal resolution. These techniques come with several layers of complexity in their implementation. This has limited their adoption as well as their adaptability to multi-color, multi-modal, and long-term imaging in living neurons. Developing machine learning (ML) assisted frameworks for optical nanoscopy allows real-time optimization of multi-modal live-cell imaging at the nanoscale as well as for quantitative high throughput super-resolution image analysis. We use ML-assisted microscopy to characterize activity-dependent remodelling of neuronal proteins. Our ML approaches not only automate image analysis but also increase multi-dimensional analysis performance of synaptic nanostructures.

Protein mobility in the synaptic bouton

Sofiia Reshetniak[1,2], Jan-Eike Ußling[1], Eleonora Perego[3], Burkhard Rammner[1], Thomas Schikorski[4], Eugenio F. Fornasiero[1], Sven Truckenbrodt[1,2], Sarah Köster[3,5], Silvio O. Rizzoli [1,5]

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The synaptic bouton is one of the most intensely studied cellular compartments. The nature and the copy numbers of the molecules involved in synaptic vesicle recycling are known relatively well, and the mobility of some elements, such as the synaptic vesicles themselves, has been thoroughly investigated. Nevertheless, just like for other cellular systems, the dynamic organization of the synaptic bouton remains elusive and we only have a sketchy view of the overall dynamics of the synaptic proteins, and the mechanisms regulating the protein composition of the synaptic bouton are still unclear.

In this study we combined fluorescence recovery after photobleaching (FRAP), particle tracking, electron microscopy, and modeling to determine protein mobility in synaptic boutons and axons of primary hippocampal neurons. We analyzed 45 different proteins, including synaptic vesicle proteins, endo- and exocytosis cofactors, cytoskeleton components, and trafficking proteins. To account for synaptic geometry during FRAP results analysis, we modeled protein diffusion in an “average” synapse, relying on a realistic 3D space created using data from 3D electron microscopy reconstructions of synapses of primary hippocampal neurons.

Our results suggest that simple and highly robust mechanisms, based on the synapse geometry and on binding to the synaptic vesicles, can account for the movement and distribution of many synaptic proteins, thereby also explaining how the composition of this compartment can be maintained over time. Our work resulted in a first visualization of overall protein motion in the synapse, which illustrates measured mobility of synaptic proteins in their realistic copy numbers within realistic synapse geometry. These results provide valuable insight in synaptic biology and should enable future modeling studies of the synapse.

Novel correlative imaging approaches for cellular structures

Felix Lange[1,2], Paola Agüí González[2], Nhu T. N. Phan[2], Stefan Jakobs[1,2], Silvio O. Rizzoli[2]

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Cellular structure and function are currently investigated by a variety of imaging techniques, with resolutions ranging from sub-nanometres to millimetres. The best approaches to an understanding of the cellular structure are typically connected to the use of electron microscopy, in which dense cellular elements are visualised with high precision in both, 2D and 3D. A downside to this approach is that specific organelles are typically identified only based on their morphology, since most electron microscopy applications are performed without labelling the organelles in a specific fashion. Specific subcellular localization of proteins for electron microscopy can be limited by a low number of epitopes in the sample being accessible for antibody labelling and usually requires substantial optimization of the staining protocols. A correlation with fluorescence microscopy (CLEM) enables the investigation of the location and function of specific cellular components by combining the information of both imaging modalities. Thus, a protein of interest can be studied within its ultrastructural context. While CLEM allows to re-assign functional information with decent precision within the corresponding ultrastructural context, it is still unable to address the chemical composition of cells, which is the domain of secondary ion mass spectrometry (SIMS). As SIMS is inherently unable to pinpoint specific cellular components, it has been correlated in several studies to either electron or fluorescence microscopy. However, only a correlation of all three techniques (CLEM-SIMS) would result in an optimal investigation of cell structure, function and composition. We generated here a protocol for CLEM-SIMS, based on transmission electron microscopy (TEM), conventional epifluorescence microscopy and nanoSIMS. The protocol was easily applied, and enabled the use of the three technologies at maximal performance parameters that can be carried out on a single prepared specimen. We suggest that CLEM-SIMS could provide hitherto inaccessible and novel insights into cellular function beyond the scope of conventional correlation approaches.

Symposium

S2: Neuronal circuit mechanisms of socio-sexual behavior

S2-1 Social touch promotes interfemale communication via activation of parvocellular oxytocin neurons
Yan Tang, Diego Benusiglio, Arthur Lefevre, Louis Hilfiger, Alexandre Charlet, Valery Grinevich

S2-2 Neural mechanisms underlying sexual behavior
Susana Lima

S2-3 Anatomical and functional characterization of the spinal circuits underlying ejaculation
Constanze Lenschow

S2-4 Circuits for care - neural control of parental behavior
Johannes Kohl

S2-5 Neural correlates of play behavior in midbrain circuits
Jean Simonnet, Miguel Concha-Miranda, Michael Brecht

S2-6 Sensory integration and movement decision in swarm forming locusts
Inga Petelski, Yvonne Hertenberger, Einat Couzin-Fuchs

Social touch promotes interfemale communication via activation of parvocellular oxytocin neurons

Yan Tang[1], Diego Benusiglio[2], Arthur Lefevre[2], Louis Hilfiger[3], Alexandre Charlet[3], Valery Grinevich[2]

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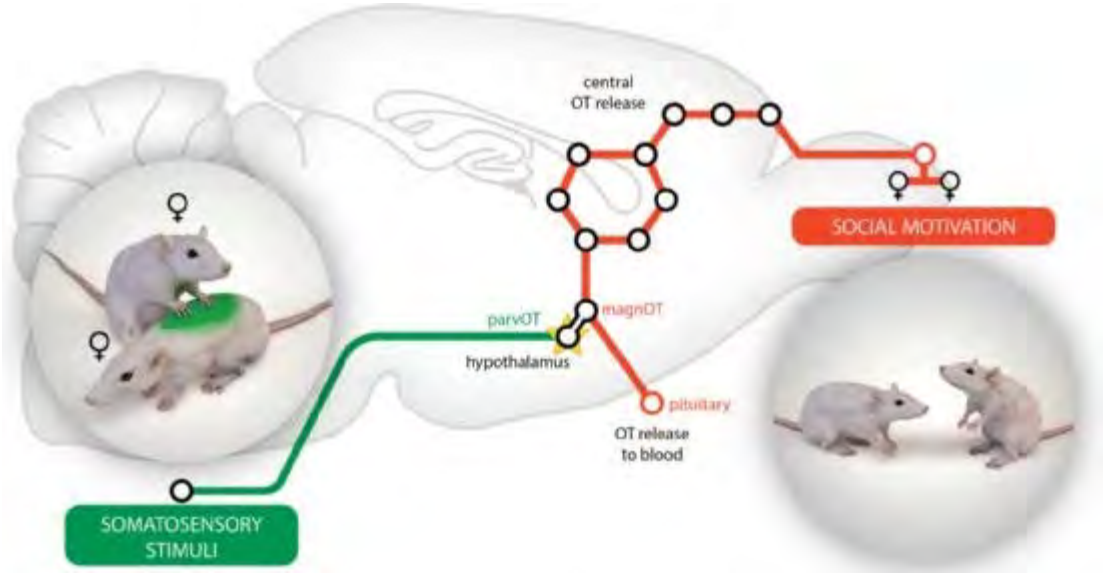
Oxytocin (OT) is a great facilitator of social life but, although its effects on socially relevant brain regions have been extensively studied, OT neuron activity during actual social interactions remains unexplored. Most OT neurons are magnocellular neurons, which simultaneously project to the pituitary and forebrain regions involved in social behaviors. In the present study, we show that a much smaller population of OT neurons, parvocellular neurons that do not project to the pituitary but synapse onto magnocellular neurons, is preferentially activated by somatosensory stimuli. This activation is transmitted to the larger population of magnocellular neurons, which consequently show coordinated increases in their activity during social interactions between virgin female rats. Selectively activating these parvocellular neurons promotes social motivation, whereas inhibiting them reduces social interactions. Thus, parvocellular OT neurons receive particular inputs to control social behavior by coordinating the responses of the much larger population of magnocellular OT neurons.



Neural mechanisms underlying sexual behavior

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Sex is intrinsically rewarding but can also be costly, by increasing the risk of predation or infection. Therefore, it is not surprising that natural selection reinforced mechanisms which ensure that sexual behavior is initiated when fertilization is most likely and inhibited after consummation. In many species, this is achieved by placing ovulation and sexual receptivity under the control of sex hormones in females, and by the establishment of a refractory period after ejaculation in males. We study sexual behavior in mice, using a combination of electrophysiological and genetically encoded imaging and anatomical tools to understand how the coordinated activity of different neuronal populations underlies the flexible, state dependent modulation of this fundamental behavior.

Anatomical and functional characterization of the spinal circuits underlying ejaculation

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During sexual behavior, copulation related sensory information and modulatory signals from the brain must be integrated and converted into the motor and secretory outputs that characterize ejaculation (Lenschow and Lima, Current Opinion in Neurobiology, 2020). Studies in humans and rats suggest the existence of interneurons in the lumbar spinal cord that mediates that step: the spinal ejaculation generator (SEG). My work aimed at gaining mechanistic insights about the neuronal circuits controlling ejaculation thereby applying cutting-edge techniques.
More specifically, we mapped anatomically and functionally the spinal circuit for ejaculation starting from the main muscle being involved in sperm expulsion: the bulbospongiosus muscle (BSM). Combining viral tracing strategies with electrophysiology, we specifically show that the BSM motoneurons receive direct synaptic input from a group of interneurons located in between lumbar segment 2 and 3 and expressing the peptide galanin. Electrically and optogenetically activating the galanin positive cells (the SEG) lead to the activation of the motoneurons innervating the BSM and the muscle itself. Finally, inhibition of SEG cells using DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) in sexual behaving animals is currently conducted to reveal whether ejaculation can be prevented.

Circuits for care - neural control of parental behavior

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Parenting is essential for the survival and wellbeing of offspring in many species, but we lack a circuit-level understanding of how this behaviour is orchestrated. Using viral tracing, in vivo imaging, optogenetic manipulations and behavioural profiling in mice, we recently discovered that a genetically specified class of hypothalamic neurons forms projection-defined subpopulations, each controlling specific aspects of parenting. This functional organization provides a new model for how discrete elements of a social behaviour are generated at the circuit level. Our current goal is to understand how physiological states (such as pregnancy, hunger and sleep) alter the form and function of this and other circuits for instinctive behaviours.

Neural correlates of play behavior in midbrain circuits

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Play is a very common behavior in humans and many other species, even more common than sex or fighting in adults. Play behaviors are generally executed repeatedly without any obvious purpose but still, they have a crucial role in the development of social abilities as well as in brain development. The neurobiological substrate of play remains yet to be determined as we still don't know what part of the brain, or what brain state or activity pattern generates or enables play behaviors.

Social play behaviors such as rough and tumble play resemble attack, defense and escape behaviors, and they require communication between players. In the midbrain, the superior colliculus (SC) and the periaqueductal gray (PAG) have been involved in enabling escape or attack behaviors as well as in the generation of ultrasonic vocalizations in rodents.

Building on those evidence, we hypothesized that play could arise from specific midbrain circuit activity patterns. We are now checking this hypothesis by recording neurons in the SC and the PAG of young male rats (using chronic implants of tetrode drives or neuropixel probes) while tickling and playing with them.

I will here present the first preliminary results we obtained by focusing on the activity patterns during different phases of our interactions with the animal. Many neurons of the SC and the PAG seem to be modulated along the experimental phases, however we are still unsure whether those activity patterns are specific to play, or just to general sensory motor processes (touch, vision, audition, movement). We are currently trying to reproduce our experiments to include more controls, and we started exploring a potential link between midbrain activity patterns and the generation of ultrasonic vocalizations during play.

Sensory integration and movement decision in swarm forming locusts

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Plague-forming locusts are currently threatening the food supply of at least 20 million people in Asia, Middle East and East Africa. Despite improved technologies for control and prediction, 2020 resulted in the largest swarms of the desert locust, Schistocerca gregaria, in decades. Understanding the process by which these voracious insects sense and select feeding sites will be essential for understanding how the behavior of individuals scales to the collective action of swarms. Due to the ephemeral, and thus unpredictable nature of their nutritional environment, locusts have adapted to both isolated and highly social lifestyles: if individuals have plentiful access to food they will tend to remain “solitarious”, exhibiting a tendency to avoid conspecifics; if food becomes scarce, and individuals are forced to aggregate on remaining vegetation, they transition to the “gregarious” swarm-forming morph. Little is presently known about how locusts utilize asocial (e.g. sight and/or smell of food) and social cues (e.g. presence or action of conspecifics) when making foraging decisions. Given the complexity of their sensory environment, where the presence of social odors may mask food odors, and where the presence of conspecifics may indicate both the presence of food and also areas of increased competition for resources, it is essential to establish a connection between the neuronal representation of salient cues and the movement decisions made by individuals. We demonstrate that, when gregarious, the desert locust is highly attracted to feeding conspecifics (as opposed to a control where insects are present but are not feeding), and that this attraction relies on food-associated odors also being present at that locality; that is, neither sight nor smell alone is sufficient to induce strong attraction. By contrast, solitarious insects differ in their response to the environment, often discount social cues, and approach areas where food-odor is presented alone. To develop a mechanistic understanding of the sensory integration of social and asocial cues by both gregarious and solitarious locusts, we employ calcium-imaging of the projection neurons in the antennal lobe when a food-associated odor (3-hexen-1-ol) and/or a social odor (colony extract) is presented. We find that the neural response to a social odor differs between solitarious and gregarious morphs with the latter being reduced. Despite the fact that the neural representation of a social odor is weak in gregarious insects when food odor is also present, the antennal lobe activity in response to the mixture is much greater than the one when food odor is presented alone. This suggests a sensory-based mechanism that can account for gregarious insects’ strong attraction to feeding conspecifics, and may also indicate an adaptation that allows them to detect food sources that would otherwise be masked by the strong social odors present in dense swarms.

Symposium

S3: Modulation and Plasticity of Inhibition in Neocortical Circuits

S3-1 Circuit motifs of neocortical VIP interneurons
Jochen Staiger

S3-2 Disinhibitory control of contextual modulation and cortical dynamics
Julia Veit, Hillel Adesnik

S3-3 Inference of inhibitory and excitatory connectivity from responses in mouse visual cortex using a stabilized supralinear network model
Simon Renner, Nataliya Kraynyukova, Yannik Bauer, Gregory Born, Martin Spacek, Georgi Tushev, Laura Busse, Tatjana Tchumatchenko

S3-4 Learning-related plasticity of inhibition in neocortical layer 1
Johannes Letzkus

S3-5 Transient developmental increase of prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice
Jastyn A. Pöpplau, Sebastian H. Bitzenhofer, Mattia Chini, Annette Marquardt, Ileana L. Hanganu-Opatz

S3-6 Inhibition in critical periods for plasticity in the developing auditory cortex
Tania Rinaldi Barkat

Circuit motifs of neocortical VIP interneurons

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Vasoactive intestinal polypeptide (VIP) expressing cells are one of three major subpopulations of cortical GABAergic neurons. Although they represent only about 1-2% of all cortical neurons, there is growing interest in them due to their behavioral relevance with respect to transmitting global saliency information to cortical circuits for sensory-guided motor behavior. However, whether this is their predominant functional contribution to cortical processing and whether there are specific types or even subtypes of VIP neurons is still a matter of debate. By using Rabies tracing, we have shown that a wide range of long-distance projections converge upon VIP cells in barrel cortex. By applying optogenetic stimulation and single cell reconstructions in VIP-Cre/tdTomato mice, we have obtained evidence that almost all recorded VIP cells are innervated by the lemniscal and paralemniscal pathways. Using neuropharmacology, we found that all L2/3 VIP cells show nicotinic receptor-mediated responses to acetylcholine application; furthermore, only some 50% respond to serotonergic stimulation via 5HT3a-receptor mediated fast depolarization whereas a 100% were slowly depolarized via 5HT2-receptors. They may feed forward this combined sensory and saliency information to their target cells, which we found to consist of intra- and translaminar Martinotti cells as well as non-Martinotti cells. In future experiments utilizing intersectional mouse lines, we aim to better understand these connections by possibly probing presynaptic VIP cell types, which are however largely elusive to date.

Disinhibitory control of contextual modulation and cortical dynamics

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Neural synchronization on fast timescales has been linked to critical aspects of sensation, cognition and action, and impairments in synchronization are associated with neurological disease. Although the strength and spatial scale of neural synchronization varies dramatically with sensory and behavioral context, the circuit mechanisms that regulate the magnitude and spatial spread of neural oscillations are largely unknown. As in humans, monkeys, and cats, we found that in mouse visual cortex (V1) the stimulus properties and behavioral state powerfully modulate gamma band synchronization. To reveal the underlying circuit that mediates this dependence, we used multi-site multi-electrode electrophysiology and optogenetic perturbations in awake mice. We found that through disinhibition, vasoactive intestinal peptide (VIP) interneurons potently control the stimulus and behavioral state dependence of gamma band network synchronization. VIP neurons control the correlation of pyramidal cell spiking on fine time scales, scaling down or even eliminating the phase coupling of many neurons to the local network depending on the stimulus statistics and brain state. Based on these data, we propose that cortical disinhibition by VIP interneurons fine-tunes the strength and spatial architecture of synchronously spiking neural assemblies, which may facilitate the downstream generation of coherent visual percepts.

Inference of inhibitory and excitatory connectivity from responses in mouse visual cortex using a stabilized supralinear network model

Simon Renner[1,2,5], Nataliya Kraynyukova[3,5], Yannik Bauer[1,2], Gregory Born[1,2], Martin Spacek[1], Georgi Tushev[3], Laura Busse[1,4,6], Tatjana Tchumatchenko[3,6]

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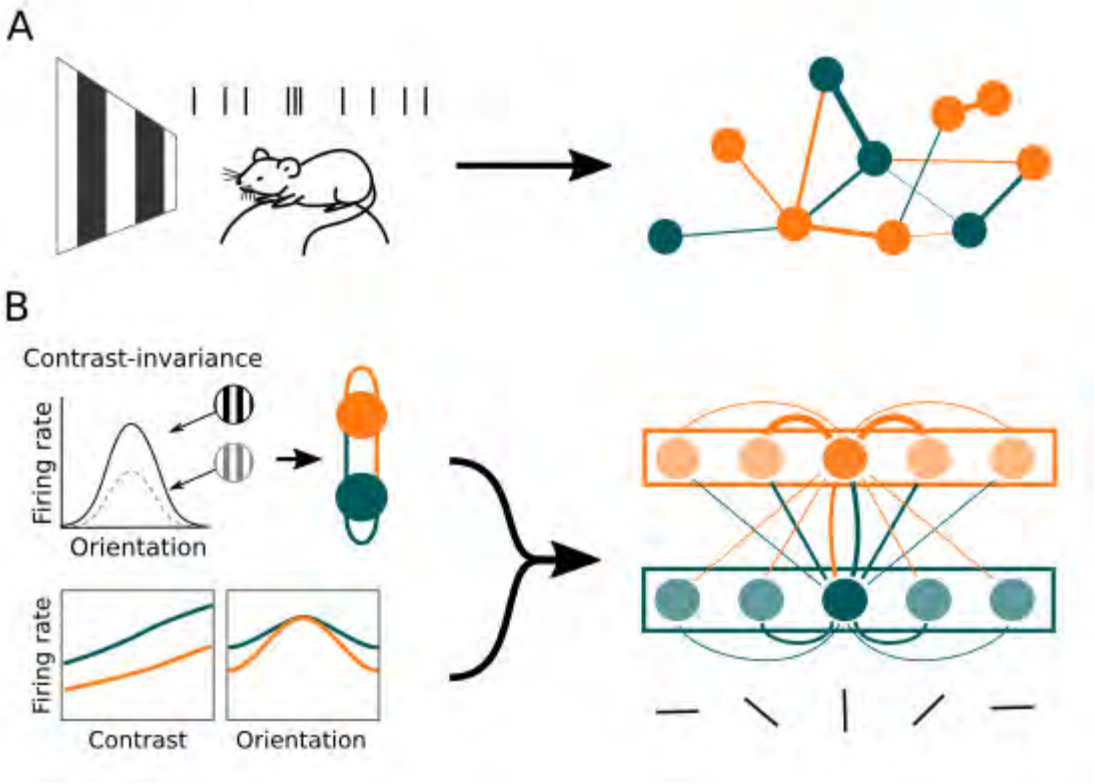
Functional connections between inhibitory and excitatory neurons shape the computational repertoire of cortical circuits. At the same time, observed cortical computations themselves place restrictions onto the underlying connectivity, resulting in a reciprocal relationship between connectivity and computation. Relating connectivity to computation is thus of utmost importance to understanding cortical function. In visual cortex, modeling efforts have successfully based computational properties like signal normalization on functional connectivity regimes, while connectomics studies have connected function and physiological connectivity *in-vitro*. However, a computational framework relating cortical *in-vivo* responses to functional connectivity has been missing.

Here, we combine a state-of-the-art cortical model with extracellular recordings of responses from mouse visual cortex (V1) and thalamus (dLGN) to infer functional connectivity between cortical inhibitory and excitatory cell populations as well as excitatory thalamic inputs. To this end, we leverage the experimental finding that the width of orientation tuning curves in V1 is independent of contrast, termed contrast-invariance, in order to invert a stabilized supralinear network model (SSN) of V1. After confirming contrast-invariance in recordings from mouse V1 obtained with silicon probes, we use a two-step process to feed the inverted SSN with contrast and orientation responses from V1 as well as dLGN.

Indicating the general validity of our inference procedure, we find that the resulting inferred connectivity profiles share many features with previous findings from the literature, like broad input to inhibitory interneurons and stabilizing of recurrent excitation via recurrent inhibition. We also find inhibitory to excitatory connections to be the strongest overall and thalamic input connections to be weaker than cortical connections. Our findings thus emphasize the critical role of inhibition for cortical computation and provide a framework to relate firing responses to functional connectivity. The framework also allows predicting the effects of firing rate manipulations on functional connectivity, providing an opportunity to study plasticity effects in functional connectivity, which we plan to investigate in further studies.


A
B
Contrast-invariance
Firing rate
Orientation
Firing rate
Contrast
Orientation

Learning-related plasticity of inhibition in neocortical layer 1

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To perceive the world around us, mere representation of sensory bottom-up evidence is insufficient. Instead, perception critically depends on continuous comparison between bottom-up information and our internally-generated models of the environment that are formed through experience. Neocortical layer 1 is a major site of convergence for a variety of brain-wide afferents carrying such experience-dependent top-down information. In turn, processing of top-down signals could be strongly and dynamically modulated by local inhibition and disinhibition supplied by layer 1 interneurons. Using a selective genetic marker for a subpopulation of layer 1 interneurons (Ndnf) in combination with in vivo 2-photon calcium imaging, electrophysiology, viral tracing and optogenetics, we find that Ndnf positive layer 1 interneurons in mouse auditory cortex provide inhibition widely to other interneuron types as well as to the distal dendrites of pyramidal neurons, thereby controlling the local circuit across different layers. These connections recruit strong Gaba-B receptor signaling, and both in vitro and in vivo data indicate that, in addition to somato-dendritic inhibition, this mechanism can also robustly affect the release probability of long-range afferents from the higher-order thalamus by activation of presynaptic Gaba-B receptors, dynamically modulating both the strength and the frequency transfer function of this major top-down projection. Moreover, the sensory responses of these layer 1 interneurons are highly plastic in response to both associative and non-associative learning paradigms. Our results thus identify Ndnf positive layer 1 interneurons as a key cell type that encodes the behavioral relevance of sensory information, and in turn adjusts a variety of circuit functions.

Transient developmental increase of prefrontal activity alters network maturation and causes cognitive dysfunction in adult mice

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Rhythmic fast frequency oscillations in prefrontal cortex (PFC) have been identified as neural network attributes of cognitive processing. In line with our recent data, they emerge at neonatal age as result of locally synchronized firing of layer 2/3 pyramidal neurons and progressively accelerate with age. However, it is still a matter of debate whether the early oscillatory activity is necessary for cortical circuit formation and behavioral performance at adult age. Here, we directly test the contribution of early activity in shaping cortical network refinement by manipulating neonatal oscillations through chronic stimulation of layer 2/3 pyramidal neurons. A mildly increased level of activity in the PFC during a defined period of neonatal development causes premature dendritic growth of layer 2/3 pyramidal neurons and altered interneuronal densities immediately after the stimulation period. These transient morphological changes lead to permanent circuit dysfunction of the adult circuit that shows weaker gamma synchronization in response to stimuli and abnormal inhibitory feedback of fast-spiking parvalbumin interneurons. Consequently, manipulation of early oscillatory activity causes at adulthood an excitation/inhibition imbalance shifted towards inhibition. These functional changes ultimately lead to impaired social interactions and poorer recognition and working memory. Our results highlight that early patterns of prefrontal oscillatory activity shape the development of neuronal networks and the behavioral performance of adults.

Inhibition in critical periods for plasticity in the developing auditory cortex

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Critical periods are time windows of heightened plasticity in postnatal brain development. They are specific to sensory features and do not all happen at the same time. In the auditory system for example, the critical period for pure tone precedes the critical period for frequency modulated sweep (FMS) by about two weeks. The role of inhibition in these critical periods is not known. We used in vivo electrophysiological recordings in combination with molecular and sensory manipulations to elucidate the biological constraints on critical period timing in the mouse auditory system. Enhancing γ-aminobutyric acid (GABA) function before the critical period for pure tone accelerated it, but had no effect on the critical period for FMS. During the FMS critical period however, an immunohistochemical analysis revealed a decreased parvalbumin (PV) expression in cortical layer 4, paralleled functionally with a transient increase in response to FMS. Exposing mice to continuous white noise prevented the change of PV expression and delayed the FMS critical period onset, suggesting a causal role between a decrease in PV expression and this late critical period. Together, these results suggest that it is a change in the excitatory/inhibitory ratio per se – it being positive for the critical period for pure tone and negative for the one for FMS – and not an increase in inhibition only that triggers an opportunity for change in a neural circuit, as the one expressed during critical periods. Our findings shed new light on the dependence of sensory features on inhibition and on the mechanisms at play in developmental plasticity.

Symposium

S4: Neuronal Autophagy - Implications for Disease and Therapy

S4-1 Hyperactive LRRK2 kinase alters neuronal autophagy by disrupting the axonal transport of autophagosomes
Erika L.F. Holzbaur, C. Alexander Boecker, Juliet Goldsmith, Dan Dou

S4-2 Autophagy in Alzheimer's Disease
Ralph Nixon

S4-3 Sigma-1 receptor-mediated autophagy
Maximilian G. Christ, Heike Huesmann, Heike Nagel, Lisa Hueske, Andreas Kern, Albrecht M. Clement, Christian Behl

S4-4 Modulating autophagy by the endosome-associated protein RME-8/DNAJC13
Albrecht M. Clement

S4-5 Axonal autophagy as a pathogenic mechanism in motoneuron disease
Patrick Lüningschrör, Markus Damme, Georg Werner, Beyenech Binotti, Anja Capell, Jahn Reinhard, Kaltschmidt Christian, Sendtner Michael

S4-6 Synaptic vesicle pools at endbulb of Held active zones upon development and lack of activity
Anika Hintze, Esther Semmelhack, Carolin Wichmann

Hyperactive LRRK2 kinase alters neuronal autophagy by disrupting the axonal transport of autophagosomes

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Neurons rely on autophagy, a critical homeostatic mechanism, to maintain cellular health over the decades of human life. Deficits in autophagy cause the accumulation of protein aggregates and dysfunctional mitochondria, and are characteristic of major neurodegenerative diseases including Parkinson's disease. Due to the unique morphology of neurons, effective neuronal autophagy is heavily dependent on robust axonal transport of autophagosomes, which are constitutively formed in the distal axon and then rapidly transported to the soma. Parkinson's disease-associated mutations in LRRK2 have been shown to hyperactivate LRRK2 kinase activity, causing increased phosphorylation and altered function of Rab GTPases, which are key regulators of intracellular organelle trafficking. Here, we ask how the hyperactivating G2019S mutation in LRRK2 affects essential axonal trafficking pathways in neurons. While we saw no disruption of lysosomal trafficking or microtubule dynamics, we found that expression of LRRK2-G2019S caused robust defects in autophagosome transport that were consistently observed in an overexpression model, cortical neurons from a LRRK2-G2019S knock-in mouse, and human iPSC-derived neurons gene-edited to express the G2019S mutation. Hyperactivation of LRRK2 by overexpression of Rab29, a known activator of LRRK2 that has also been genetically linked to Parkinson's disease, resulted in a similar disruption of axonal autophagosome transport. Mechanistically, we found that hyperactive LRRK2 recruits the motor adaptor JIP4 to the autophagosomal membrane, inducing abnormal activation of kinesin and causing an unproductive tug-of-war between mis-regulated anterograde and retrograde motors bound to autophagic vesicles, thus inhibiting processive unidirectional motility. Defective autophagosome transport was accompanied by impaired organelle acidification along the axon, suggesting that the observed transport deficit impairs effective autophagosomal cargo degradation in neurons. Notably, defects in autophagosome transport and maturation could be reversed by pharmacological inhibition of LRRK2 kinase activity. Together, our findings demonstrate that increased LRRK2 kinase activity is sufficient to induce defects in autophagosome transport and maturation, further implicating defective autophagy in the pathogenesis of Parkinson's disease. Supported by the Michael J. Fox Foundation (Grant #15100) and the German Research Foundation (DFG; BO 5434/1-1).

Autophagy in Alzheimer's Disease

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In Alzheimer's Disease, massive "storage" of waste proteins in neuronal autolysosomes, reminiscent of the pathology in lysosomal storage diseases, reflects a continuum of lysosomal system functional deficits first appearing before amyloid is deposited and culminating in an explosive autophagy-associated cell death. The recent understanding of the biology underpinning actions of AD related genes has identified primary lysosomal system dysfunction in AD as a mechanism that cripples neuronal functions critical for synaptic plasticity and neuron survival in addition to promoting accumulation of toxic proteins, including Aß and tau. The progressive failure of autophagy in AD can be traced to lysosomal dysfunction rather than to deficits at earlier steps in the pathway. Modulation of autophagy is now being actively investigated as a possible therapy for AD and other proteinopathies. Striking amelioration of diverse deficits by remediating lysosomal dysfunction in AD mouse models underscores the pathogenic significance of this dysfunction and indicates the considerable promise of autophagy modulation in the therapy of AD, PD, and related diseases of aging.
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Sigma-1 receptor-mediated autophagy

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Dysfunction of autophagy and protein homeostasis is linked to pathogenesis of human neurodegenerative diseases and modulation of autophagy has become one key pharmacological target. Due to the pleiotropic neuroprotective effects of sigma-1 receptors (Sig-1R) in various experimental paradigms, Sig-1R activation is recognized as a potential approach for prevention and therapy of neurodegeneration. Interestingly, mutations in the Sig-1R gene are associated with different neurodegenerative diseases like ALS and ALS-associated mutations cause a disturbed autophagy. Here we analyzed the effects of Sig-1R activation by PRE-084, a selective Sig-1R agonist, and by ANAVEX2-73, a muscarinic receptor ligand and Sig-1R agonist, on autophagy and proteostasis. We describe on the molecular level that pharmacological Sig-1R activation a) enhances autophagic flux *in vivo* and *in vitro*, and b) increases proteostasis capacity, ameliorating paralysis caused by protein aggregation in *C. elegans*. Our findings showing a direct impact of Sig-1R activation on proteostasis maintenance underlines Sig-1R as a potential target for different neurodegenerative disorders linked to protein aggregation. Moreover, they may fuel detailed studies on the molecular mechanisms of autophagy modulation by Sig-1R activation and its potential role in autophagic endoplasmatic reticulum remodeling (ER-phagy) where Sig-1R is highly expressed. Employing Sig-1R KO and mutant cells, as well as additional Sig-1R activating compounds, we aim to further understand and study the role of Sig-1R at the ER and the link between Sig-1R, autophagy, proteostasis and neurodegeneration.

Modulating autophagy by the endosome-associated protein RME-8/DNAJC13

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The maintenance of protein homeostasis is of vital importance for cellular function under steady-state conditions and is continuously challenged particularly upon exposure to acute or chronic insults. The endosome as a cellular hub for protein sorting is central in regulating lysosome-linked degradative processes. We identified the gene *receptor-mediated endocytosis 8* (*rme-8*; human ortholog: *DNAJC13*) in a functional proteostasis screen in *C. elegans*. RME-8/DNAJC13 belongs to the HSP40/DNAJ-domain containing protein family, is associated with the endosome, and is linked to familial forms of Parkinson disease. RME-8/DNAJC13 stabilizes proteostasis as the accumulation of aggregation-prone proteins, including alpha-synuclein, is aggravated upon *rme-8/DNAJC13* knockdown. We provide evidence that RME-8/DNAJC13 positively modulates autophagy in *C. elegans* as well as in cell lines, including differentiated human dopaminergic LUHMES cells. The cellular analysis revealed that DNAJC13 is involved in the trafficking of ATG9A which is an important component for the delivery of lipids towards the growing phagophore. In addition, Parkinson disease-linked DNAJC13(N855S) is not able to increase autophagic flux in contrast to the wildtype protein. These data demonstrate a novel function of RME-8/DNAJC13 in cellular homeostasis by modulating autophagy and point towards a possible disease mechanism related to Parkinson-associated mutants.

Axonal autophagy as a pathogenic mechanism in motoneuron disease

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The maintenance of synaptic integrity in highly polarized cells such as motoneurons requires specific mechanisms that trigger selective degradation of proteins and organelles in the presynaptic compartment. In most forms of motoneuron disease (MND), synaptic dysfunction and synapse elimination precede motoneuron loss, suggesting that the axon and neuromuscular junctions represent an early target in MND pathology. The autophagosomal/lysosomal system is highly regulated on multiple levels in different axonal compartments of motoneurons. Pathological dysregulation of these pathways is responsible for distinct MNDs.

Our research has focused on two pathogenic mechanisms mediated by Plekhg5 and Tmem106b. Plekhg5 regulates the autophagy of synaptic vesicles in axon terminals of motoneurons via its function as a GEF for Rab26, a small GTPase that specifically directs synaptic vesicles to autophagosomes. Tmem106b functions in modulation of the axonal transport of Lamp1-positive organelles and axonal sorting at the axon ignition segment.

Mutations in the human *PLEKHG5* gene are the cause of a wide range of MND. Besides, a variant in the *PLEKHG5* gene has been identified as a disease modifier in a family with TDP43-linked ALS with a rapidly progressing, early-onset disease. Here, we show that depletion of Plekhg5 in mice results in an MND with late-onset characterized by swollen axon terminals of motoneurons with synaptic vesicle accumulations. Plekhg5-depleted cultured motoneurons show defective axon growth and impaired autophagy of synaptic vesicles, which can be rescued by constitutively active Rab26. Furthermore, Plekhg5 loss has opposing effects in the early and late stages of ALS in mice. Deletion of Plekhg5 in SOD1-G93A expressing mice prepones the disease onset, but decelerates the disease progression, resulting in prolonged survival.

As a second mechanism, we show that the lysosomal membrane protein Tmem106b plays an essential role in the maintenance of the proximal part of the axon. Genetic variations in *TMEM106B* are associated with FTLD in GRN- and C9orf72-expansion carriers. We show that Tmem106B-deficient mice develop proximal axonal swellings caused by drastically enlarged Lamp1-positive vacuoles, increased retrograde axonal transport of lysosomes, and accumulation of lipofuscin and autophagosomes. Giant vacuoles specifically accumulate at the axon initial segment in motoneurons, but not in peripheral nerves or at axon terminals. These pathological alterations result in impaired motor performance predominantly of muscle groups that are innervated by the facial nerve. Taken together, we provide mechanistic insight into how Tmem106B affects lysosomal proteolysis and the degradative capacity in the proximal compartment of motoneurons.

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Synaptic vesicle pools at endbulb of Held active zones upon development and lack of activity

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Endbulbs of Held are the first central synapses of the mammalian auditory pathway. They are formed by the auditory nerve fibers that project onto bushy cells that are located in the anteroventral cochlear nucleus of the lower brainstem (Brawer and Morest, 1975). One endbulb of Held contains hundreds of individual active zones (AZs). During development, endbulbs evolve a fast signal transmission with high temporal fidelity that is essential for their role in auditory processing tasks. The lack of the hair cell specific protein otoferlin ($Otof^{-/-}$) results in an almost abolished exocytosis in the murine cochlea (Roux et al., 2006) and downstream in alterations of endbulb morphology (Wright et al., 2014). In humans, mutations in the OTOF gene result in DFNB9 non-syndromic hearing loss (Varga et al., 2003, Yasunaga et al., 1999).
We hypothesize that the number and distribution of synaptic vesicles (SVs) at endbulb of Held AZs changes upon development or the lack of activity.
In order to analyze morphological SV pools at individual endbulb AZs, we performed high-pressure freezing and freeze-substitution (HPF/FS) followed by electron tomography. HPF/FS leads to a rapid immobilization of the tissue and allows us to determine the number and distances of SVs in a near-to-native state. We compared ultrastructural parameters such as SV numbers of 10-day, 21-day and 6-month-old C57BL/6J wild-type (wt) and $Otof^{-/-}$ mice. We found a comparable vesicle pool size between wt and $Otof^{-/-}$ of 10-day and 21-day old mice. However, the SV number increased upon maturation towards adulthood at wt AZs, but decreased at $Otof^{-/-}$ AZs. The average number of docked SVs remained unaltered in all groups. Our results indicate a correlation between synapse activity and the number of SVs at individual AZs.

Symposium

S5: Tanycytes - walk between worlds

S5-1 Implication of leptin receptors expressed in hypothalamic tanycytes in the central control of energy homeostasis
Manon Duquenne, Cintia Folgueira Cobos, Cyril Bourrouh, Marion Millet, Anisia Silva, Emilie Caron, Jérome Clasadonte, Ruben Nogueiras Pozo, Jean-Sébastien Annicotte, Stéphane Gasman, Julie Dam, Vincent Prévot

S5-2 Microglial insulin signalling plays a role in the progression of dietary-induced obesity
Irina Vladimirova Milanova, Nikita L Korpel, Felipe Correa-da-Silva, Eric Fliers, Susanne E la Fleur, Andries Kalsbeek, Chun-Xia Yi

S5-3 Hypothalamic dopamine system and its interaction with peripheral tissues to control energy balance
Ruben Nogueiras

S5-4 Hypothalamic control of median emminence barrier function
Jens Brüning

S5-5 To infer tanycyte-neuron communications through single-cell transcriptomics data
Fanny Valérie Langlet

S5-6 Characterisation of intracellular signal transduction of adrenoceptors and $GABA_B$-receptors in tanycytes
Natascha Klaus, Helge Müller-Fielitz, Marcus Stahr, Markus Schwaninger

Implication of leptin receptors expressed in hypothalamic tanycytes in the central control of energy homeostasis

Manon Duquenne[1], Cintia Folgueira Cobos[2], Cyril Bourrouh[3], Marion Millet[4], Anisia Silva[5], Emilie Caron[1], Jérome Clasadonte[1], Ruben Nogueiras Pozo[2], Jean-Sébastien Annicotte[3], Stéphane Gasman[4], Julie Dam[5], Vincent Prévot[1]

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The control of energy balance that allows for the maintenance of body mass requires a continued dialogue between the periphery and the hypothalamus in the brain. The access of peripheral hormones to that structure is essential to the proper functioning of neural circuits that regulate energy balance. However, little is known about the transport mechanisms of circulating metabolic signals into the hypothalamus. The median eminence, a hypothalamic structure forming the floor of the 3rd ventricle, contains specialized ependymoglial cells called tanycytes. Tanycytes have been shown to shuttle metabolic signals such as leptin into the cerebrospinal fluid, via transcytosis. Identifying the molecular mechanisms involved in this transport is essential to our understanding of the phenomenon of central hormone resistance found in obese and type 2 diabetes patients. After an infusion of a recombinant fusion protein (TAT-Cre) into the 3rd ventricle of leptin receptor gene-floxed mouse model ($LepR^{loxP/loxP}$), we investigated the role of the LepR in tanycytes on the central control of energy homeostasis in mice. Our results show that selectively impairing LepR expression in tanycytes increases body weight, adiposity, cholesterolemia, triglyceridemia and decreases noradrenaline serum concentration. It's associated with an increase of food intake, peripheral but not central leptin anorectic effect and glucose intolerance. Pancreas and adipose tissue activity of our model is also affected. Altogether, these data demonstrate for the first time the key role of tanycytes in the central control of energy regulation in-vivo, the involvement of LepR expression in tanycytes for circulating leptin action in the metabolic brain.

Microglial insulin signalling plays a role in the progression of dietary-induced obesity

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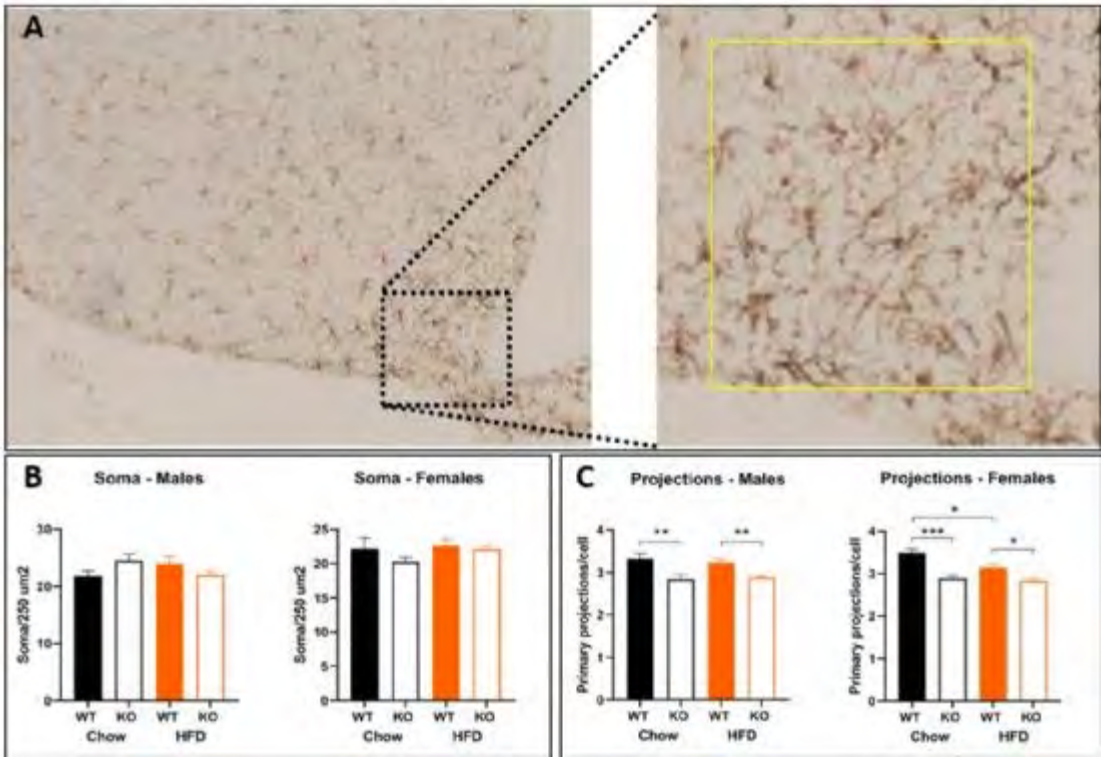
Background: Obesity and type 2 diabetes mellitus (T2DM) are highly prevalent metabolic disorders which are among the leading causes of death worldwide. One of the hallmarks of obesity and T2DM is insulin resistance. Microglia – the resident immune cells in the brain responsible for keeping a healthy activated by an obesogenic diet. Microglia express insulin receptors, however, we have very limited understanding of the involvement of microglial insulin signaling in the pathogenesis of obesity and T2DM. We hypothesize that reduced insulin signaling affects microglial immune function, which could ultimately result in dysfunction of neighboring neurons and impaired CNS control energy homeostasis.

Methods: We induced microglia-specific knock-out of the insulin receptor gene *in vivo* in $InsR^{fl/fl}$-$Cx3Cr1^{CreERT2}$ mice (InsR-KO). Male and female InsR-KO mice and $InsR^{wt/wt}$-$Cx3Cr1^{CreERT2}$ controls (InsR-WT) were fed with high-fat diet (HFD) or Chow diet for 10 weeks. We evaluated the activity, energy expenditure (EE) and respiratory exchange rate (RER) of the animals for 5 days in metabolic cages. The animals were perfused and fixed in paraformaldehyde. Coronal slices were used to evaluate microglial cell number and primary branching in the arcuate nucleus of the hypothalamus (Figure 1A). Statistical analysis was performed with one- or two-way ANOVA. Data are presented as mean±SEM.

Results: Following 10 weeks of HFD, both male and female mice showed higher body weight (BW) gain in InsR-WT and InsR-KO animals, compared to the respective control group ($p<0,0001$). We observed no difference between in InsR-WT and InsR-KO animals in males and females fed Chow or HFD. Both Chow-fed InsR-WT and InsR-KO male mice show a higher RER during the dark phase compared to the light phase, and this difference is abolished in HFD-fed animals. However, both InsR-WT and InsR-KO females show a higher RER during the dark phase, irrespective of the diet. All animals have a higher activity and energy expenditure during the dark phase, compare to the light phase. We found no difference in number of microglial soma in any of the groups (Figure 1B), however we found a reduced number of primary projections in InsR-KO animals, compared to InsR-WT, irrespective of the sex or diet (Figure 1C).

Conclusions: We observed a decrease in microglial primary projections in InsR-KO, compared to InsR-WT animals, which could be indicative of higher microglial activation in the absence of microglial insulin signaling.


A
B
Soma - Males
Soma - Females
Soma/250 um2
WT
KO
Chow
HFD
C
Projections - Males
Projections - Females
Primary projections/cell
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Hypothalamic dopamine system and its interaction with peripheral tissues to control energy balance

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The dopamine system is widely known to modulate the brain reward system, affecting feeding behavior. Dopamine receptors are also expressed in the hypothalamus and has been shown to regulate metabolic actions. In particular, we will show how the pharmacological or chemogenetic stimulation of the dopamine receptor 2 (D2R) expressing cells in the lateral hypothalamic area (LHA) and the zona incerta (ZI) affects body weight and systemic metabolism. This mechanism seems to be important in some physiological situations. In addition to preclinical findings, we will also show that patients undergoing treatment with the D2R agonist cabergoline also show changes in their metabolism.

Hypothalamic control of median emminence barrier function

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Components of the median emminence barrier (tanycytes, vascular endothelial cells) form an important compartment that regulates access of circulating factors and hormones to metabolism-regulatory neurons in the hypothalamus. However, the mechanisms underlying the state-dependent regulation of this barrier remain incompletely defined. We demonstrate that energy state- and sleep-dependently regulated neurons that express the melanin concentrating hormone (MCH) provide dense projections to the median eminence (ME) in close proximity to tanycytes and fenestrated vessels. Chemogenetic activation of MCH neurons enhances permeability of the ME via increasing fenestrated vascular loops and enhances leptin action in the arcuate nucleus of the hypothalamus (ARC). Similarly, optogenetic stimulation of MCH neuron projections in the ME enhances leptin's acute anorexigenic effect. Unbiased phosphoRiboTrap-based assessment of cell activation upon chemogenetic MCH neuron activation reveals MCH neuron dependent activation of endothelial cells. MCH neurons express the vascular endothelial growth factor (VEGF)-A, and blocking VEGF-R signaling abrogates the leptin-sensitizing effect of MCH neuron activation. Our experiments reveal, that MCH neurons directly regulate permeability of the ME barrier in a VEGFA-dependent manner, linking the activity of energy state sensing neurons to the regulation of hormone accessibility to the ARC.

To infer tanycyte-neuron communications through single-cell transcriptomics data

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Tanycytes are specialized ependymoglial cells lining the wall and the floor of the third ventricle next to common ependymal cells from which they are morphologically distinct. They are known to be involved in a variety of functions such as metabolism regulators, traffic controllers at the interface between blood and brain or a neural stem cells niche. However, tanycyte heterogeneity as well as integration within hypothalamus networks remain largely undervalued.
To characterize tanycyte sub-populations and communication with neural cells at a single-cell level, we induced the expression of tdTomato in ependymal cells using Rosa26-floxed stop tdTomato mice. Single-cell RNA sequencing was then performed on tdTomato-positive cells isolated by FACS of adult mice in three different metabolic conditions (i.e. fed, 12h-fast and 24h-fast mice). Differential gene expression (DGE) analysis was conducted on integrated data between two conditions set (fed vs 12h-fast, fed vs 24h-fast and 12h-fast vs 24h-fast). Different tools were also used for inference of cell pseudotimes (Monocle3), RNA velocity (Velocyto) and intercellular network communication (CellChat).
The results highlight extensive cell heterogeneity along the third ventricle, as well as high gene expression dynamics according to the energy status of the individual. Pseudotime trajectories also provide new clues for gliogenesis in adult mice. In conclusion, this work shows the power of single cell transcriptome to analyze the complexity of heterogeneous neural structures and to understand the interplay among key cell types in environment sensing and energy homeostasis.

Characterisation of intracellular signal transduction of adrenoceptors and $GABA_B$-receptors in tanycytes

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The wall of the basal third ventricle is lined by specialized glia cells called tanycytes. Due to their localization, they play a potential role in the communication between the brain and the periphery by contacting the cerebrospinal fluid, the fenestrated capillaries in the median eminence, the blood vessels of the blood-brain-barrier, and neurons in the hypothalamus. Tanycytes react to changes in concentration of glucose, amino acids, and hormones. Stimulation with ATP, acetylcholine, or thyroid releasing hormone (TRH) leads to an increase in intracellular calcium concentrations ($[Ca^{2+}]_i$) through activation of $G_{\alpha q}$-coupled receptors. Furthermore, tanycytes express a variety of $G_{\alpha s}$- and $G_{\alpha i}$-coupled receptors, modulating the concentration of intracellular cAMP ($[cAMP]_i$).

To characterize the signal transduction of $G_{\alpha s}$- and $G_{\alpha i}$-coupled receptors in tanycytes further, the fluorescent cAMP indicator Flamindo2 was expressed in mouse tanycytes using a recombinant adeno-associated virus. Two weeks after injecting the vector into the lateral ventricle, the role of $GABA_B$-receptors and adrenoceptors was characterized ex vivo using acute brain slices. Activation of the $GABA_B$-receptors using the agonist baclofen reduced $[cAMP]_i$ in tanycytes via the $G_{\alpha i/o}$-coupled pathway. Epinephrine, an agonist at all adrenoceptors, led to an increase of $[cAMP]_i$ via $G_{\alpha s}$-coupled β-adrenoceptors. At low concentrations of epinephrine, the effect of the $G_{\alpha i/o}$-coupled α_2-adrenoceptors prevailed, leading to a reduction of $[cAMP]_i$. Clonidine, an α_2-adrenoceptor agonist, decreased $[cAMP]_i$, and this effect was antagonized by preincubation with pertussis toxin. In contrast, the effect of baclofen was not inhibited by pertussis toxin. Although the $G_{\alpha q/11}$-coupled α_1-adrenoceptor mRNA was found in tanycytes, the agonist phenylephrine did not support functional expression of this receptor.

Tanycytes in the hypothalamus act as important sensors by different G-protein-coupled receptors. The AAV-based expression of Flamindo2 allows investigating changes of cAMP in tanycytes in real time and in acute brain slices. The physiological relevance of the verified expression $GABA_B$-receptors and α_2- and β-adrenoceptors in tanycytes remains to be elucidated by further studies.

Symposium

S6: The entorhinal micronetwork - how connectivity determines function

S6-1 Processing of hippocampal output signals within the mEC
Alexei V. Egorov

S6-2 New insights into hippocampal/entorhinal circuitry
Gulsen Surmeli, Sau Yee Tsoi, Merve Oncul, Ella Svahn, Mark Robertson, Christina McClure

S6-3 Intrinsic and extrinsic connectivity of the lateral and medial entorhinal cortex
Menno P. Witter

S6-4 Excitation-inhibition dynamics regulate signal output in the perirhinal-entorhinal cortex and modulate the window of opportunity for information processing in the rhinal cortices
Natalie Cappaert, Janske Willems, Wytse Wadman

S6-5 Functional, dynamic and structural diversity of parvalbumin-expressing interneurons in the mouse dentate gyrus.
María del Ángel Ocaña Fernández, Marlene Bartos

Processing of hippocampal output signals within the mEC

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The deep layers of the medial entorhinal cortex (mEC) are an important hub for relaying neuronal activity from the hippocampus to downstream networks. We investigated the activation of the receiving mEC layer V (LV) network by evoked and naturally occurring output patterns in mouse hippocampal-entorhinal cortex slices. Stimulation-evoked inputs as well as sharp wave-ripple complexes (SPW-Rs) directly excite both types of excitatory neurons in layer V (LVa and LVb). Excitatory connections between LVb and LVa neurons, however, are very sparse, suggesting separation of hippocampal output signals into two distinct excitatory pathways. This configuration minimizes the influence of mEC LVb cells on information transfer to telencephalic structures which is mediated by LVa neurons. Local processing of intermediate/ventral hippocampal output signals within mEC LV is asymmetric, favoring excitation of far projecting LVa neurons over locally projecting LVb neurons. This finding indicates a preferential routing of SPW-R-encoded information to remote neocortical networks, rather than circulating activity within the entorhinal-hippocampal loop. In apparent contrast, projections originating from the dorsal hippocampus show a clear preference for excitation of LVb over LVa neurons, suggesting signal propagation favoring (entorhinal-hippocampal) feedback at the dorsal level. These findings suggest a new model for the mEC which acts as a bifurcation gate for hippocampal network activity and differentially relays hippocampal activity along the dorsoventral axis.

New insights into hippocampal/entorhinal circuitry

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Entorhinal cortex (EC) deep layers are a conduit for hippocampal-neocortical communication that is critical for long-term storage of experience dependent memory. Unlike the superficial layers of EC that convey highly processed cortical inputs that contribute to hippocampal computations, EC layer 5a (ECL5a) is an output layer that passes hippocampal outputs onto cortical and subcortical areas. Here we provide evidence against the view that ECL5a is merely a final step of a feedforward loop. We demonstrate that ECL5a provides a copy of its cortical and subcortical outputs to hippocampal CA1. Projections from medial ECL5a have a unique topography directly targeting pyramidal cells and interneurons in distal and proximal CA1. Our findings suggest that medial ECL5a back-projections actively participate in modulating CA1 networks.

Intrinsic and extrinsic connectivity of the lateral and medial entorhinal cortex

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The entorhinal cortex is considered a cortical entity within the cortical-hippocampal memory system. It is envisioned as the main gateway between the neocortex and the hippocampus. The concept of two entorhinal areas, the lateral and medial entorhinal cortex, originally based on cytoarchitectural differences, is well supported by main differences in inputs. However, whether or not intrinsic circuits and the interactions of inputs with these intrinsic networks are similar, is less well understood. In my presentation, I will reappraise the organization of intrinsic and extrinsic networks of the entorhinal cortex, emphasizing that local inter- and intralaminar networks show a remarkable similarity between the two subdivisions, at least in rodents, with only a few exceptions. The latter differences likely have important functional consequences. In addition, I will argue that comparative data strongly indicate that the cortical input streams to either entorhinal subdivision are very different. These data lead to the hypothesis that the lateral entorhinal cortex is the main entorhinal multimodal integrative structure with a unique set of external sensory-derived inputs, allowing its network to represent a continuously changing extrinsic environment.

Excitation-inhibition dynamics regulate signal output in the perirhinal-entorhinal cortex and modulate the window of opportunity for information processing in the rhinal cortices

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The perirhinal (PER) and entorhinal cortex (EC) receive input from the agranular insular cortex (AiP) and the subcortical lateral amygdala (LA) and function as a gateway for information transmission between (sub)cortical areas and the hippocampus. Information transfer through the PER/EC network however, is not always guaranteed. It is hypothesized that this network actively regulates the (sub)cortical activity transfer to the hippocampal network and that the inhibitory system is involved in this function. In this study we determined the integration of synaptic activity evoked by neocortical and amygdala electrical stimulation in PER-LEC deep layer principal neurons and PV interneurons in mouse acute brain slices. The data revealed that both deep layer PER-LEC principal neurons and PV interneurons receive synaptic input from the neocortical agranular insular cortex (AiP) and the lateral amygdala (LA). Furthermore, simultaneous stimulation of the AiP and LA never reached the firing threshold in principal neurons of the PER-LEC deep layers. PV interneurons however, mainly showed linear summation of simultaneous AiP and LA inputs and reached their firing threshold earlier when the two inputs were stimulated simultaneously. The effect of early PV firing was seen in the forward shift of the evoked inhibitory conductance in principal neurons after simultaneous AiP and LA stimulation. This data suggests that activity from the neocortex can be modulated by the LA in PER-LEC deep layer principal neurons and PV interneurons. By a forward shift in the inhibition, a precise temporal window for coincidence and enhancement of synaptic inputs is created which likely plays a crucial role in information processing.

Functional, dynamic and structural diversity of parvalbumin-expressing interneurons in the mouse dentate gyrus.

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The high diversity of interneurons in the brain grants a wide range of information processing capabilities to neuronal networks and allows a precise spatiotemporal control of principal cell activity. Previous work identified a large diversity of GABAergic inhibitory interneurons based on morphological physiological and neurochemical properties. One functionally dominant group of interneurons are parvalbumin (PV)-expressing, fast-spiking perisoma-inhibiting cells (PVIs). Here we examined the PVI diversity in the mouse dentate gyrus (DG), a hippocampal area critically involved in the representation of space and context by sparsely active granule cell (GC) populations. Their activity is precisely controlled by PVI-mediated perisomatic inhibition (Elgueta and Bartos, 2019, Nat Commun. 10:1-15). Using whole-cell patch clamp recordings of visually identified tdTomato expressing PVIs in acute slice preparations of PV-Cre.tdT mice combined with intracellular labelling, we identified two subtypes of DG-PVIs, characterized by marked correlation between soma location at the inner granule cell layer (gcl)-hilar boarder (PVIis) and at the outer gcl-molecular layer boarder (PVIos) with their morphological, physiological and molecular characteristics. (1) Reconstructions of the somato-dendritic domain revealed a higher number of basal dendritic arbors and larger vertical extend of apical dendrites in PVIis than PVIos, pointing to higher dendritic complexity and the potential of integrating a larger number of synaptic inputs. (2) PVIis had a more positive resting membrane potential, exhibited a lower threshold for action potential generation and discharge at higher maximal frequency compared to PVIos. (3) Antibody labelling revealed a higher proportion of PVIis expressing mGluR5, a key receptor involved in the induction of long-term plasticity (LTP) at their GC input synapses. (4) PVIis show higher levels of GC input-mediated LTP. (5) By using paired GC-PVI recordings, we found a higher connection probability at GC-PVIis than at GC-PVIo synapses, indicating a stronger feedback excitation onto PVIis. On the contrary, perforant path-mediated EPSCs, evoked by extracellular stimulation of the middle molecular layer were elicited after shorter latencies, with larger mean amplitude and faster decay time constants at PVIos, indicating a stronger recruitment by feedforward excitation. Finally, PVI-GC paired recordings revealed similar functional properties of unitary IPSCs, however, failure rate and the coefficient of variation of individually evoked IPSCs were lower at PVIi-GC synapses with pronounced paired-pulse depression. In contrast, PVIo-GC synapses lacked significant dynamic changes. Thus, our data suggest that PVIis provide reliable phasic feedback, whereas PVIos unreliable and fluctuating feedforward inhibition to the DG network. Further investigations are, however, required to examine the in vivo activity of the two PVI types and their role in controlling GC activity in the behaving animal.

Symposium

S7: Advanced optics for neuroscience

MINFLUX nanoscopy and related matters

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I will show how an in-depth description of the basic principles of diffraction-unlimited fluorescence microscopy (nanoscopy) [1-3] has spawned a new powerful superresolution concept, namely MINFLUX nanoscopy [4]. MINFLUX utilizes a local excitation intensity minimum (of a doughnut or a standing wave) that is targeted like a probe in order to localize the fluorescent molecule to be registered. In combination with single-molecule switching for sequential registration, MINFLUX [4-6] has obtained the ultimate (super)resolution: the size of a molecule. MINFLUX nanoscopy, providing 1–3 nanometer resolution in fixed and living cells, is presently being established for routine fluorescence imaging at the highest, molecular-size resolution levels. Relying on fewer detected photons than popular camera-based localization, MINFLUX nanoscopy is poised to open a new chapter in the imaging of protein complexes and distributions in fixed and living cells.

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Mind the gap: Super-resolution imaging of the extracellular space of the brain

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The extracellular space (ECS) forms an important but understudied frontier in neuroscience. It consists of the narrow gaps that surround all brain cells, which are filled with interstitial fluid and extracellular matrix molecules, occupying around one fifth of the volume of the brain. It likely provides the molecular cues and physical rails that incite and guide morphogenic processes and the migration of immune cells like microglia. However, mapping the dynamic landscape of the ECS with enough spatial resolution has been impossible to accomplish until now for lack of appropriate tools. In my presentation, I will review our technical progress in labeling and imaging the ECS vis-à-vis fluorescently labeled neurons, astrocytes and microglia cells in living brain slices using a of STED microscopy and lattice light sheet calcium imaging.

Holographic manipulation of neuronal circuits

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Genetic targeting of neuronal cells with activity reporters (calcium or voltage indicators) has initiated the paradigmatic transition whereby photons have replaced electrons for reading large-scale brain activities at cellular resolution. This has alleviated the limitations of single cell or extracellular electrophysiological probing, which only give access to the activity of at best a few neurons simultaneously and to population activity of unresolved cellular origin, respectively. In parallel, optogenetics has demonstrated that targeting neuronal cells with photosensitive microbial opsins, enables the transduction of photons into electrical currents of opposite polarities thus writing, through activation or inhibition, neuronal signals in a non-invasive way.

These progresses have in turn stimulated the development of sophisticated optical methods to increase spatial and temporal resolution, light penetration depth and imaging volume. Today, nonlinear microscopy, combined with spatio-temporal wave front shaping, endoscopic probes engineering or multi scan heads design, enable in vivo in depth, simultaneous recording of thousands of cells in mm3 volumes at single-spike precision and single-cell resolution. Joint progress in opsin engineering, wave front shaping and laser development have provided the methodology, that we named circuits optogenetics, to control single or multiple target activity independently in space and time with single-neuron and single-spike precision, at large depths.

Here, we will review the most significant breakthroughs of the past years, which enable reading and writing neuronal activity at the relevant spatiotemporal scale for brain circuits manipulation, with particular emphasis on the most recent advances in circuit optogenetics.

Optical observation and manipulation reveal that mitochondria fuel local translation during synaptic plasticity

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Since synapses can be very remote from the soma, synaptic proteins are often translated locally. This local translation accounts for protein turnover and synaptic plasticity. The source of the necessary energy supply is not known.

Using modern optical methods not only for imaging but also for optical manipulation, we show that the energy supply for local translation in dendrites is based on mitochondria and not on glycolysis.

Stimulated emission depletion (STED) microscopy reveals that mitochondria in dendrites are present as stable compartments. In order to change local energy demands, we use spatially targeted photolysis (“uncaging”) of neurotransmitters to induce structural plasticity and local protein synthesis. Local inhibition of mitochondria by targeted excitation of the phototoxic protein Killer Red allows a comparison of structural plasticity and synaptic translation in regions devoid of mitochondria with control regions. We find that depletion of mitochondria eliminates structural plasticity and synaptic translation whereas synaptic translation in other dendritic areas is not disturbed. The mitochondrial compartments serve thus as local energy supply. We can thereby demonstrate the importance of the presence of local mitochondria for local protein synthesis during plasticity.

cFos does not simply reflect an increase in neuronal activity.

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The activity-dependent expression of immediate early genes, such as cFos, is widely used as an indicator of which neurons participated in a behavioral task. It is generally believed, that if a neuron was strongly activated or underwent an event leading to synaptic potentiation, then cFos will be expressed. However, surprisingly little is known about how cFos expression is related to the neuronal firing pattern and which molecular cascades are important. We therefore investigated the relationship between neuronal activity and cFos expression and used pharmacological manipulation to investigate the pathways leading to cFos. When high potassium was used to depolarize all cells in organotypic rat hippocampal slice cultures, we found that cFos expression is not uniform but depends on neuronal type and is especially low in the CA2 region. cFos expression is abolished by blocking either CREB, MEK or calcineurin signaling pathways (AND logic) or by preventing neurons from firing action potentials with tetrodotoxin (TTX) during high potassium stimulation. We next used the channelrhodopsin ChrimsonR to precisely drive action potentials with red light flashes throughout the slices in the presence of blockers of fast synaptic transmission. At a high frequency (50 Hz), 30 action potentials are sufficient to induce cFos in DG, CA3 and CA1 hippocampal regions and 300 action potentials maximally drove cFos. cFos expression was also frequency dependent. There was a clear U-shaped dependence of cFos expression on firing frequency with high cFos driven at high and low frequencies but not by intermediate frequencies. In contrast to high potassium stimulation, cFos expression after both low and high frequency firing was not blocked by inhibiting either CREB, MEK or calcineurin alone. Action potentials were required as cFos expression after low frequency stimulation was abolished, when sodium channels were blocked with TTX. We conclude, that increasing cFos expression does not simply indicate increasingly active neurons. Rather, cFos expression is highly dependent on neuronal identity and increases after both short high intensity bursts of activity or after prolonged low frequency firing. In addition, the molecular pathways leading to cFos expression after stimulation-induced firing differ from those seen using high potassium to globally depolarize not only neurons but other cells in the slices.

Symposium

S8: The choice is yours: multicircuit regulation of motivated behaviors

S8-1 Diversity of dopamine circuits in reward and aversion
Stephan Lammel

S8-2 Brain state dependent responses of midbrain dopaminergic neurons to the aversive stimulus
Gabriela Izowit, Magdalena Walczak, Gniewosz Drwiega, Kamil Pradel, Wojciech Solecki, Tomasz Blasiak

S8-3 Processing of internal states in insular cortical circuits gatesmotivated behaviors
Nadine Gogolla, Alexandra S. Klein

S8-4 Lateral Habenula control of reward-guided tasks
Manuel Mameli

S8-5 Role of orexin in drinking and binge-like eating behavior
Nadine Faesel, Michael Koch, Markus Fendt

S8-6 Dynamic regulation of social and feeding behaviors by lateral septal circuits
Tatiana Korotkova, Francisco J. de los Santos, Letizia Moscato, Hanna van den Munkhof, Robson Scheffer Teixeira

Diversity of dopamine circuits in reward and aversion

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The mesocorticolimbic dopamine (DA) system, composed of DA neurons in the ventral tegmental area (VTA) projecting to nucleus accumbens and prefrontal cortex, has been intensively studied because of its importance in reward processing and motivated behavior. Importantly, while VTA DA neurons were thought to represent a homogeneous cell population, recent research has demonstrated a much greater diversity of DA cell type and function than had been previously supposed. Accordingly, VTA DA neurons encode much more than reward and also contribute to aversive behaviors. How DA neurons could mediate both reward and aversion is an important goal of the research in my lab. In my presentation, I will discuss recent work that has elucidated the circuit architecture and function of distinct mesoaccumbal DA subcircuits underlying reward learning and motivated behaviors.

Brain state dependent responses of midbrain dopaminergic neurons to the aversive stimulus

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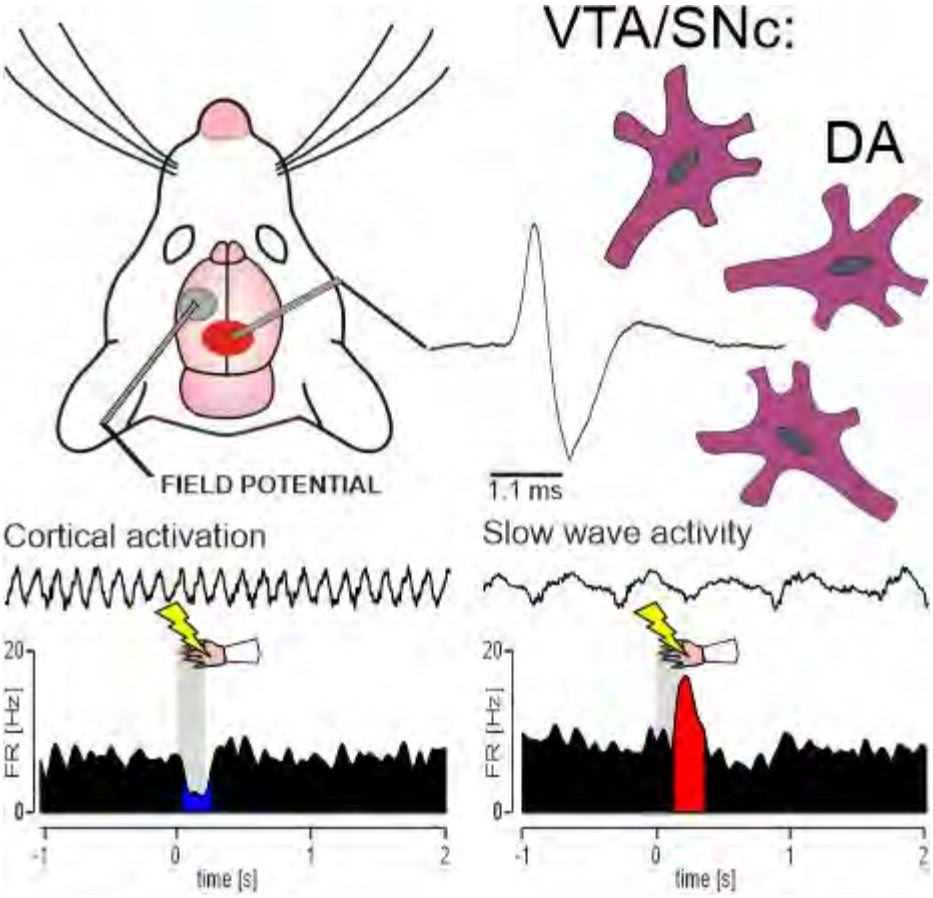
Midbrain dopaminergic neurons (DA) has been repeatedly shown to generate burst of action potentials in response to a reward or cue associated with it, and cease to fire in response to reward omission or aversive stimuli. For a long time it has been assumed that, in this manner, DA neurons of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) uniformly encode the information about value of perceived stimuli. The dopamine signal generated in this way affects the neuronal plasticity in many networks of the brain, enabling the animal to learn based on the value of results of the actions taken. However, this unified concept was challenged a few years ago after the identification of DA neurons that are excited by both rewarding and aversive stimuli, regardless of their value. This resulted in division of midbrain DA neurons into two distinct subpopulations: one encoding a value and the other encoding the salience of the stimuli. It has also been shown that the general state of the brain modulates the electrical activity of DA neurons, but it remains unknown whether this factor may influence signalling of value and/or salience. Therefore, we aimed our experiments to determine whether DA neurons can encode information about both value and salience of perceived stimuli in brain state dependent manner. For this purpose, we have recorded responses of VTA/SNc dopaminergic neurons to electrical footshocks across alternating brain states of urethane anaesthetized rats. Combining optogenetic tagging and extracellular in vivo recordings, we examined 75 midbrain DA neurons. We have observed previously described populations of value- and salience-coding neurons (43% and 23% out of all recorded cells, respectively). However, we have also discovered subpopulation of DA neurons that was not described so far. It is a relatively large subpopulation of DA neurons (28%) that changes their type of response to an aversive stimulus depending on ongoing brain state. Majority of those neurons were inhibited by footshocks applied during cortical activation but with the appearance of slow wave activity, they changed their type of response to excitation. It can be hypothesised that this subpopulation may be involved in ‘dual-coding’ of both value and salience of stimulus depending on the general state of the brain. It is plausible that during behaviours characterised by cortical activation, i.e. exploration or freezing, information about negative value of external stimuli is favoured due to involvement in learning of avoidance behaviours. On the contrary, during deep sleep or low arousal states characterised by slow wave activity, recognizing of external stimuli as salient may be crucial for animal’s survival and guide orienting animals’ attention, boost cognitive processing and result in a general increase of alertness and arousal. Recognizing that ongoing brain state determines how certain DA neurons respond to stimuli may provide new scientific ideas about how information about value and salience can be processed.


VTA/SNc:
DA
FIELD POTENTIAL
1.1 ms
Cortical activation
Slow wave activity
20
FR [Hz]
0
-1
0
1
2
time [s]

Processing of internal states in insular cortical circuits gates motivated behaviors

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The insular cortex integrates external sensory cues with internal affective and bodily states to predict future outcomes. It further acts as an important top-down regulator of ongoing behaviors through projections to diverse subcortical structures, such as the amygdala, the striatum, or the thalamus. In my talk, I will highlight recent results from our lab that identify an important role for the insular cortex in processing diverse bodily and emotional states which strongly affect the responsiveness to sensory cues and gate motivated behaviors. Collectively, our data suggest that the insular cortex provides top-down control of motivated behaviors based on the current affective and bodily states.

Lateral Habenula control of reward-guided tasks

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Weighing alternatives during reward pursuit is a vital cognitive computation that, when disrupted by stress, yields aspects of neuropsychiatric disorders. To examine the neural mechanisms underlying these phenomena, we employed a behavioral task in which mice were confronted to a reward and its omission (i.e. error). The experience of error outcomes engaged neuronal dynamics within the lateral habenula (LHb), a subcortical structure supporting appetitive behaviors and susceptible to stress. High incidence of errors predicted low strength of habenular excitatory synapses.
Accordingly, stressful experience increased error choices while decreasing glutamatergic neurotransmission onto LHb neurons. This synaptic adaptation required a reduction in postsynaptic AMPA receptors (AMPAR), irrespective of the anatomical
source of glutamate. Bidirectional control of habenular AMPAR transmission recapitulated and averted stress-driven cognitive deficits. Thus, a subcortical synaptic mechanism vulnerable to stress underlies behavioral efficiency during cognitive performance.

Role of orexin in drinking and binge-like eating behavior

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Since its discovery, the orexin (hypocretin) neuropeptide system is known for its crucial role in regulating ingestive behaviors. Here, we focus on the involvement of orexin in drinking and binge-like eating behavior. For that, the effects of heterozygous and homozygous orexin deficiency in female and male mice were tested in two behavioral experiments: (1) Drinking behavior after intracerebroventricular (ICV) injections of the dipsogenic peptide angiotensin II (ANG II), and (2) binge-like eating in a paradigm in which mice had intermittent access (24-h, weekly) or continuous access to a high-energy diet (HED) for three weeks, followed by testing for anxiety-like behavior.

Experiment (1): ICV ANG II injections (100 ng) stimulated water intake in male mice, but not in female mice. These effects were independent of the orexin genotype. However, a higher dose of ANG II (500 ng) also induced drinking in female wild-type mice, but not in female orexin-deficient mice. Therefore, the dipsogenic effects of ANG II are sex-dependently influenced by orexin deficiency.

Experiment (2): In the intermittent group, mice of all genotypes and sexes consumed significantly more food during the weekly 24-h HED presentations, compared to the continuous control group. Moreover, mice of the intermittent group expressed increased levels of anxiety. Preliminary data suggest that orexin deficiency affected binge-like eating in a sex-dependent manner, i.e., it reduced binge-like eating in females, but not in males. Furthermore, the increased anxiety-like behavior observed in the intermittent group seemed to be mainly driven by males of each genotype, while female orexin-deficient mice partly did not show higher levels of anxiety.

In conclusion, our results indicate and further substantiate an important and sex-dependent role of orexin in ingestive behaviors, which may be of translational relevance for human conditions like polydipsia and/or binge eating disorder.

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Dynamic regulation of social and feeding behaviors by lateral septal circuits

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Feeding and social behaviors are crucial for the survival. Neural circuit mechanisms underlying the interactions between both sets of behaviors remain largely elusive. Further, little is known about how the brain computes choices when competing stimuli for mutually exclusive basic behaviors, e.g. social interaction vs. feeding, occur simultaneously. Aggression and feeding-related behaviors are regulated by the lateral septum (LS), which is connected with hypothalamic areas as well as with the prefrontal cortex and the hippocampus. We have recently shown that fast oscillations in the pathway from somatostatin-expressing LS neurons to the lateral hypothalamus promote food-seeking (Carus-Cadavieco et al., Nature 2017). Here we investigated functions of two cell populations in the LS, somatostatin (Sst)-, and neurotensin-expressing (NT) cells, in feeding and social interactions. Using 1-photon calcium imaging, optogenetics and chemogenetics in behaving mice, we found that activity of NT and Sst cells selectively changes during different stages of feeding and social behaviors. Further, optogenetic or chemogenetic activation of these cells resulted in cell-type specific behavioral changes, including a decrease of food intake and an increase of spontaneous social interactions upon activation of NT cells. Subsecond analysis of behavior using MoSeq, an unsupervised machine learning algorithm, upon chemogenetic activation of NT cells, revealed changes of multiple feeding and social-related behavioral modules on a subsecond scale.
These results suggest that NT and Sst cells in the lateral septum complementary regulate feeding-related and social behaviors.

We gratefully acknowledge funding by the ERC Consolidator Grant 2017 (HypFeedNet, TK), the Max Planck Society (TK) and the DFG (RTG-NCA, 233886668/GRK1960, FS).

Symposium

S9: Revealing the evolutionary trajectory of the first nervous systems: genomics, structure and dynamics

S9-1 The ctenophore nervous system: from Hertwig hand drawings to genetic studies, what do we really know?
Mari-Luz Hernandez Nicaise

S9-2 Placozoan behaviour is coordinated by neuropeptide-like signaling.
Elizabeth Amy Williams

S9-3 Testing the Ca^{2+}sensor hypothesis of otoferlin
Han Chen, Qinghua Fang, Nils Brose, Tobias Moser

S9-4 From Single Neurons to Behavior in the Jellyfish Aurelia aurita
Raoul-Martin Memmesheimer, Fabian Pallasdies, Sven Ole Goedeke, Wilhelm Braun

S9-5 Neural control of 2-d skin patterning in two species of cephalopods
Xitong Liang, Gilles Laurent

S9-6 Scalability in the nervous system of Hydra
Christophe Dupre, Florian Engert, Jeff Lichtman

The ctenophore nervous system: from Hertwig hand drawings to genetic studies, what do we really know?

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To Be announced

Placozoan behaviour is coordinated by neuropeptide-like signaling.

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Placozoans are small, free-living marine animals with a simple body plan of three cell layers. These early-branching metazoans have only a few identified cell types and lack muscle or nerve cells. Despite this, placozoans express genes encoding several neuropeptide-precursor-like proteins. We investigated the function of the small peptides encoded by these precursors as signaling molecules in the placozoan *Trichoplax adhaerens*. We found specific expression of several neuropeptide-like molecules in non-overlapping cell populations, suggesting an unsuspected cell-type diversity in *T. adhaerens*. Using live imaging, we discovered that treatments with different peptides elicited a number of different effects on the animals' shape, patterns of cilia-driven movement, and velocity. The behavioural changes caused by peptide treatment in some cases resembled the animals' typical behavioural responses to different environmental stimuli. Together, our results indicate that peptidergic signaling can coordinate behaviour even in the absence of a nervous system.

Testing the Ca^{2+}sensor hypothesis of otoferlin

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The ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) are highly specialized to achieve indefatigable afferent transmission at rates of hundreds of Hertz and with submillisecond temporal precision. IHC ribbon synapses differ fundamentally from 'conventional synapses' of the central nervous system. The protein machinery mediating exocytosis at 'conventional synapses' is composed of neuronal SNARE proteins and SNARE regulatory proteins whereby Synaptotagmins work as Ca^{2+} sensors of exocytosis. However, mature IHCs lack synaptotagmin1/2, indicating an alternative Ca^{2+} sensor of exocytosis. Otoferlin, a protein and deafness gene product with six C_2 domains specifically expressed in IHCs, is the likely candidate for Ca^{2+} sensor in IHCs and we further tested this hypothesis.

Based on clinical findings and protein structure predictions, we generated three otoferlin mouse mutants with predicted alterations of Ca^{2+} binding to the C_2E and C_2F domains and analyzed them by whole cell patch-clamp recordings of IHC presynaptic function, immunohistochemistry and auditory brainstem response (ABR) recordings. In the C_2F-DDA mutant, two potential Ca^{2+} binding aspartates of the most C-terminal C_2F domain were substituted by alanines. According to immunohistochemistry results, otoferlin remained expressed in IHCs but was abnormally localized to the apical IHC part. ABR indicated that DDA mice are deaf. While the Ca^{2+} currents of IHCs were retained, exocytosis was abolished. In C_2E-TDA mutant, three potential Ca^{2+} binding aspartates at the C_2E domain were substituted by alanines. Immunohistochemistry showed that otoferlin was successfully expressed in IHCs of DDA mice with a normal subcellular distribution. Patch-clamp revealed normal Ca^{2+} currents but greatly reduced exocytosis, suggesting a strong connection between Ca^{2+} binding and exocytosis. Finally, OTOFIT mutants (Ile1573Thr) carry a pathogenic missense mutation near a C_2E top loop leading to progressive human hearing impairment. OTOFIT mice lacked detectable ABR and exocytosis was abolished in their IHCs despite normal Ca^{2+} current. Immunohistochemistry and real-time PCR revealed that otoferlin was dramatically decreased in both protein and RNA level in OTOFIT mouse IHCs.

The newly generated mutants show that the normal expression and function of otoferlin are indispensable for IHC exocytosis. The strong synaptopathy phenotype in the OTOFIT mutant modeling the human Ile1573Thr substitution seems to contrast the human phenotype and calls for further studies of this mutant e.g. using behavioral assays of hearing. Exocytosis was completely abolished also in C_2E-TDA mutants, despite apparently normal expression and distribution of otoferlin, supporting the notion of otoferlin serving as a Ca^{2+} sensor of exocytosis.

From Single Neurons to Behavior in the Jellyfish Aurelia aurita

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Understanding how neuronal activity leads to behavior in animals is a central goal in neuroscience. Since jellyfish are anatomically relatively simple animals with a limited behavioral repertoire, modeling their nervous system opens up the possibility to achieve this goal. Further, such modeling provides insight into the origins of nervous systems, as both their taxonomic position and their evolutionary age imply that jellyfish resemble some of the earliest neuron-bearing, actively-swimming animals. We develop the first neuronal network model for the nerve nets of jellyfish. The study focuses on the neuro-muscular control of the swimming motion in a moon jelly Aurelia aurita in its medusa stage of development. Incorporating the available experimental observations and measurements, we develop a bottom-up multi-scale computational model of single neurons, nerve nets, steered muscles and the bell. The proposed single neuron model disentangles the contributions of different currents to a spike. The network model identifies factors ensuring non-pathological activity and suggests an optimization for the transmission of signals. Using fluid-structure hydrodynamics simulations, we then explore how the nervous system generates and shapes different swimming motions; the predictions are compared with results from experiments. Our model bridges the scales from single neurons to behavior, allowing for a comprehensive understanding of jellyfish motor control.

Neural control of 2-d skin patterning in two species of cephalopods

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Coleoid cephalopods (octopus, squid, and cuttlefish) have the ability to change their skin patterns instantaneously for camouflage or communication. Their skin patterns are generated in great part by an extensive array of variable-sized pigment cells (chromatophores), controlled individually by radial muscles. Using this system, the cuttlefish Sepia officinalis generates skin 2d textures that match certain statistics of its surrounding visual environment. How thousands to millions of chromatophores are controlled in parallel and coordinated to generate different textures is an open and fascinating question. A different cephalopod, the bobtail squid Euprymna berryi, camouflages by covering itself with sand. Its chromatophores change size mostly synchronously, switching entire animal between transparency and dark pigmentation. To study the neural basis underlying these divergent chromatophore dynamics, we compare the neuronal and network properties of chromatophore motor control between these two species. By tracing their axons in descending nerves, we identified chromatophore motoneurons in both species. Although those motoneurons show similar electrical properties, the ratio of motoneurons to chromatophores is ~9-fold higher in Sepia than in Euprymna. Electrical stimulation further suggested a somatotopic organization of motoneurons in Sepia posterior chromatophore lobes, while such maps were absent in Euprymna. We developed the first preparation to carry out whole-cell recordings and calcium imaging on chromatophore motoneurons, in conjunction with observing the dynamic activity of the chromatophores, elicited by stimulating the upstream visual system. This approach begins to uncover the principles of organization of neural circuits generating high-dimensional motor output, and may reveal how such neural circuits have diverged adaptively during evolution.

Scalability in the nervous system of Hydra

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The nervous system can change in size dramatically while keeping its functions intact, which is illustrated by the fact that many species of vastly different sizes exhibit very similar behaviors. In rodents for instance, the brain size can span two orders of magnitude, with the mouse brain having about 70 million neurons whereas the capybara brain has about 1600 million neurons. Uncovering the mechanisms underlying brain scalability can help understand fundamental principles of brain function, since brain size differences among closely related species are frequently observed throughout the evolutionary tree. This problem is difficult to study in rodents because of the complexity of their brain, which makes a side-by-side comparison very challenging. Fortunately, many other animal species can change in size too, some of them placed very early on the evolutionary tree. Among them, the freshwater invertebrate Hydra is an ideal model to pursue such questions since its nervous system is very simple and the same animal can change in size repeatedly by up to an order of magnitude. We aim at reconstructing the nervous system of Hydra using electron microscopy in order to describe how it is built and how it changes when the animal grows and shrinks.

Symposium

S10: The undiscovered country - Plasticity in the enteric nervous system

S10-1 The enteric nervous system as a source of neurogenesis?
Peter H. Neckel

S10-2 R-Spondin1 regulates Wnt signalling in the enteric nervous system and leads to enteric neurogenesis *in vitro*
Melanie Scharr, Karin Seid, Melina Fischer, Simon Scherer, Lothar Just, Peter Neckel

S10-3 Control of enteric nervous system development: from syndromic HSCR genes identification to post-transcriptional regulation
Nadege Bondurand

S10-4 Enteric neurons sensitivity: adaptive changes in the enteric nervous system
Gemma Mazzuoli-Weber

S10-5 Peptide neuromodulation counterbalances detrimental temperature influences on electrical spread
Margaret Louise DeMaegd, Wolfgang Stein

S10-6 Dynamic heterogeneity of neuro-glial circuits in the gut wall
Werend Boesmans

The enteric nervous system as a source of neurogenesis?

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The enteric nervous system (ENS) is the third division of the autonomic nervous system and with approximately 500 million neurons the largest part of the peripheral nervous system. Together with enteric glial cells, these neurons form ganglionated plexus within the walls of the gastrointestinal tract. The ENS exhibits a highly complex histoarchitecture with over 20 different enteric neuronal subtypes defined by morphology, neurotransmitter chemistry, or function. THis enables the ENS to serve various functions ranging from controlling motility to regulating blood flow, nutrient uptake, or immune response. Although the proper functioning of the ENS is vital for the organism from the first breath, its changes, plasticity, or regenerative capacities over the course of aging as well as the underlying molecular regulatory pathways are hardly elucidated.

In this talk, I will give insights into the architecture of the ENS and its changes over the course of aging. I will put special emphasis on the poorly understood cell pool of enteric progenitor cells, which can be isolated from rodent and human intestines even at high ages and successively proliferated and differentiated to form new-born neurons in vitro. Intriguingly, although adult neurogenesis in the gut is an arguably rare and scarcely elucidated phenomenon, the sheer existence of neurogenic cell populations in the aged intestine holds the promise of future cell replacement or neuroregenerative therapies for enteric neuropathies, such as Hirschsprung's disease, achalasia, or diabetic gastroparesis. I will also present our previous and current results that strongly indicate an involvement of the canonical Wnt pathway and its regulators R-Spondin1 and Dkk1 in the regulation of the enteric progenitor cell niche. These findings led us to the discovery of the Wnt-receptor Fzd4 as a marker for enteric neural progenitor cells from pediatric patients, which underlines how firmly Wnt signaling is rooted in the cellular homeostasis of the ENS across species. Additionally, I will present data in work on how heparin sulfate proteoglycans are a part of the ENS microenvironment in mice and men and how the extracellular matrix could possibly influence neurogenesis in a Wnt-dependent manner.

R-Spondin1 regulates Wnt signalling in the enteric nervous system and leads to enteric neurogenesis *in vitro*

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Many attempts have been made over the last three decades to isolate, expand and differentiate fetal, postnatal and adult progenitor cells from the enteric nervous system of rodents and men, *in vitro*. Since this cell population is not well characterized yet, insights into signalling pathways that regulate survival, maintenance, cell cycle, and maturation of these progenitor cells are indispensable. Published data of our group demonstrated that canonical Wnt signalling is involved in controlling postnatal proliferation and differentiation processes of enteric progenitor cells and that the Wnt-receptor frizzled-4 is a useful marker for the isolation of postnatal progenitor cells from the human gut. Due to its pivotal role in regulating stem cell proliferation and differentiation, Wnt signalling is tightly regulated by agonistic R-Spondin proteins. R-Spondins are ligands for the Leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5), known to enhance active Wnt signalling. Further, R-Spondins and LGR5 are expressed by other adult stem cell niches at sites of active proliferation. Hence, our research focus on the hypothesis, whether R-Spondin1 has a similar co-stimulating influence on enteric progenitor cells.

For this purpose, we isolated enteric progenitor cells from the muscular layer of the intestine of newborn and adult wild-type mice. Afterwards, we carried out neurosphere assay, immunofluorescence co-labelling studies as well as gene expression analysis after R-Spondin1 stimulation. These cell cultures consist of a heterogeneous cell population such as differentiated smooth muscle cells, which themselves could contribute to an enteric neuronal progenitor niche by expressing several Wnt ligands as well as R-Spondins. To exclude such a Wnt/R-Spondin bystander-effect mediated by these cells, we further carried out R-Spondin stimulation assays with FACS-purified neural crest derivatives. In addition, we confirmed the pro-proliferative R-Spondin1 effect in human enteric progenitor cells derived from paediatric gut samples, thus adding clinical relevance to our findings.

In detail, at the cellular level, activation of Wnt signalling by R-Spondin1 alone lead to a significant increase of total number and cellular mass of proliferating enteric neurospheres. In addition, after differentiation, R-Spondin1 strongly increases the number of newborn as well as the total amount of enteric neurons derived from mice and humans *in vitro*. Furthermore, corresponding immunreactivity for the R-Spondin receptor LGR5 was observed in submucosal, myenteric ganglia of small, and large intestine samples from adult mice, human gut samples, as well as in proliferating murine and human enteric neurospheres. Finally, gene expression analysis further showed LGR receptors- and R-Spondin ligands expression as well as an upregulation of Wnt target genes after R-Spondin1 stimulation.

In conclusion, the observed mitogenic activity of R-Spondin1 alone points towards an intrinsic Wnt production mediated not only by non-neuronal cells but also by enteric progenitor cells to maintain their proliferative niche in an autocrine fashion. Future next generation sequencing analysis will help to verify how

R-Spondin influences the proliferative gene expression patterns of enteric progenitor cells.

Control of enteric nervous system development: from syndromic HSCR genes identification to post-transcriptional regulation

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The enteric nervous system (ENS) controls several intestinal functions, including motility, blood flow and epithelial secretion. Organized into interconnected ganglia that are distributed along the length of the gut, the ENS is composed of large numbers of neurons and glial cells, which are derived from the neural crest. The ENS development relies on the highly regulated cellular processes of migration, survival, proliferation and differentiation of the enteric neural crest cells. Perturbations to enteric neural crest cell number, migratory behavior or rate of differentiation result in aganglionosis of the distal bowel, a disorder known as Hirschsprung disease (HSCR), the commonest enteric neuropathy (1 in 5000 neonates), leading to severe constipation or intestinal obstruction. Several monogenic syndromes with a variable predisposition to HSCR have been delineated, including Waardenburg-Hirschsprung disease (WS4, HSCR+ pigmentation defects and deafness). Dozens of HSCR associated genes are now described, including RET, EDNRB/EDN3 and the transcription factor SOX10.

Here, I will describe the involvement of SOX10 in WS4 in human and various animal models and how genetic and developmental analyses in mice highlighted its importance during various phases of enteric nervous system (ENS) development, vagal and enteric neural crest cells survival and differentiation in particular. Focus on various gastrointestinal alterations associated to SOX10 mutations in human and identification of novel syndromic HSCR genes will then be described.

More recently, we gained interest into post-transcriptional modifications and studied the role of A-to-I RNA editing in various neural crest derivatives including ENS, melanocytes and Schwann cells. Current knowledge and ongoing experiments aiming at better understanding the role of this post-transcriptional modification in Schwann cells and ENS will be presented.

Enteric neurons sensitivity: adaptive changes in the enteric nervous system

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The first part of this talk will introduce the concept of chemo- and mechanosensitivity within the enteric nervous system (ENS). The second part will elucidate some examples of adaptive changes in the ENS in response to different factors such as nutrients, aging and diseases.

The gastrointestinal tract is able to control and regulate all its functions independently from the central nervous system. This is achieved via the ENS, a network of around 200 million neurons located inside the gut wall. In order to regulate gastrointestinal functions the enteric neurons must be capable to sense, to transduce and to respond to a variety of sensory stimuli. These stimuli can be divided in two categories: chemical and mechanical stimuli. Chemical stimuli can indirectly activate (through epithelial or enteroendocrine cells) enteric neurons. However, some ENS can also directly sense and respond to carbohydrates, aminoacids, lipids, low PH and low osmolarity. Regarding mechanosensitivity, in the last decade, it was shown that a subpopulation of enteric neurons is mechanosensitive. Interestingly, these neurons respond to mechanical stimulation but belong to neurochemically, electrophysiologically and functionally different classes. Indeed, it has been shown that interneurons and even motor neurons are able to directly respond to mechanical stimulation. Thus, they are multifunctional. This concept was validated across different species and gut regions.
Chemical and mechanical stimuli activate enteric neurons, which then respond coordinating gastrointestinal reflex functions, as motility. These response circuits are not fixed; they can be modulated by different factors. For instance, nutrients can have short and long-term effects on the ENS. Aging and diseases can induce enteric neuroplasticity.
All in all the ENS is able to reshape their structural and functional properties to maintain the homeostatic control of gut function.

Peptide neuromodulation counterbalances detrimental temperature influences on electrical spread

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Acute temperature changes can disrupt neuronal activity, leading to loss of motor control and failure of vital behaviors in both vertebrates and invertebrates. Acute warming of the isolated crustacean stomatogastric nervous system from 10°C to 13°C disrupts rhythmic pattern generation underlying mastication. Specifically, the acute warming silences a key motor neuron, the Lateral Gastric neuron (LG). Such temperature sensitivity *in vitro* contrasts with activity *in vivo*, which can continue over a much larger temperature range (10-20°C). It has been recently shown that peptide neuromodulation restores rhythmic bursting in LG and thereby restores rhythmic pattern generation for mastication. However, it is not known how peptide neuromodulation achieves temperature compensation.

Neuronal activity depends on adequate electrical spread throughout the neuropil - a process sensitive to shunting when ion channel conductances increase. We hypothesize that an overall increase in conductances in response to elevated temperatures disrupts rhythmic bursting in LG by shunting the membrane and reducing electrical spread in the neuropil. Peptide neuromodulation restores rhythmic bursting by counterbalancing membrane shunt and re-establishing electrical spread.

To test these hypotheses, we quantified electrical spread in LG during an acute temperature increase from 10°C to 13°C, and when modulated by the neuropeptide, *Cancer borealis* tachykinin-related peptide Ia (CabTRP Ia) using two approaches; fluorescent Calcium imaging and two-electrode current- and voltage-clamp. We found that electrical spread and membrane resistance decreased as the system warmed, but was restored in the presence of CabTRP Ia. Our results indicate that peptide neuromodulation increases electrical spread by opposing membrane shunt.

CabTRP Ia activates an NMDA-like current called the modulator-induced current (I_{MI}). To assess whether I_{MI} activation by CabTRP Ia enabled temperature compensation, we selectively introduced I_{MI} into LG using dynamic clamp. We found that I_{MI} introduction was sufficient to increase electrical spread and restore rhythmic bursting in LG at an elevated temperature. Altogether, our results support a general mechanism for temperature compensation whereby peptide activation of an NMDA-like current increases electrical spread in the neuropil to support temperature-robust neuronal activity

Dynamic heterogeneity of neuro-glial circuits in the gut wall

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Gastrointestinal function relies on delicate interactions between different cellular ensembles located within and outside the gut wall. The enteric nervous system (ENS), which is strategically positioned within the bowel, is a pivotal hub integrating the activity of intestinal cell types with signals from the gut lumen. Although the ENS harbours dedicated neural circuits that regulate stereotypic gut functions such as intestinal motility, it is organised in a seemingly anarchic mosaic composed of different types of enteric neurons and glia. To investigate the spatial configuration of enteric neural networks, elucidate the foundations of the heterogeneity of ENS constituents, and to delineate the wiring schemes of the amorphous neuro-glia circuitry we use a combination of lineage tracing methods, in vivo and ex vivo physiological assays, genetic sparse labelling and live cell imaging. Our studies identify lineage relationships and cellular assemblies underpinning ENS function and reveal that motility control is hard-wired in the enteric neural networks. At the same time, we show that ENS components and their combined output are dynamic and exhibit an extensive level plasticity, which is tuned by the regional micro-environment and cues from the gut lumen.

Symposium

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Postsynaptic cAMP is insufficient for LTP induction at hippocampal CA3-CA1 synapses

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The cyclic nucleotide cAMP has been implicated as a key second messenger in mediating activity-dependent synaptic plasticity. In the hippocampus, cAMP-dependent induction of plasticity is thought to express presynaptically at the dentate gyrus-CA3 synapse and postsynaptically in the Schaffer collateral synapse, by activating the cAMP-PKA-CREB pathway. Most studies have used forskolin stimulation of endogenous adenylyl cyclases combined with inhibition of phosphodiesterases to induce synaptic potentiation. While successful at inducing synaptic potentiation, pharmacological manipulations affect all cells in the preparation, making it difficult to localize the mechanism of action.

We took advantage of recent developments in optogenetic tools to study the effects of selectively raising cAMP in only the presynaptic or only the postsynaptic compartment of Schaffer collateral synapses. We investigated cAMP signaling in individual postsynaptic CA1 neurons by expressing the soluble photoactivatable adenylyl cyclase bPAC (Stierl *et al.*, 2011 JBC 286:1181). The endogenous adenylyl cyclases are, however, membrane-associated. Therefore, we also used a membrane-targeted cyclase (PACm) with reduced dark activity, large dynamic range and fast kinetics.

Surprisingly, light stimulation of bPAC or of the newly developed PACm in the postsynaptic CA1 neuron did not change the amplitude of EPSCs, nor the threshold for inducing LTP. While the application of forskolin increased the frequency of miniature EPSCs, light-induced cAMP elevation did not replicate these effects, suggesting that cAMP elevation alone is not sufficient to enhance synaptic strength. Additionally, light-induced cAMP elevation in the postsynaptic compartment did not trigger immediate early gene expression (cFos), unlike forskolin, which induced robust cFos expression.

Using Booster-PKA, a recently published PKA sensor (Watabe *et al*., 2020 ACS Sens. 5:719), we confirmed that light activation of bPAC and PACm increases intracellular cAMP levels and induces PKA activation, which should lead to cFos expression. Forskolin activation of endogenous cyclases induced cFos expression. However, this was only slightly reduced but not blocked by pre-treatment with PKA and EPAC inhibitors, suggesting that cAMP and PKA are not sufficient to induce cFos expression. We observed that blocking action potentials completely abolished forskolin-induced cFos expression. The same was true when blocking HCN channels prior to FSK stimulation.

Taken together, our data show that elevation of cAMP in individual neurons does not increase synaptic transmission, nor does it induce cFos expression, even though downstream targets of cAMP are activated (PKA, HCN channels). Thus, forskolin-induced long-term plasticity and expression of cFos are not cell autonomous phenomena, but a result of increased network activity caused by global (HCN-mediated) depolarization.

The role of the membrane contact sites in neurodegeneration

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The Membrane-bound organelles inside the cell communicate either by vesicles or by making close contact with each other through contact sites. The communication through the contact sites helps in the exchange of ions, proteins, lipids, calcium, etc. Mitochondria and Endoplasmic reticulum also interact with each other through a specialized region called Mitochondria associated membrane, also known as MAM. Recent studies indicate that the role of MAMs is not just limited to the exchange of materials, rather they are involved in many different biological processes such as lipid metabolism, autophagy, ER stress, mitochondrial morphology etc and its abnormal functioning can cause neuropathological and neurodegenerative disorders.

In the present study, we are investigating the role of a MAM protein called *Tomm70* in Neurodegeneration. *Tomm70* interacts with a sterol transporter ER protein called *Lam6* or *Ltc1* at the MAM. Using biochemical and microscopic methods, we have found that a single point mutation in the C-terminal region of *Tomm70* causes partial disruption of interaction with *Lam6*. We are now evaluating the impact of this disruption of interaction on lipid metabolism and thereby on MAM and whether it causes neurodegeneration or not.

Postnatal development of electrophysiological and morphological properties in layer 2/3 and layer 5 pyramidal neurons in the mouse primary visual cortex

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Homeostatic plasticity plays a major role in the development of the brain. In the first postnatal month, at the time around eye-opening, the cortex of rodents develops rapidly represented in morphology and physiology. We characterized the electrophysiological properties of pyramidal neurons in layers 2/3 and 5 in acute slices of C57Bl/6 mouse primary visual cortex. Separating our results into 3 postnatal stages: young - before eye opening (p10-14), juvenile - after eye opening (p25-p29) and adult (p60-p70), we found significant differences in intrinsic membrane properties. The membrane resistance decreased in both layers with age resulting in faster time constants. In layer 2/3 of juvenile mice the resting membrane potential was significantly more negative and firing threshold depolarized gradually with age. All in all, these changes lead to a reduced excitability of cortical neurons likely as homeostatic compensation for the increased sensory input after eye opening. Neurons were further filled with biocytin to investigate changes in dendritic morphology during postnatal development. In layer 2/3, we found an increase in apical and oblique dendritic lengths after eye opening. Spine densities increased in all branches but were reduced again in adult layer 5 cells. While the growth of spine density is correlated with increased sensory input after eye opening, the loss of spines might follow due to specification processes. Together, we have characterized electrophysiological and morphological properties of neurons from supra- and infragranular layers of visual cortex around eye opening and in adult mice. The observed changes are in line with a homeostatic compensation in response to the increased sensory input as well as maturation of passive and active cellular properties.

Moving beyond in vivo experiments - encoding of spatiotemporal gratings in electric fish in experimental and simulated data

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Sensory flow is a crucial source of information for guiding appropriate behavioural responses. The gymnotiform wave-type electric fish is well known for its ability to actively sense objects in the water using its electric sense. This fish emits a continuous quasi-sinusoidal electric organ discharge (EOD) which is detected by specialized electroreceptors distributed throughout the body surface. This sense, which allows the fish to successfully navigate its environment and locate prey, is influenced by the object's size, shape, material, lateral distance, relative speed and water conductivity. Here, we characterize the electroreceptor responses in *Apteronotus leptorhynchus* to moving gratings at different sizes, speeds, and lateral distances in experiments and simulations, and establish some of the spatiotemporal dynamics involved in the neural encoding. Electrophysiological recordings are compared to simulated electroreceptor responses, to augment experimental data. Their generation takes finite element simulations of the electric image based on the previous electric image model of Babineau et al. (Journal of Experimental Biology, 2006) that are fed into a leaky integrate-and-fire model with adaptation current. We then show the results of the receptor model simulation using conditions that go beyond current experimental limits. By combining experimental and simulated data, we gain a more complete understanding of the spatiotemporal dynamics involved in the neural encoding of moving stimuli by the electroreceptors in the electric fish *Apteronotus leptorhynchus*. Experimentally, we show that electroreceptor responses correlate negatively with increasing lateral distance and become indiscriminable within a few millimeters, correlate positively with increasing speed, and become difficult to discriminate at gap sizes below 2mm. Using the model, we create different neuronal population sizes to mimic the receptive fields of postsynaptic neurons and investigate the impact of population size on the electrosensory encoding of such objects. Our findings provide a fundamental starting point for further electrosensory flow research in electric fish. Furthermore, our technique opens the door to study how the electric sense encodes any sort of stimuli in the electric fish computationally.

The Johnston's organ of desert ants and its central projections

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The Johnston's organ (JO) in the insect antenna is a multisensory organ involved in several navigational tasks including wind-compass orientation, flight control, graviception, and, possibly, magnetoreception. Here we present the three dimensional anatomy of the JO and its neuronal projections into the brain of the desert ant *Cataglyphis*, a marvelous long-distance navigator. The JO of *Cataglyphis nodus* workers consists of 40 scolopidia comprising three sensory neurons each. The numbers of scolopidia slightly vary between different sexes (female & male) and castes (worker & queen) with the highest number found in males. Individual scolopidia attach to the intersegmental membrane between pedicel and flagellum of the antenna and line up in a ring-like organization. Three JO nerves project along the two antennal nerve branches into the brain. Anterograde double staining of the antennal afferents revealed that JO receptor neurons project to several distinct neuropils in the central brain. The T5 tract projects into the antennal mechanosensory and motor center (AMMC), while the T6 tract bypasses the AMMC via the saddle and forms collaterals terminating in the posterior slope (PS) (T6I), the ventral complex (T6II), and the ventrolateral protocerebrum (T6III). Double labeling of JO and ocellar afferents revealed that input from the JO and visual information from the ocelli converge in tight apposition in the PS. The general JO anatomy and its central projection patterns resemble situations in honeybees and Drosophila. The multisensory nature of the JO together with its projections to multisensory neuropils in the ant brain likely serves synchronization and calibration of different sensory modalities during the ontogeny of navigation in *Cataglyphis*.

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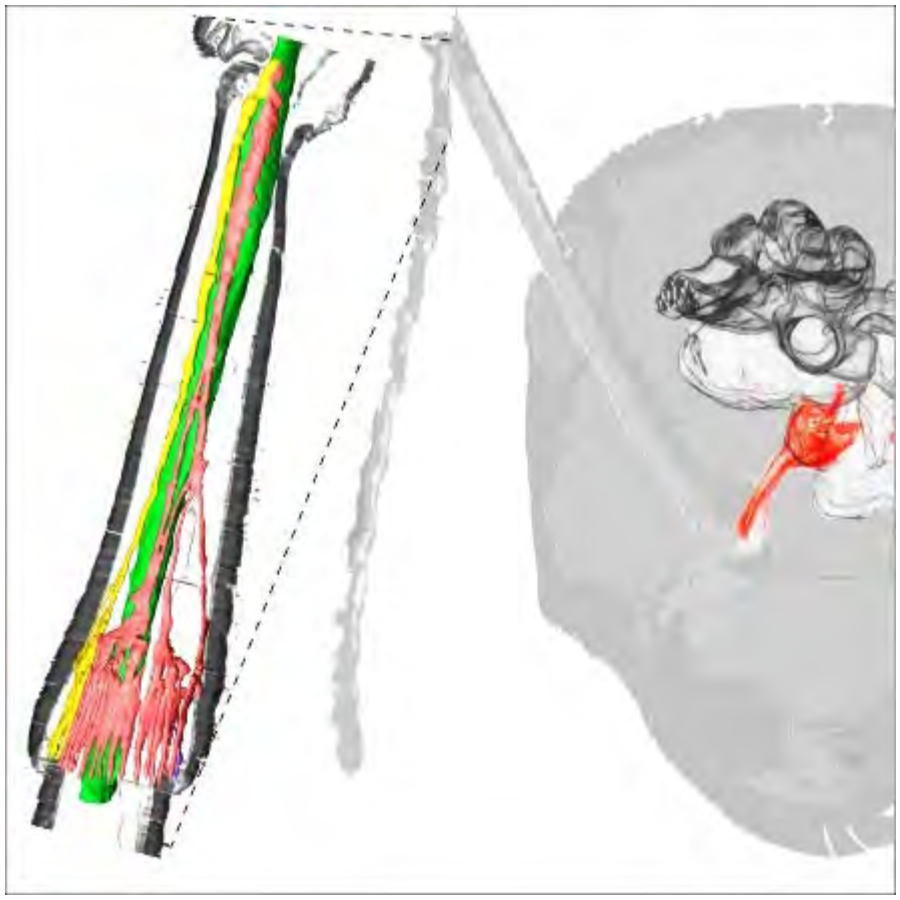

Optogenetically controlled aggregation of calcium channels in the auditory cortex causes deterministic population dynamics and suppressed impulse responses

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Neuronal communication is critically mediated by the release of chemical transmitters from presynaptic vesicles. This neurotransmitter release is controlled by voltage gated calcium channels (VGCCs) which trigger the influx of calcium ions to the presynaptic bouton upon action-potential-induced membrane depolarization. However, intracellular calcium is strictly controlled and immediately buffered from the intracellular space due to its substantial function as a second messenger molecule. The transient and local action of calcium requires a close proximity between vesicular calcium sensors and the VGCC pore, known as nanodomains, to efficiently initiate vesicle fusion and transmitter release. The probability that enough channels are perfectly situated in nanometer distances in the moment when an action potential arrives in the presynapse is not 100%. This lack of determinable neurotransmitter release by action potential is part of what gives us a probabilistic firing rate. Network oscillations emerge naturally out of a system like this. When neuronal VGCCs were acutely aggregated using the optogenetic clustering of an cryptochrome mutant , CRY2olig[1], under blue light, this probabilistic firing mode was shifted to a deterministic one[2]. Upon VGCC clustering, cultured primary hippocampal neurons generated a strong and reliable paired-pulse depression of consecutive responses while control neurons showed almost no reduction in response amplitudes during the train of stimulation. While this method was used in vitro to render a single cell from a more random to a more predictable firing mode, the question we sought to answer was how this would affect a neuronal network. For this purpose, we have combined the usage of CRY2olig with a transgenic mouse model that allows for the optogenetic clustering of VGCCs in vivo. In particular, this knock-in line expresses a Citrine tag, a YFP/GFP derivate, at the N-terminus of the neuronal VGCC type $Ca_V2.1$[3]. This Citrine tag, and in turn the $Ca_V2.1$ channel, were coupled to Cry2olig via a feed-back-controlled anti GFP-intrabody which was transduced to the primary auditory cortex (A1) in a lentivirus. We recorded local field potentials down the depth of the A1 using a 32-channel shaft electrode and transformed the output into current source density (CSD) profiles over consecutive measurements under ketamine-xylazine anesthesia. Cortical response to auditory stimuli in the form of click trains and amplitude modulated tones was recorded, as well as spontaneous brain activity. We demonstrated through cortical layer activity and average rectified CSD profiles that there is a significant suppression of the impulse response to click stimuli. Interestingly, response

to amplitude modulated tones and spontaneous activity did not show this same level of suppression. With applying the more elaborate spectral analysis of power and phase coherence of oscillations in layer-specific neuronal activity, we aim to disentangle the influences of VGCC dynamics on the dominant impulse response, provided by click stimulation, and on the more temporally dispersed coordinated response characteristics, represented in response to amplitude modulation.

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Top-down acetylcholine enables social discrimination via unlocking action potential generation in olfactory bulb vasopressin cells

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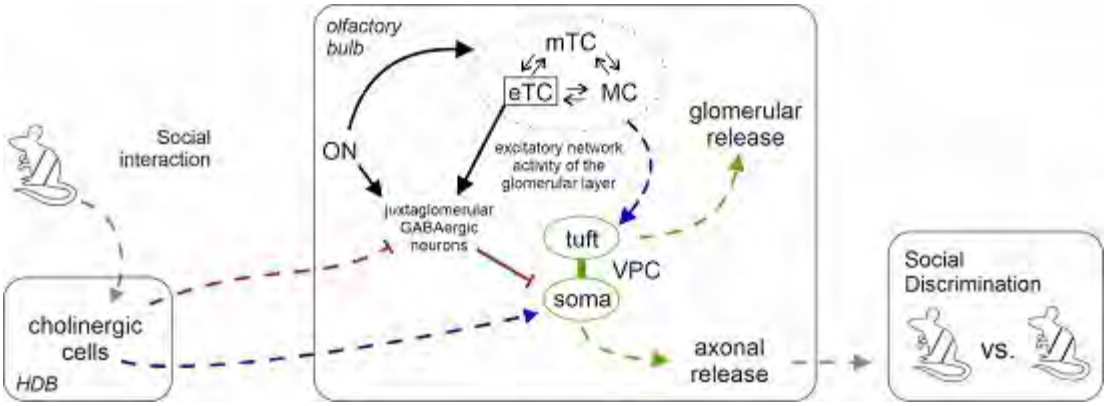
Identification of a conspecific's identity, e.g. an intruder or littermate, is essential for adequate behavioral responses in social mammals, e.g. aggressive behavior or grooming. In rodents, the social discrimination paradigm is performed to quantify the ability of individual conspecific identification. In this paradigm, social discrimination is quantified as the duration of investigating a novel conspecific compared to a known one. Social discrimination in rats requires activation of the intrinsic bulbar vasopressin system, since a preference towards a novel rat is abolished with microinjection of a vasopressin receptor antagonist into the olfactory bulb. However, we previously found that olfactory-nerve inputs alone inhibit, thus cannot excite bulbar vasopressin cells *in-vitro*. Therefore, it is unclear how this system comes into operation. Here we show that a higher number of bulbar vasopressin cells was activated ($pERK^+$) by stimulation with a conspecific compared to rat urine, indicating that vasopressin cell activation depends on more than olfactory cues during social interaction. *In-vitro* slice electrophysiology combined with pharmacology showed that acetylcholine, especially the muscarinic signaling, enables olfactory-nerve evoked action-potentials in vasopressin cells. Furthermore, immunohistochemistry indicated that centrifugal cholinergic input from the horizontal limb of the diagonal band of Broca plays a role as a higher number of activated ($pERK^+$) cholinergic neurons was observed with an exposure of a conspecific than rat urine. Finally, we were able to show that muscarinic activation of the bulbar vasopressin system is also involved in vasopressin-dependent social discrimination, since recognition of a known rat could be blocked by local application of a muscarinic antagonist and rescued by additional application of vasopressin. As both, vasopressin and acetylcholine were shown to be not needed for simple odor discrimination, we hypothesize that the bulbar vasopressin system requires additional inputs, i.e. top-down acetylcholine, to be activated to discriminate complex similar odors, like body odors, during certain contexts, such as social interaction. In summary, we demonstrated that top-down cholinergic modulation of bulbar vasopressin cell activity is crucial for individual social discrimination in rats.



Respiration paces prefrontal neuronal activity during intense threat

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Respiration, subdivided in inspiration and expiration, has been shown to affect human emotion recognition via nasal airflow (Zelano et al., 2016. *J. Neurosci.* **36**, 12448–12467). Furthermore, recent studies performed on rodents revealed that respiration plays a key role in higher order cognitive processes, namely by entraining the medial prefrontal cortex (mPFC) neuronal activity during freezing induced by Pavlovian auditory conditioning (Moberly et al., 2018. *Nat. Commun.* **9**). We recently found that the mPFC activity is reliably entrained by respiration during despair-like behavior in a tail suspension test (TST) (Biskamp et al., 2017. *Sci. Rep.* **7**), suggesting that respiration-related rhythms (RR) might aid prefrontal processing during threat. To address this hypothesis, we performed local field potential and single unit recordings in the mPFC of mice while monitoring the respiration during different behavioral states. We found that respiration paces the activity of a majority of mPFC neurons during immobility, whether this immobility was emotionally neutral, as in the home cage, or linked to threat during TST. Nonetheless, we observed that neurons fire preferentially during inspiration when the mice where in their home cage, and switched toward firing more during the transition from expiration to inspiration when the mice were subjected to TST stress. Furthermore, immobility during the TST induced a robust increase in the percentage of cells coupled to the respiration, but solely in the superficial layers 2/3, when compared to neutral immobility. This localized change in RR entrainment suggests that a different macro-circuit of the mPFC is recruited by the respiration during intense threat. The respiration frequency and amplitude is strongly modulated by cognitive states, and unlike other sensorial afferences, projections from the olfactory bulb by-pass the thalamus to directly project to brain regions involved in emotions and cognition. These characteristics, along with increasing electrophysiological evidences, indicate that the RR role in the brain extends beyond olfactory processing.

The effects of breathing rate on theta-gamma coupling

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Nasal breathing has been shown to induce respiration-related network oscillations far beyond olfactory regions. These rhythms modulate specific frequency bands of cortical gamma oscillations while other gamma subbands are coupled to theta-oscillations. (1) Cross-frequency-coupling between theta and gamma activity is especially strong during rapid eye movement (REM) sleep. At the same time, both theta-gamma coupling and the rate of nasal breathing are highly variable. We therefore analyzed the relation between breathing rate and theta-gamma coupling in the parietal cortex during REM-sleep in mice.
We simultaneously recorded local field potentials and respiration rate in 22 mice, using a customized full-body plethysmograph. (Figure A). REM-sleep was staged manually. Built-in and custom-written routines in MatLab were used for spectral analysis, cross-frequency-coupling and continuous estimation of breathing rate.
Our results show that theta-gamma coupling closely correlates with breathing rate in REM-sleep. The dependence of theta-gamma coupling on respiration rate follows a V-shaped curve with maximal coupling at breathing rates around 4-6 Hz. (Figure B). Interestingly, breathing rate differentially relates to theta-gamma coupling in two distinct sleep patterns, defined as phasic and tonic REM (Figure C). (2)
Our data reveals a new interaction of respiration with brain activity. Thus, respiration and other cortical network patterns are linked to each other in a more complex fashion than previously assumed. At the same time, the relation between breathing rate and theta-gamma coupling differs between phasic and tonic REM-sleep, lending a new, independent support for their physiological distinctiveness.

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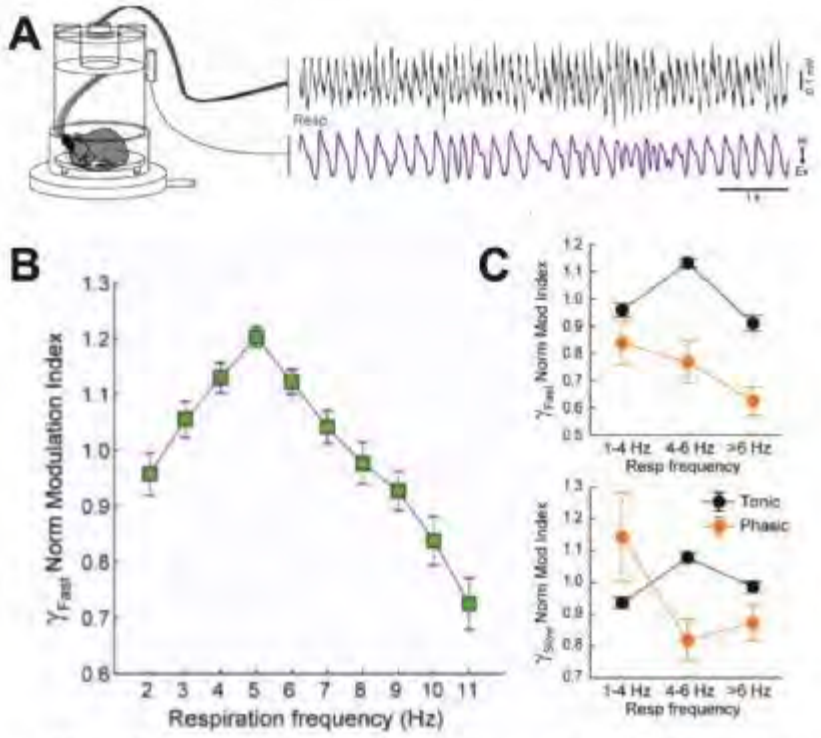

modified after Hammer et al., Theta-Gamma coupling depends on breathing rate. bioRxiv 2020.10.22.349936; doi: https://doi.org/10.1101/2020.10.22.349936

Optogenetic silencing of neurotransmitter release with a naturally occurring invertebrate rhodopsin

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Optogenetic silencing is a powerful method for functionally dissecting neuronal circuits and understanding the contribution of defined neuronal populations to behavioral processes. However, silencing of long-range axonal projections has posed a formidable challenge for modern neuroscience. Existing optogenetic tools suffer from low efficacy and off-target effects when applied to presynaptic terminals, while chemogenetic approaches for synaptic silencing lack spatiotemporal precision. Here we present eOPN3, a targeting-enhanced type-II mosquito rhodopsin that can effectively suppress presynaptic neurotransmitter release through activation of the $G_{i/o}$ signaling pathway. We show that expression of eOPN3 in CA3 pyramidal cells of organotypic hippocampal slices yields efficient membrane targeting and trafficking to distal axons projecting to area CA1. Activation of eOPN3 in the somatodendritic compartment of CA3 cells leads to mild GIRK-channel mediated hyperpolarization and weak reduction in action potential firing. In contrast, brief activation of eOPN3 at synaptic terminals strongly reduces the amplitude of EPSCs between pairs of CA3 and CA1 cells without affecting presynaptic CA3-cell action potential firing. Illumination of eOPN3-expressing Schaffer collateral afferents during field stimulation showed that the decrease in synaptic output was reversible. Moreover, similar to natural presynaptic inhibition via $GABA_B$-receptor mediated $G_{i/o}$ signaling, eOPN3 activation displayed high-pass filtering properties during high firing rates. Two-photon calcium imaging at individual presynaptic boutons revealed a decrease in action potential-evoked Ca^{2+} influx upon eOPN3 activation. This decrease was independent of GIRK-channel activation, indicating a direct, $G_{i/o}$-mediated inhibition of presynaptic voltage-gated Ca^{2+} channels by eOPN3, similar to native $G_{i/o}$-coupled receptors. In behaving mice, eOPN3 activation triggered robust pathway-specific behavioral effects. Here we used eOPN3 to inhibit dopaminergic substantia nigra afferents in the dorsal striatum, leading to an ipsiversive rotational bias in freely moving mice. We therefore conclude that eOPN3 can be used to selectively suppress neurotransmitter release at synaptic terminals with high spatiotemporal precision, opening new avenues for functional interrogation of long-range neuronal circuits *in vivo*.

Symposium

S12: Emerging views on microglia and oligodendrocytes in Alzheimer's disease

S12-1 Oligodendrogenesis declines during healthy aging in the mouse central nervous system and is mediated by neuronal BDNF-TrkB signalling
Madeline Nicholson, Rhiannon Wood, Jessica Fletcher, Simon Murray

S12-2 Oligodendrocyte dysregulation in Alzheimer’s disease
Xin Qi

S12-3 Aging-associated oligodendrocyte dysfunction drives amyloid deposition in Alzheimer’s disease
Constanze Depp, Andrew Octavian Sasmita, Ting Sun, Lena Spieth, Stefan Berghoff, Klaus-Armin Nave

S12-4 Innate immune activation in neurodegenerative disease
Michael Thomas Heneka

S12-5 Synaptic remodeling in response to acute microglia activation using light-induced engagement of microglia in the adult brain
Carla Cangalaya, Anna Godfried, Weilun Sun, Susane Wegmann, Stoyan Stoyanov, Klaus-Dieter Fischer, Alexander Dityatev

S12-6 Therapeutic Modulation of TREM2 Function
Christian Haass

Oligodendrogenesis declines during healthy aging in the mouse central nervous system and is mediated by neuronal BDNF-TrkB signalling

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Age is the primary risk factor for neurodegenerative disease and most pathologies are associated with impairments to myelin. Although the generation of oligodendrocytes and myelin continues throughout life, there is a decline in the efficiency of oligodendrogenesis and total myelin content with age. However, the underlying temporal properties of the onset and progression of this decline during healthy aging remain unknown, as does whether distinct CNS regions undergo differential courses of decline. A complete understanding of oligodendrogenesis during healthy aging could inform therapeutic strategies to promote myelination in the adult CNS.

To assess oligodendrogenesis, the thymidine analogue EdU was administered for 6-weeks to cumulatively label newly formed oligodendrocytes in adult C57Bl/6 mice beginning at 2-months (young-adulthood), 12-months (early aging) and 18-months (aging). Immunohistochemistry and confocal microscopy identified oligodendroglial lineage cells in 3 distinct regions; the optic nerve (ON, an almost fully myelinated white matter tract), the corpus callosum (CC, a partially myelinated white matter tract) and the somatosensory cortex (SC, a sparsely myelinated grey matter area). To investigate neuronal BDNF-TrkB signalling as a candidate molecular mediator of oligodendrogenesis during aging we generated an inducible, neuronal-specific conditional TrkB knock-out mouse. We induced deletion at 12-months and performed cellular analysis 5-months later.

At 2-months of age we found regionally distinct variation in oligodendrogenesis, consistent with known regional variation in myelin development. After 6-weeks of EdU, 11% of newly generated post-mitotic oligodendrocytes were identified in the CC, 6% in the SC and 0.33% in the ON. By 12-months this uniformly decreased in the brain, with <1% new EdU+ oligodendrocytes in the CC and 2% in SC, and remains ~1% in ON. Uniformly, this is maintained at 18-months. Consistent with this, the population of EdU+ dividing OPCs also declines during healthy aging to stabilise at 60% at 12- and 18-months across regions; falling from ~80% at 2-months. Further, the differentiation of OPCs declines in the CC with 58% of total EdU+ oligodendroglia identified as post-mitotic at 2-months and ~20% at 12- and 18-months, and a similar trend in the SC with 23% at 2-months and ~15% at 12- and 18-months. Interestingly, we are the first to find that neuronal TrkB expression is required during healthy aging for both survival of pre-existing oligodendrocytes and for differentiation of adult-born oligodendrocytes. In knock-out mice we observed a 25% and 50% reduction, respectively, in the SC, but not CC or ON.

These results suggest that oligodendrogenesis during young adulthood is tailored regionally to the requirements of lifelong myelination, suggesting a predominant function of de novo myelination. However,

during healthy aging production is maintained at a much reduced, but proportionally uniform level, suggesting consistent rates of oligodendrocyte turnover across the CNS. Importantly, these results have established a role for neuronal TrkB signalling in mediating oligodendrocyte survival and differentiation in the cortex during healthy aging. Together, these results establish the homeostatic capacity for oligodendrogenesis across the CNS during healthy aging. Fascinatingly, they further identify a regional difference in the underlying molecular program driving lifelong myelination.

Oligodendrocyte dysregulation in Alzheimer's disease

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Myelin degeneration and white matter loss resulting from oligodendrocyte (OL) death are early events in Alzheimer's disease (AD) that lead to cognitive deficits, however, the underlying mechanism remains unknown. Here, we find that mature OLs in both AD patients and an AD mouse model undergo NLRP3-dependent Gasdermin D-associated inflammatory injury, concomitant with demyelination and axonal degeneration. The mature OL-specific knockdown of dynamin-related protein 1 (Drp1; a mitochondrial fission GTPase) abolishes NLRP3 inflammasome activation, corrects myelin loss, reduces axonal degeneration, and improves cognitive ability in AD mice. Moreover, Drp1 hyperactivation in mature OLs induces a glycolytic defect in AD models by inhibiting hexokinase 1 (HK1; a mitochondrial enzyme that initiates glycolysis), which then triggers NLRP3-associated inflammation. These findings suggest that OL glycolytic deficiency plays a causal role in AD development, and that the Drp1-HK1-NLRP3 signaling axis may be a key mechanism and therapeutic target for white matter degeneration in AD.

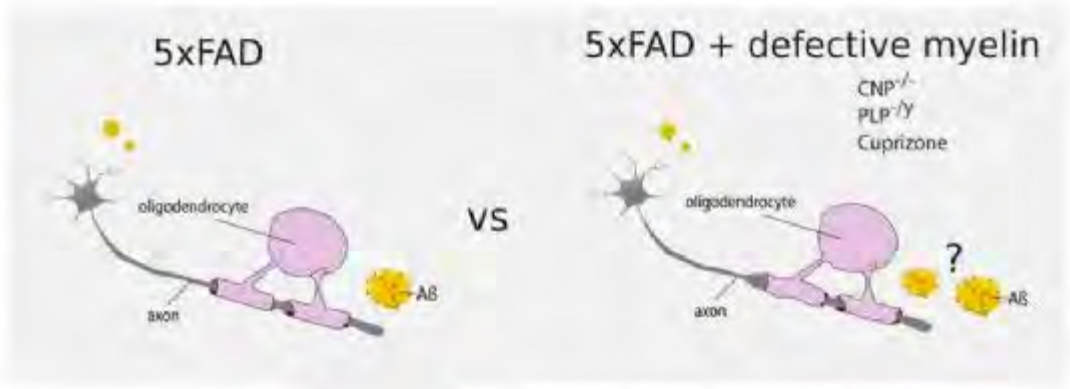
Aging-associated oligodendrocyte dysfunction drives amyloid deposition in Alzheimer's disease

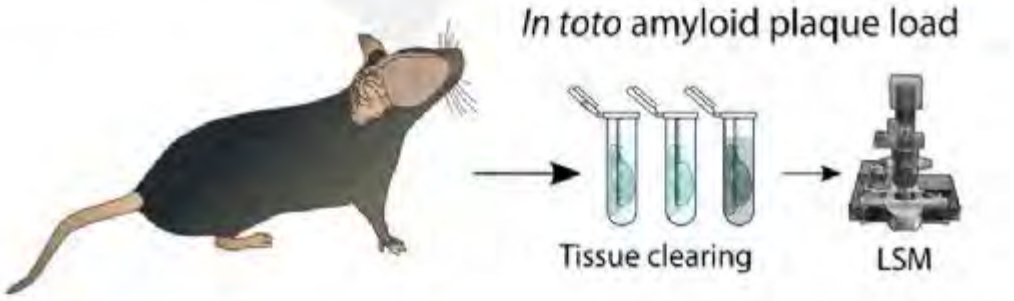
Constanze Depp[1], Andrew Octavian Sasmita[1], Ting Sun[1], Lena Spieth[1], Stefan Berghoff[1], Klaus-Armin Nave[1]

[1]Max Planck Institute for Exprimental Medicine, Department of Neurogenetics, Hermann-Rein-Straße 3, 37075 Goettingen, Germany

Brain aging constitutes the greatest risk factor for the development of Alzheimer's Disease (AD), but this association is mechanistically poorly understood. Aging clearly affects the integrity of myelin, with splittings, outfoldings and secondary inflammation in white matter tracts. In addition to the well-established function of myelin as electrical insulator, mature oligodendrocytes provide metabolic support to the axonal compartment through cytoplasmic channels in the myelin sheath. Therefore, it is likely that age-associated structural perturbations of myelin also impair axonal energy balance which enhances Amyloid-ß production.

In proof of principle experiments, we used transgenic mouse model of AD (5xFAD and APP^{NLGF}) and tested the impact of advanced white matter aging on amyloid burden. We induced aging-associated myelin dysfunction by genetic inactivation of the oligodendrocyte-specific genes CNP and PLP as well as by direct demyelination (Cuprizone intoxication model). To visualize and quantify amyloid burden in toto, we used light sheet microscopy of stained Aß plaques in combination with tissue clearing. Indeed, double mutant mice (AD + defective myelin) consistently showed enhanced plaque load in cortex, hippocampus and hippocampal white matter. Mechanistically, we show that myelin dysfunction drives the accumulation of the Aß producing machinery (ß- and γ-secretase) in axonal swellings and increases cortical APP cleavage. Surprisingly, we also found a profound loss of plaque-associated microglia in double mutant mice despite brain wide microgliosis. RNAseq analysis of microglia isolated from $CNP^{-/-}$/5xFAD and 5xFAD revealed a similarly inflammatory profile in both populations (DAM signature, adequate Trem2 expression) but $CNP^{-/-}$/5xFAD additionally showed highly increased Apolipoprotein secretion. In contrast, the lack of compact myelin in forebrain-specific shiverer mice (Emx-Cre/$MBP^{fl/fl}$) in the absence of axonal swellings decreased amyloid deposition and enhanced microglia association with amyloid plaques. Our observations suggest that in the aging brain microglia become primarily engaged with myelin dampening the protective reaction to amyloid deposition which together with heightened Apolipoprotein secretion promotes plaque-seeding. Simultaneously, axonal problems arise that alter neuronal APP metabolism in favor of amyloid production. Our work, therefore, shows that oligodendrocyte defects can drive Aß deposition via mechanisms involving both increased APP processing and altered microglia responses, and identifies myelin aging as a previously overlooked risk factor for AD.


5xFAD
oligodendrocyte
axon
Aß
vs
5xFAD + defective myelin
CNP-/-
PLP-/Y
Cuprizone
oligodendrocyte
axon
?
Aß


In toto amyloid plaque load
Tissue clearing
LSM

Innate immune activation in neurodegenerative disease

Michael Thomas Heneka[1]

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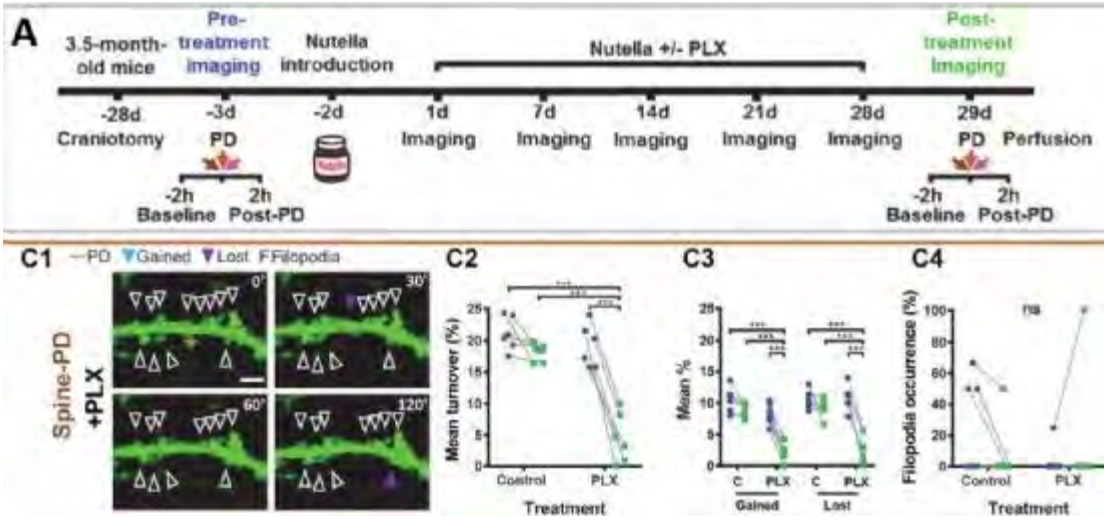
The accumulation of neurotoxic or misfolded amyloid beta peptides and/or neurofibrillary tangle formation represent key pathological hallmarks of neurodegenerative diseases including but not limited to Alzheimer's disease, frontotemporal dementia and multi-system atrophy. The brain has been considered as an immune-privileged organ, however, increasing evidence from translational, genetic, and pathological studies suggests that activation of distinct innate immune pathways are a third important disease hallmark which, once initiated, actively contributes to progression and chronicity of neurodegenerative disease.
Microglia play a pivotal role in this immune response and are activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors. This immune activation leads to the release of inflammatory mediators, but also distracts microglia cells from their physiological functions and tasks, including debris clearance and trophic factor support. NLRP3 inflammasome activation and release of ASC specks contribute to spreading of pathology and impair microglia clearance mechanisms, and together contribute to neuronal spine loss, neuronal degeneration, and ultimately to spatial memory deficits. In keeping with this immune hypothesis of neurodegeneration, inhibition of this and other immune pathways protect from neurodegeneration in cellular and murine models of neurodegenerative disease. Modulation of the microglia driven innate immune response at key signaling steps might therefore be protective and alter disease progression. However, the microglia are not a stable population, but have continuous turn over, most likely resulting in more than one generation of microglia being involved in disease progression. Moreover their turnover is increased in response to neurodegeneration. Along with the regional diversity of microglia, these phenomena need to be understood in more detail prior to targeting innate immune mechanisms for therapeutic purposes.

Synaptic remodeling in response to acute microglia activation using light-induced engagement of microglia in the adult brain

Carla Cangalaya[1,2,3], Anna Godfried[2,4], Weilun Sun[3], Susane Wegmann[6], Stoyan Stoyanov[3], Klaus-Dieter Fischer[2,4], Alexander Dityatev[1,3,4,5]

[1]ESF International Graduate School on Analysis, Imaging and Modelling of Neuronal and Inflammatory Processes, Magdeburg, Germany, [2]Institut für Biochemie und Zellbiologie, Germany, [3]German Center for Neurodegenerative Diseases (DZNE), Molecular Neuroplasticity, Magdeburg, Germany[4]Otto-von-Guericke University, Medical Faculty, Magdeburg, Germany [5] Center for Behavioral Brain Sciences (CBBS), Magdeburg, Germany [6]German Center for Neurodegenerative Diseases (DZNE), Berlin, Germany

Microglia continuously monitor synapses, but active synaptic remodeling by microglia in mature healthy brains is rarely directly observed. We performed targeted photoablation and two=photon imaging of single synapses in mature transgenic mice expressing fluorescent labels in neurons and microglia. The photodamage temporally and focally increased the duration of microglia-neuron contacts, and dramatically stimulated both the turnover of dendritic spines and presynaptic boutons as well as the generation of new filopodia originating from spine heads or boutons. The results of microglia depletion confirmed that elevated spine turnover and the generation of presynaptic filopodia are microglia-dependent processes. Moreover, systemic and local acute activation of microglial cells (with lipopolysaccharide or sarkosyl-insoluble fraction derived from AD patient brain) further exacerbated spine/bouton turnover, contact between these cells and neurons and generation of filopodia as compared to resting microglia. Our results provide a direct evidence that activated microglia not only more active in pruning dendritic spines, but also actively promote formation of new spines and filopodia. These results highlight a specific role of activated microglia in synaptic remodelling and uncover new aspects of how the activation of the immune system may impact on cognitive and behavior processes.



Therapeutic Modulation of TREM2 Function

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Alzheimer's disease (AD) is currently untreatable, and therapeutic strategies targeting the amyloid cascade have not yet been successful, indicating that novel treatment strategies are required. Recent genome wide association studies have identified a number of risk factors in genes expressed in microglia, including the Triggering receptor expressed on myeloid cells 2 (TREM2). TREM2 is essential for the transition of homeostatic microglia to disease associated microglia. TREM2 loss of function locks microglia in a homeostatic state, and affects a multitude of microglia functions such as chemotaxis, phagocytosis, cell survival, lipid- and energy metabolism. Biomarker studies revealed that TREM2 may protect humans from AD. To enhance TREM2 activity, we selectively increased the full-length protein on the cell surface via reducing its proteolytic shedding by ADAM proteases. We generated a panel of monoclonal antibodies against the stalk region of TREM2, which encompasses the cleavage site, with the aim to compete for α-secretase mediated shedding. Monoclonal antibody 4D9, which binds to an epitope close to the ADAM10/17 cleavage site, stabilized TREM2 on the cell surface, reduced its shedding and concomitantly activated phospho-SYK signaling in a dose dependent manner. Moreover, 4D9 stimulated survival of cultured macrophages, increased myelin debris uptake of primary microglia and reduced the amyloid burden in a mouse model for AD pathology. Thus, our findings demonstrate that antibodies elevating full-length TREM2 on the cell surface allow selective modulation of TREM2 dependent functions in microglia and macrophages, which may be of potential therapeutic benefit.

Symposium

S13: Sino-German joint symposium on cutting-edge neurotechnology in behavioral and systems neuroscience

S13-1 Rhythms for Cognition: Communication through Coherence
Pascal Fries

S13-2 Spying on monoamine neuromodulation with new genetically encoded fluorescent sensors
Yulong Li

S13-3 Holistic bursting cells
Xiaowei Chen, Meng Wang, Xiang Liao, Ruijie Li, Shanshan Liang, Hongbo Jia

S13-4 Reversible and pathway-selective inactivation of the mesoaccumbal circuit in primates affects motivational behavior but not reinforcement-based learning.
Wim Vanduffel, Tadashi Isa

S13-5 A Hierarchical 3D-motion Learning Framework for Animal Spontaneous Behavior Mapping
Pengfei Wei, Kang Huang, Yaning Han, Liping Wang

S13-6 Ketogenic diet modulates microglial morphological properties at steady-state and promotes resilience to repeated social defeat stress in adult mice
Fernando Gonzalez Ibanez, Kaushik P Sharma, Katherine Picard, Kanchan Bisht, Haley A Vecchiarelli, Nathalie Vernoux, Marie-Eve Tremblay

S13-7 BiPOLES: an optogenetic tool for bidirectional dual-color control of neurons
Silvia Rodriguez-Rozada, Johannes Vierock, Peter Hegemann, J. Simon Wiegert

Rhythms for Cognition: Communication through Coherence

Pascal Fries[1]

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Free viewing of natural images induces gamma-band oscillations in early visual cortex. If the gamma rhythm in a lower visual area entrains a gamma rhythm in a higher visual area, this might establish an effective communication protocol: The lower area sends a representation of the visual stimulus rhythmically, and the higher area is most excitable precisely when this representation arrives. At other times, the higher area is inhibited, which excludes competing stimuli. I refer to this scenario as the Communication-through-Coherence (CTC) hypothesis. To test the CTC hypothesis, we used polyimide thin-film based electrocorticography, realizing 252 recording locations distributed over large parts of the left hemisphere of awake macaque monkeys. We found that indeed, when two visual stimuli induce two local gamma rhythms in V1, only the one induced by the attended stimulus entrains V4. Crucially, the gamma synchronization between V1 and V4 occurs at the phase relation that is optimal for stimulus transmission, as evidenced by short behavioral reaction times. While these results strongly support the CTC hypothesis, causal evidence remained indirect. Therefore, we used optogenetics to generate depolarizing currents in pyramidal neurons of anesthetized cat visual cortex, emulating excitatory synaptic inputs under precise temporal control, while simultaneously measuring spike output. Cortex transformed constant excitation into strong gamma-band synchronization, revealing the well-known cortical resonance. White-noise input sequences enabled causal analysis of network transmission, establishing that cortical resonance selectively transmits input components that are phase-aligned with the postsynaptic resonance. Corresponding computational models composed of recurrently coupled excitatory and inhibitory units uncovered a crucial role of feedback inhibition in this process. Together, these results lend further support to the CTC hypothesis.

Spying on monoamine neuromodulation with new genetically encoded fluorescent sensors

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Monoamine neuromodulators, e.g., dopamine (DA), norepinephrine (NE) and serotonin (5-HT), play important roles in a plethora of physiological processes, including motivational behavior, movement, attention, sleep, learning and memory. Dysfunction of the monoaminergic system is associated with a range of diseases, such as Parkinson's disease (PD), attention deficit hyperactivity disorder, depression and addiction. A longstanding yet largely unmet goal is to measure the dynamics of monoamine neuromodulators reliably and specifically with high spatiotemporal precision, particularly in animals executing complex behaviors. In this talk I will introduce a series of genetically encoded GPCR-activation-based (GRAB) sensors that enable these measurements. $GRAB_{DA}$ sensors can detect endogenous DA release in mouse brain slices, resolve compartmental DA release from a single neuron in live flies, and report optogenetically elicited nigrostriatal DA release as well as mesoaccumbens dopaminergic activity during sexual behavior in freely behaving mice. Using $GRAB_{NE}$, we successfully observed looming-evoked NE release in the midbrain of live zebrafish, as well as optogenetically and behaviorally triggered NE release in the LC and hypothalamus of freely moving mice. Similarly, $GRAB_{5\text{-}HT}$ can detect 5-HT release in multiple physiological and pathological conditions in both flies and mice. These sensors enrich our understanding of monoaminergic neuromodulation in the brain. Furthermore, The GRAB strategy can be applied to develop new GRAB sensors for other important neurotransmitters and neuromodulators.

Holistic bursting cells

Xiaowei Chen[1], Meng Wang[1], Xiang Liao[2], Ruijie Li[1], Shanshan Liang[1], Hongbo Jia[3]

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[2]Center for Neurointelligence, School of Medicine, Chongqing University, Chongqing 400030, China
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Learning can change neuronal activities in neocortex. However, the spike firing properties of individual cortical neurons transformed by learning remain unknown. By combining two-photon Ca2+ imaging and single-cell electrophysiology in behaving mice following auditory associative training. We find a learning transformed sparse set (~5%) of layer 2/3 neurons in the primary auditory cortex, each of which reliably exhibits high-rate burst firing responses to the trained sound. In mice trained with complex chords, we strikingly discover distinct subsets of neurons that exhibit bursting responses specifically to a chord but neither to any constituent tone nor to the other chord. Together, our results demonstrate an integrated representation of learned complex sounds in a small subset of cortical neurons and their pivotal role in sensory processing.

Reversible and pathway-selective inactivation of the mesoaccumbal circuit in primates affects motivational behavior but not reinforcement-based learning.

Wim Vanduffel[1], Tadashi Isa[2]

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[2]Kyoto University, Division of Neurobiology and Physiology, Department of Neuroscience, Yoshida-Konoe-cho, Sakyo-ku, 606-8501 Kyoto, Japan

Refined causal methods to investigate brain circuits are well established in rodent research. Unfortunately, these powerful tools are only slowly adopted by the primate neuroscience community, mainly because primates are less genetically tractable than rodents. Especially relevant are perturbation tools which affect pathways between anatomically connected regions rather than a brain region in isolation. The latter approach can impact the entire out- and input from/to that region, while pathway selective manipulations reveal considerably more specific information leaving non-targeted outgoing and incoming pathways unaffected. Tadashi Isa was the first to apply such pathway-selective inactivation in the spinal cord of monkeys using a double-infection viral vector procedure (Kinoshita et al., 2012). In my talk, I will present results of a recent collaborative study (Vancraeyenest et al., 2020), in which we used the double-infection technique to reversibly inactivate the connections between the ventral tegmental area and the nucleus accumbens, two major hubs of the reward system in monkeys. Rodent studies demonstrated the role of this mesoaccumbal circuit in both reinforcement-based learning and motivational behavior. Given the profound differences between the primate and rodent dopaminergic system, however, it remains unclear whether the same holds true for monkeys. We showed that selective, unidirectional and reversible blockage of the primarily dopaminergic mesoaccumbal circuit in monkeys increased network-level functional connectivity, as measured with resting state whole brain fMRI. These counterintuitive increased functional connectivity effects were especially pronounced in fronto-temporal cortical circuits. Surprisingly, these global network changes were not associated with deficits in reinforcement-based learning during an object discrimination reversal task. In contrast, sustained mesoaccumbal inactivation greatly reduced motivation for performing a motivation-based decision-making task. Thus, contrary to our predictions, the mesoaccumbal pathway in primates is critical for high-effort motivational behavior, but not for all forms of reinforcement-based learning. More generally, pathway-selective perturbations are becoming increasingly more sophisticated, also for primate brain research. These methods will enable us to dissect the functional role of very specific brain circuits underlying perception, cognition and action. Ultimately, reversible up-and downregulation of specific brain pathways may lead to considerably more refined therapeutic interventions when fighting psychiatric and neurological disorders. Indeed, it may reduce unwanted side effects associated with traditional therapies targeting a specific brain region, because pathways unrelated to the disease can remain functional.

Kinoshita, M., Matsui, R., Kato, S., Hasegawa, T., Kasahara, H., Isa, K., Watakabe, A., Yamamori, T., Nishimura, Y., Alstermark, B., Watanabe, D., Kobayashi, K., and Isa, T. (2012). Genetic dissection of the circuit for hand dexterity in primates. Nature 487, 235-238.

Vancraeyenest, P., Arsenault, J.T., Li, X., Zhu, Q., Kobayashi, K., Isa, K., Isa, T., and Vanduffel, W. (2020).

Selective Mesoaccumbal Pathway Inactivation Affects Motivation but Not Reinforcement-Based Learning in Macaques. Neuron 108, 568-581 e566.

A Hierarchical 3D-motion Learning Framework for Animal Spontaneous Behavior Mapping

Pengfei Wei[1], Kang Huang[1,2], Yaning Han[1,2], Liping Wang[1]

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[2]University of the Chinese Academy of Sciences, Beijing 100049, China

Objective quantification of animal behavior is crucial to understanding the relationship between brain activity and behavior. For rodents, this has remained a challenge due to the high-dimensionality and large temporal variability of their behavioral features. Inspired by the natural structure of animal behavior, the present study uses a parallel, and multi-stage approach to decompose motion features and generate an objective metric for mapping rodent behavior into the animal feature space. Incorporating a three-dimensional (3D) motion-capture system and unsupervised clustering into this approach, we developed a novel framework that can automatically identify animal behavioral phenotypes from experimental monitoring. We demonstrate the efficacy of our framework by generating an “autistic-like behavior space” that can robustly characterize a transgenic mouse disease model based on motor activity without human supervision. The results suggest that our framework features a broad range of applications, including animal disease model phenotyping and the modeling of relationships between neural circuits and behavior.

Ketogenic diet modulates microglial morphological properties at steady-state and promotes resilience to repeated social defeat stress in adult mice

Fernando Gonzalez Ibanez[1], Kaushik P Sharma[2], Katherine Picard[1], Kanchan Bisht[2], Haley A Vecchiarelli[3], Nathalie Vernoux[1], Marie-Eve Tremblay[3]

[1]Centre de Recherche CHU de Quebec-Université Laval, Quebec, QC, Canada

[2]Center for Brain Immunology and Glia, Department of Neuroscience, University of Virginia, Charlottesville, VA, USA.

[3]Department of Medical Sciences, University of Victoria, BC, Canada

Psychological stress is a major risk factor for the development of major depressive disorder (MDD), characterized by depressed mood, loss of interest and enjoyment, along with cognitive decline. Signs of neurodegeneration in the form of synaptic loss have been observed in clinical and preclinical studies of MDD.

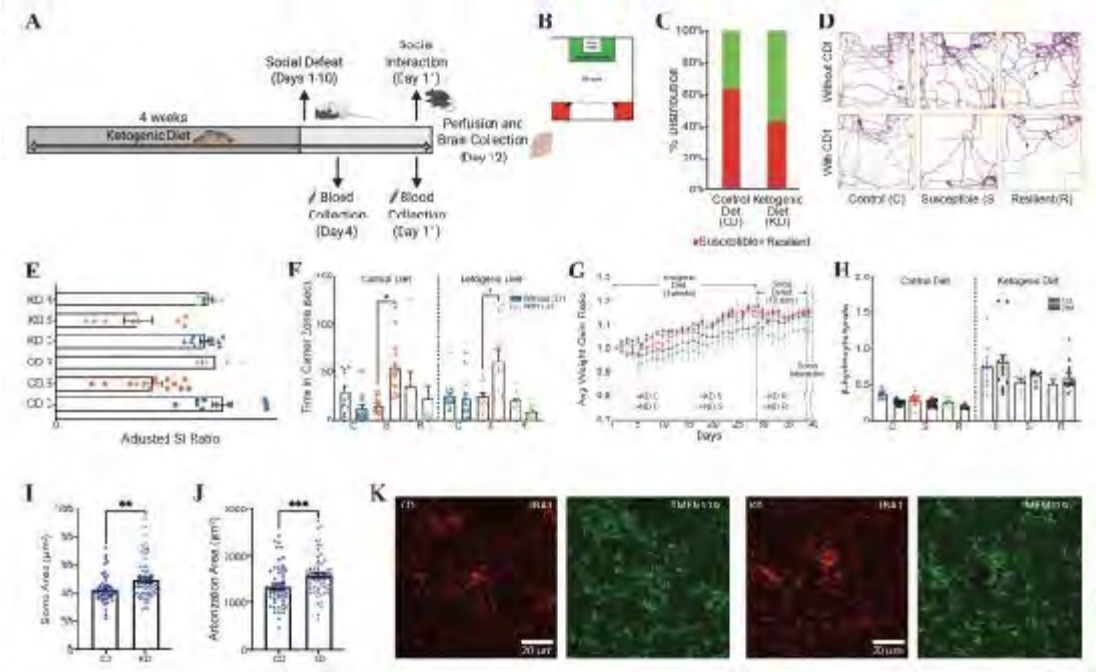
Ketogenic diet (KD; high-fat, low-carbohydrate) has emerged as a lifestyle change that may promote stress resilience. Patients following KD report reduced anxiety under stressful conditions, while preclinical studies in mice show increased sociability among other behavioural adaptations to stress. KD has been successfully used clinically for different types of cancers, autism and epilepsy. This study investigated the possible resilience-promoting properties of KD and aimed to unravel underlying mechanisms by focusing on microglia specifically.

Microglia are the resident immune cells of the brain. They contribute to synaptic development, maturation, function and plasticity in the healthy brain. Pathological elimination of synapses by microglia is emerging as a key mechanism implicated in synaptic loss and cognitive decline across different neuropsychiatric and neurodegenerative conditions including MDD. Previous studies in mouse models of chronic stress-induced depression revealed altered microglial density and morphology, as well as increased phagocytosis of synaptic elements.

Using 2 month-old adult male C57BL/6 mice, we studied the effects of KD versus normal diet (ND) exposure for 4 weeks. The consequences of chronic stress under KD versus ND were investigated by comparing non-stressed controls with animals undergoing 10 days of repeated social defeat (RSD), a model that induces social withdrawal and anhedonia. After RSD, mice underwent a social interaction test (SIT) to classify them as resilient or susceptible to stress. The analyses were performed in the ventral hippocampus CA1 stratum radiatum, previously shown to be affected by MDD and chronic stress.

Our results confirm that RSD is an effective model for inducing depression-like behaviour in mice. Susceptible mice spent less time interacting during SIT (2-way ANOVA with Bonferroni post hoc test, $p= 0.0001$). KD mice also displayed increased resilience to stress. In particular, 57.14% of KD mice (n=28) vs 36.36% of ND (n=22) mice were found to be resilient to stress.

Preliminary analyses of TMEM119/IBA1 double-stained sections were performed for the non-stressed control group (blind to diet treatment) (KD=4 animals; ND=3 animals). No change in the density and distribution of microglia (IBA1+/TMEM119+) or density of peripheral immune cells (IBA1+/TMEM119-) were observed between diet groups. Microglial morphology analysis (n=19 cells/animal) revealed an increase in the soma area (Mann-Whitney test p=0.0021) as well as arborization area (p=0.0009) with KD, suggesting an increased production of trophic factors or cytokine mediators, along with increased microglia-synapse interactions, respectively. Further analyses of stress susceptible versus resilient animals, together with ultrastructural studies, will expand our understanding of KD on microglial function. Ongoing analysis using scanning electron microscopy will allow us to perform ultrastructural characterization of microglial function, their interactions with other cell types, synaptic elements, as well as provide valuable intracellular information regarding their phagocytic activity and markers of cellular stress.



BiPOLES: an optogenetic tool for bidirectional dual-color control of neurons

Silvia Rodriguez-Rozada[1], Johannes Vierock[2], Peter Hegemann[2], J. Simon Wiegert[1]

[1]Research Group Synaptic Wiring and Information Processing, Center for Molecular Neurobiology Hamburg, University Medical Center Hamburg-Eppendorf, Falkenried 94, 20251 Hamburg, Germany

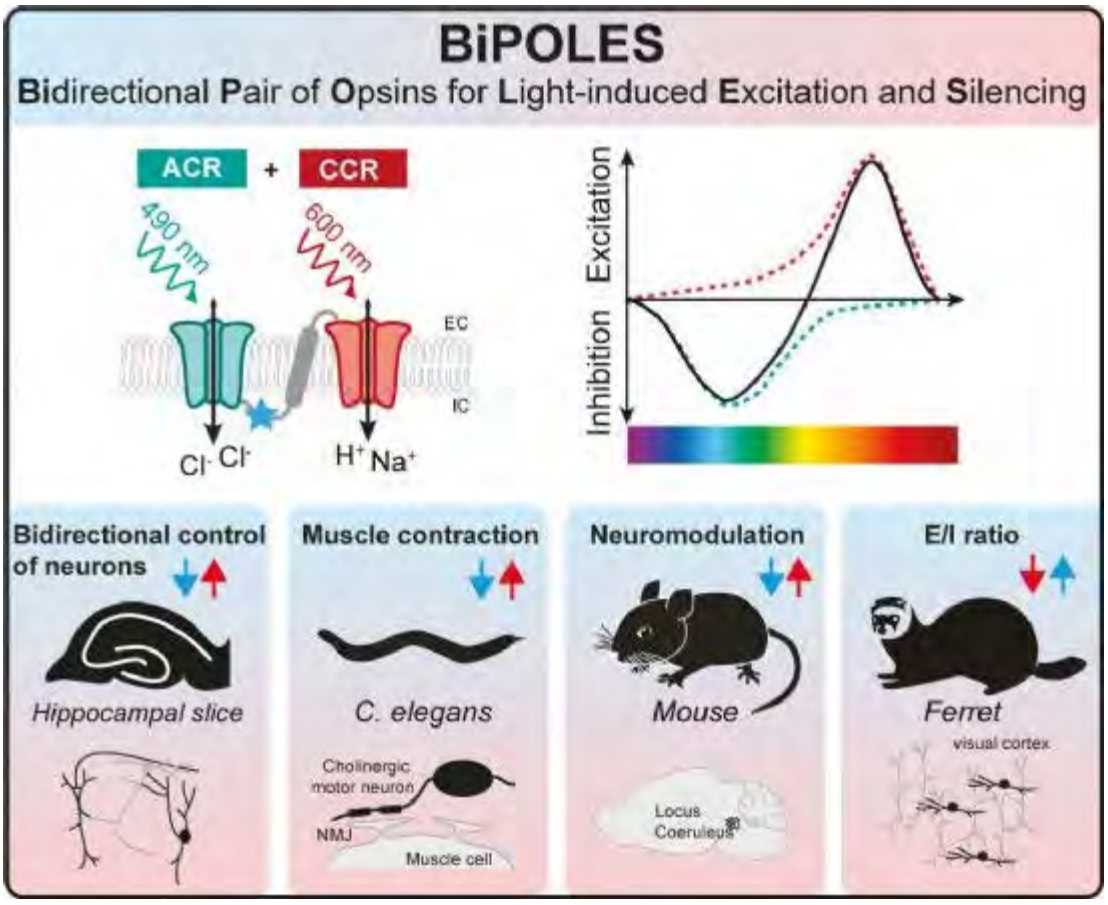
[2]Institute for Biology, Experimental Biophysics, Humboldt University Berlin, D-10115 Berlin, Germany

Perturbation of neural activity by optogenetic means is a powerful approach to probe the function of selected neuronal populations from the synaptic to the behavioral level. Despite the numerous studies that in recent years have successfully employed optogenetic tools to tackle a wide range of scientific questions, there are still some remaining challenges in the field of optogenetics.

To prove necessity and sufficiency of a particular neuronal population for a specific function, it is desirable to both faithfully inhibit and activate this exact same population of neurons. In principle, this could be accomplished by co-expressing an excitatory and an inhibitory opsin in the brain region of interest. However, it is not trivial to achieve equal subcellular distribution and a defined ratio between excitatory and inhibitory action, which is required to precisely control activation and silencing in all transduced cells. To overcome this challenge and other limitations of currently available bicistronic tools used for bidirectional control of neurons, we developed BiPOLES, a fusion protein consisting of the blue-light-sensitive anion-conducting channelrhodopsin GtACR2 and the red-light-sensitive cation-conducting channelrhodopsin Chrimson, allowing bidirectional control of the same set of neurons using light of different wavelengths.

A second, long-standing challenge when manipulating neuronal circuits using optogenetic tools is the independent activation of two distinct neuronal populations. All rhodopsins, even the red-shifted ones, are activated to a certain extent by blue light, which leaves only a narrow spectral and energetic window for independent control of a blue- and a red-light sensitive opsin without cross-activation. In BiPOLES the blue-light-activated inhibitory photocurrents from GtACR2 shunt any residual Chrimson-mediated blue-light activated excitatory photocurrents, thereby restricting optical excitation in BiPOLES-expressing cells exclusively to the orange/red spectrum. This feature enables independent two-color excitation of two distinct neuronal populations at light intensities spanning multiple orders of magnitude, when BiPOLES is combined with a second blue-light-sensitive channelrhodopsin.

In summary, our new optogenetic tool, BiPOLES, expands the possibilities for optical manipulation of neuronal networks. BiPOLES allows multiple new applications including reliable bidirectional control of neurons and dual-color spiking of two distinct populations, as demonstrated in organotypic hippocampal slices, worms, mice, and ferrets.


BiPOLES
Bidirectional Pair of Opsins for Light-induced Excitation and Silencing
ACR
+
CCR
490 nm
600 nm
EC
IC
Cl- Cl-
H+ Na+
Excitation
Inhibition
Bidirectional control of neurons
Hippocampal slice
Muscle contraction
C. elegans
Cholinergic motor neuron
NMJ
Muscle cell
Neuromodulation
Mouse
Locus Coeruleus
E/I ratio
Ferret
visual cortex

Symposium

S14: Post-translational modifications of proteome in neuronal development

S14-1 Studying Ubiquitin & Ubiquitin-like proteins - Global site-specific neddylation profiling reveals that NEDDylated cofilin regulates the actin cytoskeleton in developing neurons
Annette Monika Vogl, Lilian Phu, Raquel Becerra, Sebastian A. Giusti, Erik Verschueren, Trent B. Hinkle, Martín D. Bordenave, Max Adrian, Amy Heidersbach, Patricio Yankilevich, Fernando D. Stefani, Wolfgang Wurst, Casper C. Hoogenraad, Donald S. Kirkpatrick, Damian Refojo, Morgan Sheng

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Studying Ubiquitin & Ubiquitin-like proteins - Global site-specific neddylation profiling reveals that NEDDylated cofilin regulates the actin cytoskeleton in developing neurons

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The Ubiquitin-like protein (UBL) NEDD8 is covalently conjugated to target proteins via a cascade of enzymatic reactions, called neddylation. Neddylation is the post-translational protein modification most closely related to ubiquitination. Beyond the Cullin proteins, the scaffold proteins of the Cullin-RING ligase complexs (CRLs), little is known about other NEDD8 targets. Unambiguous detection of endogenous NEDD8-modification sites is limited by the C-terminal sequence similarity of NEDD8 and Ubiquitin that prevents their discrimination by classic mass spectrometry approaches. Using a CRISPR/Cas9-based strategy, we generated HEK293 cells in which endogenous Nedd8 is replaced with a Nedd8^{R74K}-knock-in variant that can be distinguished from Ubiquitin by mass spectrometry after Lys-C digestion and enrichment of K-κGG peptides, a method that we termed serial NEDD8-Ubiquitin Substrate Profiling (sNUSP). Using sNUSP, we identified 607 neddylation sites dynamically regulated by the neddylation inhibitor MLN4924 and the de-neddylating enzyme NEDP1, implying that many non-cullin proteins are neddylated. Serial profiling of Ubiquitin substrates from the same samples provided clear evidence for neddylation-dependent ubiquitination at distinct lysines from those that are neddylated. Among the candidates, we characterized lysine 112 of the actin regulator cofilin as a novel neddylation event. Global inhibition of neddylation in developing neurons leads to cytoskeletal defects, altered actin dynamics and neurite growth impairments, whereas site-specific neddylation of cofilin at K112 regulates neurite outgrowth, suggesting that cofilin neddylation contributes to the regulation of neuronal actin organization.

TRIM9 and TRIM67: Yin Yang E3 ubiquitin ligases in neuronal morphogenesis

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Class I TRIM E3 ubiquitin ligases alter multiple stages of neuronal morphogenesis, including axon branching and axon guidance, dendritic arborization, and dendritic spine density. We have demonstrated that TRIM9 and TRIM67, and in particular their ligase activity, are critical for responses to the axon guidance cue netrin, and mediate changes in the actin cytoskeleton and exocytic membrane delivery. We have demonstrated that deletion of these ligases in the mouse model results in a number of neuroanatomical defects and severe deficits in spatial learning and memory. Some of the functions of TRIM9 and TRIM67 are mediated through the antagonistic ubiquitination of the actin polymerase VASP. In constrast, regulation of exocytic t-SNAREs SNAP25 and SNAP47 requires TRIM ligase activity, but we have not detected TRIM-dependent ubiquitination of these t-SNAREs. E3 ubiquitin ligases often have multiple interacting partners and substrates. We used unbiased proximity-based biotinylation to identify the complete neuronal repertoire of their interactome, and thus the targets by which they manipulate neuronal form and function. This revealed an overlapping interactome of proteins enriched in cellular processes regulated by TRIM9 and TRIM67, including neuronal growth and arborization, cytoskeletal dynamics, and membrane remodeling. In addition, novel cellular processes such synapse structure and maintenance were identified as potential TRIM9 and TRIM67 regulation points. We validated a subset of candidates implicated in cytoskeletal dynamics, membrane remodeling, and transport. We demonstrate that one of the validated candidates, Myo16, plays a role in netrin-dependent axon branching, in coordination with TRIM9 and TRIM67. We conclude that TRIM9 and TRIM67 likely regulate neuronal form and function through a host of interaction partners and potential substrates. Future work will define how their ligase activity regulates each of these interaction partners.

Regulation of Neuronal Cell Fate Determination by Endocytic Adaptor AP-2

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AP-2 is a heterotetrameric complex comprised of α, β, μ, and σ; subunits that link clathrin and other endocytic proteins to sites of clathrin-mediated endocytosis. Full body knockout of AP-2(μ) in mice causes embryonic lethality before day 3.5 postcoitus (Mitsunari, T. et al., 2005). In contrast, depletion of AP-2(μ) in neurons results in postnatal neurodegeneration and defective synaptic vesicle recycling (Kononenko et al., 2014, Kononenko et al., 2017). However, it does not block plasma membrane retrieval during neuronal activity, questioning the canonical function of AP-2 in neurons and suggesting that AP-2 might perform different functions in mitotic versus postmitotic cells. Using a combination of biochemical, cell biology, and live imaging approaches, we show that AP-2 controls neuronal progenitor cells (NPCs) proliferation but is not required for neuronal differentiation. In wildtype NPCs, AP-2 can be found at the centrosomes, where it colocalizes with gamma-tubulin complex protein 3 (GCP3) subunit of γ-tubulin small complex (γTuSC). Using mass spectrometry analysis, we identified GCP2, and GCP3, as novel interaction partners of AP-2 complex in neuronal cells, where the interaction between the γTuSC and AP-2 was confirmed in co-immunoprecipitation studies. Deletion of AP-2μ in NPCs leads to abnormal centrosome morphology, multinucleation, cell cycle arrest, and altered microtubule dynamics. This phenotype was not reproduced in NPCs treated with clathrin inhibitor PitStop2, suggesting that the role of AP-2 at centrosomes is independent of its function in clathrin-mediated endocytosis. Surprisingly, AP-2 was not required in NPCs committed to becoming neurons, suggesting that AP-2 is a positive regulator of proliferative symmetric cell division in neuronal cells. Despite no differences found in differentiation, AP-2 KO NPCs reveal defective migratory behavior, which results in an accumulation of doublecortin-positive cells in the lateral ventricle and causes the disorganization of cortical structure in AP-2μ KO brains. Since γTuSC comprises the part of the large γ-Tubulin organizing complex, necessary for centrosome function during the cell cycle, our data suggest that AP-2 is required in neuronal mitotic cells for centrosome assembly during proliferative symmetric cell division, while AP-2 function in postmitotic neurons additionally includes the regulation of neuronal migration.

The murine ortholog of Kaufman oculocerebrofacial syndrome protein Ube3b regulates synapse number by ubiquitinating Ppp3cc

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Kaufman oculocerebrofacial syndrome (KOS) is a severe autosomal recessive disorder characterized by intellectual disability, developmental delays, microcephaly, and characteristic dysmorphisms. Biallelic mutations of UBE3B, encoding for a ubiquitin ligase E3B are causative for KOS. In this report, we characterize neuronal functions of its murine ortholog Ube3b and show that Ube3b regulates dendritic branching in a cell-autonomous manner. Moreover, Ube3b knockout (KO) neurons exhibit increased density and aberrant morphology of dendritic spines, altered synaptic physiology, and changes in hippocampal circuit activity. Dorsal forebrain-specific Ube3b KO animals show impaired spatial learning, altered social interactions, and repetitive behaviors. We further demonstrate that Ube3b ubiquitinates the catalytic γ-subunit of calcineurin, Ppp3cc, the overexpression of which phenocopies Ube3b loss with regard to dendritic spine density. This work provides insights into the molecular pathologies underlying intellectual disability-like phenotypes in a genetically engineered mouse model.

Protein synthesis in neocortex development at near-atomic resolution

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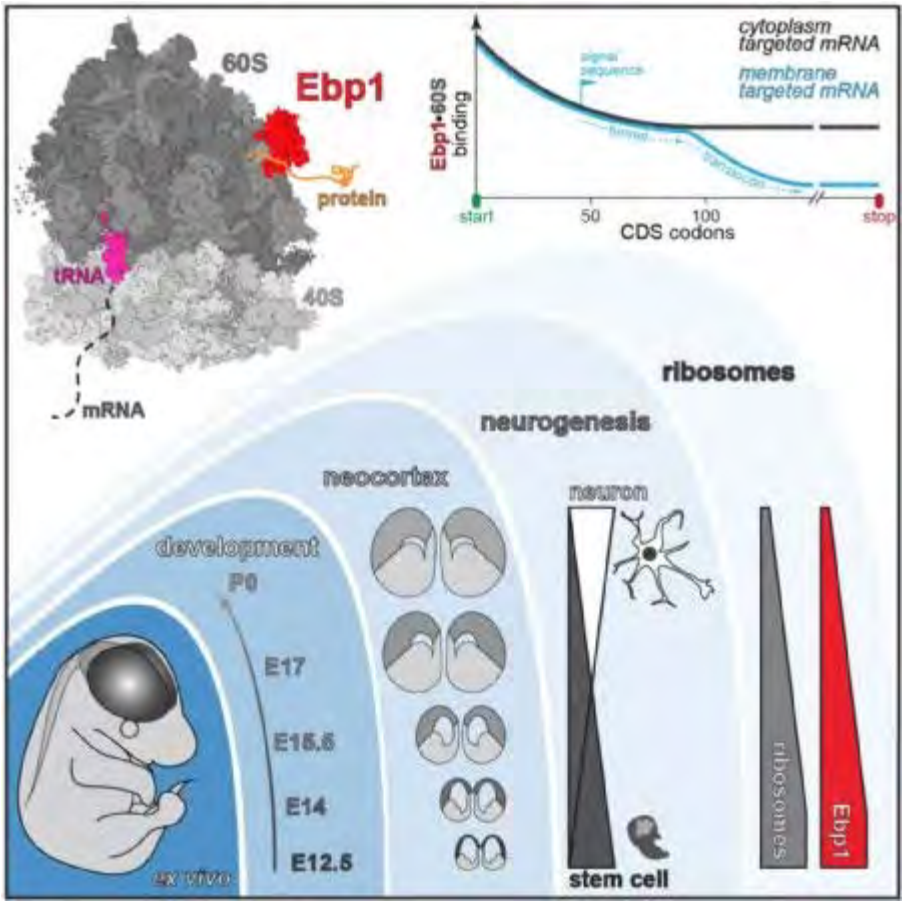
Protein synthesis must be finely tuned in the developing nervous system as the final essential step of gene expression. This study investigates the architecture of ribosomes from the neocortex during neurogenesis, revealing Ebp1 as a high-occupancy 60S peptide tunnel exit factor during protein synthesis at near-atomic resolution by cryo-electron microscopy. Ribosome profiling demonstrated Ebp1-60S binding is highest during start codon initiation and N-terminal peptide elongation, regulating ribosome occupancy of these codons. Membrane-targeting domains emerging from the 60S tunnel, which recruit SRP/Sec61 to the shared binding site, displace Ebp1. Ebp1 is particularly abundant in the early-born neural stem cell lineage and regulates neuronal morphology. Ebp1 especially impacts the synthesis of membrane-targeted cell adhesion molecules, measured by pSILAC/BONCAT mass spectrometry. Therefore, Ebp1 is a central component of protein synthesis, and the ribosome tunnel exit is a focal point of gene expression control in the molecular specification of neuronal morphology during development.


60S
Ebp1
protein
tRNA
40S
mRNA
Ebp1•60S binding
cytoplasm targeted mRNA
membrane targeted mRNA
signal sequence
start
50
100
CDS codons
stop
ribosomes
neurogenesis
neocortex
development
P0
E17
E15.5
E14
E12.5
ex vivo
neuron
stem cell
ribosomes
Ebp1

Molecular mechanisms of structural maintenance and plasticity in neurons

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Pathological changes of the dendrite architecture are hallmarks of many neurological disorders. Therefore, it is crucial to understand the mechanisms of structural aberrations. Despite the fact that dendrites are mostly stable in adult neurons, little is known on the molecular mechanisms of dendrite maintenance and even less on its relation to structural plasticity. Previously, our group identified Vascular Endothelial Growth Factor D (VEGFD) – an angio- and lymphangiogenic factor- as a crucial factor for the maintenance of dendritic morphology and the ability to form long-term memories. Recent unpublished findings show that VEGFD acts like a molecular brake on neuronal morphology: normal expression of VEGFD maintains the dendritic architecture while VEGFD downregulation allows dendritic remodeling. How VEGFD mediates this phenomenon is, however, not known. We characterized the effect of VEGFD on the cytoskeleton by performing atomic force microscopy as well as time-lapse live imaging of actin and microtubules via fluorescent genetically-encoded markers in hippocampal neurons. Furthermore, we monitored the VEGFD-regulated dynamics of dendrite structure. Using a phosphoproteomic screen of cytoskeleton elements and their regulators we generated a list of potential VEGFD-regulated target proteins. We functionally characterized these proteins with gain and loss of function approaches and pharmacological tools and identified an actin-binding protein as a potential mediator of VEGFD signaling.
Our study revealed the mechanisms of VEGFD-mediated dendrite stabilization and thereby contributes to our understanding of pathological dendrite aberrations.

Symposium

S15: Gene and cell based therapies to counteract neuroretinal degeneration

S15-1 Novel AAV capsids for intravitreal retinal gene therapy
Stylianos Michalakis

S15-2 Transcriptomic landscape of Double Strand Break pathways:
towards precise *in vivo* genome editing
Giovanni Pasquini, Knut Stieger, Volker Busskamp

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S15-4 Deciphering factors that affect the outcome of gene and cell therapies for IRDs
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S15-5 Proposing the targeting of Müller cells for complement modulating gene addition therapy in a mouse model for Stargardt disease type 1
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S15-6 Development of clinical readout parameters for novel gene and cell-based therapies to counteract neuroretinal degeneration
Wolf Harmening

Novel AAV capsids for intravitreal retinal gene therapy

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Recent advances in molecular biological techniques made it possible to develop causative therapies for inherited retinal disorders (IRD). Some of the most promising options are gene-specific approaches which use adeno-associated virus (AAV)-based vectors to express a healthy copy of the disease-causing gene in affected cells of a patient. However, AAV vectors come with some limitations such as the requirement for invasive (subretinal) injection for the treatment of photoreceptors. Here, I will introduce our approach to overcome this limitation by engineered novel AAV vector variants via capsid diversification. In particular, I will present data on the development of novel AAV capsid variants that enable efficient targeting of photoreceptors via less invasive intravitreal administration. Using directed evolution of AAV2 peptide display libraries, we identified novel AAV variants that mediate panretinal transduction after a single intravitreal injection and lateral spreading after subretinal injection in mice. Widespread retinal transduction was replicated in larger mammals, after intravitreal injection in dogs and non-human primates. Translatability was confirmed by transduction of human photoreceptors in human retinal explant cultures and by proof-of-concept gene supplementation studies in relevant IRD mouse models. These novel AAV capsids expand our current toolbox for preclinical and clinical AAV-based retinal gene delivery.

Transcriptomic landscape of Double Strand Break pathways: towards precise *in vivo* genome editing

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Mutations in more than 200 retina-specific genes have been associated with inherited retinal diseases. In vivo genome editing represents a promising emerging field in the treatment of monogenic disorders, as it aims to correct disease-causing mutations within the genome. In general, genome editing relies on highly specific endonucleases to apply DNA cuts and on the intrinsic capacity of the cells to repair the double-strand breaks (DSBs). Three DSB-repair pathways are found in mammalian cells: Non-homologous end joining (NHEJ); microhomology-mediated end joining (MMEJ); and homology-directed repair (HDR). While NHEJ can be used to knock out mutant alleles in dominant disorders, HDR and MMEJ are better suited for precise genome editing, or delivering templates in genomic regions. Although we know HDR being mainly upregulated by cell cycle activity and NHEJ being the predominant pathway in post-mitotic cells, not much is known about MMEJ in retinal cell types. In order to develop therapeutic in vivo genome editing, we transcriptomically scored DSB-repair pathways in post-mitotic retinal photoreceptors and neurons, and compared human cell types with relevant models. We analyzed transcriptomic in vivo and in vitro data and revealed that HDR is indeed downregulated in postmitotic neurons, whereas MMEJ and NHEJ are active. Using single-cell RNA sequencing analysis, we characterized the dynamics of DSB repair pathways in the transition from dividing cells to postmitotic retinal cells. Time-course bulk RNA-seq data confirmed DSB repair gene expression in both in vivo and in vitro samples. Transcriptomic DSB repair pathway profiles are very similar in adult human, macaque, and mouse retinas, but not in ground squirrel retinas. Moreover, human-induced pluripotent stem-cell-derived neurons and retinal organoids can serve as well suited in vitro testbeds for developing genomic engineering approaches in photoreceptors. Our study provides additional support for designing precise in vivo genome-editing approaches via MMEJ, which is active in mature photoreceptors.

Development of cell-based therapies for the treatment of inherited retinal degenerations

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Inherited forms of retinal degeneration mainly affect the light-sensing photoreceptors and the supporting retinal pigment epithelium (RPE) causing visual impairment and blindness. While gene therapy approaches target remaining cells within the retina, cell therapy strategies aim to contribute new cells to the damaged tissues. Regarding the replacement of cells in the retina via transplantation, the research field has dramatically progressed within the past decade with first clinical trials initiated for RPE transplantation. Particularly the introduction of human pluripotent stem cells (PSC) for the generation of donor cell material or the development of human disease modelling systems has shown immense potential. Thus, as one pillar of the SPP2127, cell-based technologies are being developed for characterizing and optimizing PSC-derived retinal organoid and RPE generation for therapy approaches. These studies include (i) label-free enrichment of retinal organoid-generated photoreceptors by morpho-rheological phenotyping and machine learning for transplantation into preclinical animal models, (ii) the development of iPSC-retinal organoids with patient-specific or general, chemically induced gliotic, disease environments, for mimicking photoreceptor pathologies to provide candidate targets and test-beds for interventional cell-based and/or molecular therapies like human-to-human photoreceptor transplantation or gene supplementation/editing approaches, and (iii) modification of ciliopathy-related pathways for improved RPE development and maturation as a treatment target. Such cell-based systems will be essential for defining and optimizing conditions of donor cells as well as disease environments to foster functional restoration following cell therapeutic interventions for retinal degenerative diseases.

Deciphering factors that affect the outcome of gene and cell therapies for IRDs

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Several factors influence the efficacy of gene and cell based therapies, including potential immune system reactions to viral vectors or cells, intrinsic adverse reaction of the target tissue, especially the Müller glia cells, as well as selection of optimal patient cohort for future clinical trials. Four groups in the SPP cover analysis of those outcome parameters from different angles. The first angle is the potential involvement of immune system upon therapeutic intervention. While the immune response against invading viral vectors is considered low, recent data suggest activation of the immune system up to a so far unknown level. In depth characterization of the innate and adaptive immune pathway responses upon subretinally administered AAV vectors in non-human primates and human patients enrolled in the current achromatopsia trial will elucidate the role of local and systemic immunity in subretinal gene therapy administration. The second angle to study are the retinal Müller glial cells, which are activated and become gliotic during inherited retinal degenerative diseases (IRDs). This activation typically presents with a loss of intrinsic neuroprotective support provided by these cells to retinal neurons and thus drives degeneration. Further, Müller cells are immune competent and treatment with viral vectors (AAV) carrying (d)Cas9 enzyme might modify the immunogenic peptides presented on their cell surface. Thus, the modifications of the immunoproteome upon gene therapy treatment is studied and strategies to counteract adverse activation of Müller cells are developed. Complement activation drives neuroinflammation in IRDs. Of note, key inhibitors of complement activation are predominantly expressed by Müller cells while positive regulators such as properdin (CFP) are mainly expressed in retinal neurons and pigment epithelium. The complement cascade activation can be therapeutically influenced by involving expression of anti-human CFP mAbs in Müller cells as a novel immunoprophylaxis strategy. Finally, a very important angle for assessing therapy outcome is the selection of the right patient for applying the right therapy. The effect of the type of mutation within a gene may influence the response to a therapeutic intervention, be it the interaction of the endogenous gene with the transgene or be it that the mutation is not the only problem underlying the disease phenotype. Knowing in advance, which mutation may respond best to experimental therapies will immensely ameliorate the outcome. To this end the respective project aims at the establishment and implementation of a pathogenicity scoring system combined with functional in vitro and in vivo validation of missense mutations in patients with IRDs eligible for therapeutic studies.

Proposing the targeting of Müller cells for complement modulating gene addition therapy in a mouse model for Stargardt disease type 1

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A common feature of Stargardt type 1 (STGD1) and other diseases with underlying macular degeneration is neuro-destructive para-inflammation. In STGD1, a mutation in the ATP binding cassette subfamily A member 4 (ABCA4) causes an accumulation of lipofuscin which leads to an exaggerated reaction of the complement system (CS). While an intact blood-retina barrier ensures that no circulating complement components enter retinal tissue, it still needs unequivocal proof that complement proteins are expressed by retinal cells. Our aim is to map the closed-circuit CS to understand complement derailment in disease and be able to treat it with a long-term efficient gene addition therapy. To do so, we first needed to in-depth characterize our ABCA4$^{-/-}$ mouse model to determine changes in retinal complement expression and progression of disease in the course of aging to decide which complement factor holds potential to be targeted at which time point of disease progression.

We mapped the expression patterns of complement factors in wild type and ABCA4$^{-/-}$ mice through RNAseq and qPCR. We established protocols for immunolabeling to localize the secreted complement factors in healthy, ABCA4$^{-/-}$ and postischemic retinae. The latter serves as positive control, as we could demonstrate massive intraretinal upregulation of complement activity. Analysis of RPE autofluorescence, neuronal cell loss and glial reactivity was performed on eye cup as well as retinal slice and flatmount preparations to characterize degenerative processes in the ABCA4-/- eye from animals 8, 16, 24, 32-40 weeks of age.

We show that the main inhibitory complement regulator, complement factor H (CFH), was mainly expressed in retinal pigment epithelium, microglia and vascular cells, while the only known positive regulator, properdin (CFP), was primarily detected in Müller cells, microglia and neurons. Morphometric analysis revealed higher RPE autofluorescence levels in ABCA4$^{-/-}$ than in age-matched controls, as well as enhanced microglia activation beginning in mice 24 weeks of age. No major neuronal degeneration was observed in any of the mouse strains at the ages investigated.

In collaboration with the team of Diana Pauly, we are on our way to develop a gene therapeutic approach for STGD1 that modulates the overshooting CS. We established the background knowledge needed to decide on which CS modulatory protein holds potential to dampen the retinal complement response if overexpressed in Müller cells. Ultimately, the advantage of this approach will be its independence of the underlying genotype and that it can be applied to STGD1 or other inherited retinal degeneration with complicated or unknown genetics.

Development of clinical readout parameters for novel gene and cell-based therapies to counteract neuroretinal degeneration

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Novel gene and cell-based therapies to counteract retinal degeneration that are developed now and in the near future need testing for efficacy in humans. Sensitive and efficient clinical readout parameters need to be developed to decide whether the retina and visual function is preserved or even restored after treatment. One of the scientific pillars of the SPP2127 is comprised of research groups tackling this challenge from five different angles. The first angle is high-resolution, photoreceptor-resolved in-vivo imaging in patients with retinal disease to better understand disease progression on a cellular level, and how the retinal tissue reacts to treatment. A second angle covers visual function as assessed by pupillometry, allowing a subjective response-independent, quasi-objective readout of visual function through the high coupling of the pupillary light reflex and a visual stimulus. A third angle is concerned with the change in psychophysical response to visual stimuli that allows keeping apart effects that only target certain photoreceptor classes in the retina, using a triple silent-substitution stimulation paradigm. A fourth group focuses on patient-relevant outcome measures based on subjective responses through questionnaires particular designed for impact of low and ultra-low vision impairment, and by objective readouts of daily activity records though body-worn tracking devices. Finally, a fifth angle is aiming at combining both objective, structural information of the cellular changes the retina undergoes during disease and treatment, and also the subjective changes of photoreceptor function by means of adaptive optics shaped single cell-targeted microstimulation and their psychophysical responses. In this talk, I will give an overview of the aims and progress of each of these projects.

Symposium

S16: From sensation to action: shaping neuronal representations during learning

S16-1 Active behaviours shape fundamental information processing in sensory cortex
Janelle Pakan

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S16-5 Functionally distinct hippocampal rhythms and circuits underly location-specific motor, appetitive and aversive learning
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S16-6 Glutamatergic septal inputs to VTA modulate movement onset and speed in a state-dependent manner
Petra Mocellin, Pavol Bauer, Hiroshi Kaneko, Kevin Luxem, Sanja Mikulovic, Stefan Remy

Active behaviours shape fundamental information processing in sensory cortex

Janelle Pakan[1]

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A fundamental challenge in neuroscience is how information from the external world is integrated to form a coherent, and continually updated, perception of external events. Progress towards this problem has been hindered by the underlying assumption that our five sensory systems are both anatomically and phenomenologically distinct from each other as well as from systems processing motor related information. However, in recent years, a surge in research investigating multimodal integration during active behaviors has emphasized that the senses together provide critical links to building an external reality, even at the earliest levels of cortical processing, and that combining sensory and motor inputs leads to perceptual and behavioral improvements. Here I will discuss our recent work on how information processing in sensory cortex is influenced by active behaviours, and how these relate to spatial processing, reward-related responses, and experience-dependent plasticity during learning. We have utilized advanced in vivo two-photon calcium imaging techniques in behaving mice to investigated cell-type specific responses of neurons in both primary visual cortex (V1) as well as auditory cortex and, further, investigated mechanisms of experience-dependent plasticity in V1 while mice are navigating within a VR environment. We have found a diversity of cell-type specific responses in sensory cortex during active behaviours and that spatial locations are represented directly within V1, with the influence of behaviourally relevant stimuli (such as those associated with reward) leading to enhanced plasticity. Together, this work demonstrates the import influence of motor and active behaviours on the fundamental processing of sensory stimuli, even in primary sensory cortices.

Inhibition suppresses membrane potential variability to stabilize motor cortical output

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The output of primary motor cortex (M1) is important for controlling the initiation and execution of a wide range of motor actions. A hallmark of motor cortical activity is quenched trial-to-trial response variability during movement initiation. However, little is known about the cellular mechanisms and the role of intracortical inhibition in the task-related suppression of neural variability.

To address these questions, we combined whole-cell patch-clamp recordings, 2-photon population calcium imaging and cell-type-specific manipulations in motor cortex layer 5B neurons while head restrained mice performed a cued forelimb task. We observed a consistent reduction in trial-to-trial variability of layer 5B pyramidal neuron firing rates and subthreshold membrane potential (V_m) around movement initiation, with individual neurons reaching a defined peri-movement V_m setpoint irrespective of the magnitude and direction of pre-movement fluctuations in V_m. Individual neuronal setpoints and polarity of movement-related V_m changes are determined by the ratio of synaptic excitation and inhibition. We found that the reversal potential of synaptic input during movement initiation is hyperpolarized relative to action potential threshold for most neurons, indicating a strong contribution of synaptic inhibition that stabilizes V_m dynamics around movement onset.

By employing cell-type specific 2-photon population calcium imaging in layer 5B we demonstrate the sequential recruitment of Parvalbumin (PV) and Somatostatin (SST) positive interneurons, where the onset of PV interneuron activity coincides with that of layer 5B pyramidal neurons while SST interneuron activity is delayed with respect to movement onset. These data suggest a critical role for PV interneurons in counterbalancing excitatory input during movement initiation to ensure stable trial-to-trial output.

Optogenetic loss-of-function experiments using stGtACR2 to suppress PV interneuron activity during rest strongly affected cortical network state, increasing layer 5B V_m variability and firing rates sufficiently to trigger discrete forelimb movements. During movement trials, photostimulation also increases V_m variability but this effect is partially counteracted by strong movement-related input that drives the Vm towards the setpoint observed in control trials. Together, our results show the robustness of motor cortex to perturbations and that local inhibition actively suppresses membrane potential variability to ensure trial-to-trial stability in motor cortical output.

Long-term stability of prefrontal principal cell assemblies representing contextual task rules

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Flexible adaptation of behaviour to context-related changes of rules depend on the medial prefrontal cortex (mPFC). A recent study indicated that the mPFC has the ability to store contextual memories for long terms (Kitamura et al., 2017, *Science* **356**:73–78), suggesting that the recall of these memories may support flexible behaviour. However, how the contextual task is represented by memory engrams and how stable they are over time remained largely unknown. Recent technical advances allow us to track the activity patterns of the same set of neurons on a daily basis over subsequent weeks up to months in freely behaving animals. Here we applied chronic 1-photon imaging of large neuronal populations with single cell resolution in the mPFC of Thy1-GCaMP6f transgenic mice performing a learned olfaction-guided match-to-place task. We observed that the animals' performance in the task was highly reliable and persisted over several weeks even when time windows of lacking task exposure were included (1-6 days). Analysis of calcium transients revealed that (1) a subset of principal cells were active daily on subsequent weeks during task execution. (2) Task-related neurons showed maximal activity at given locations thereby representing the entire trajectory of the experienced arena. (3) Our preliminary results indicate that the representation of the trajectory remains stable across days, with a high trial-to-trial (reliability) and day-to-day activity correlations (consistency). Thus, our data suggest that successful behavioural performance over time might be linked to a temporally stable and reliable representation of the task rule by principal cell assemblies.

Context value updating and multidimensional neuronal encoding in the retrosplenial cortex

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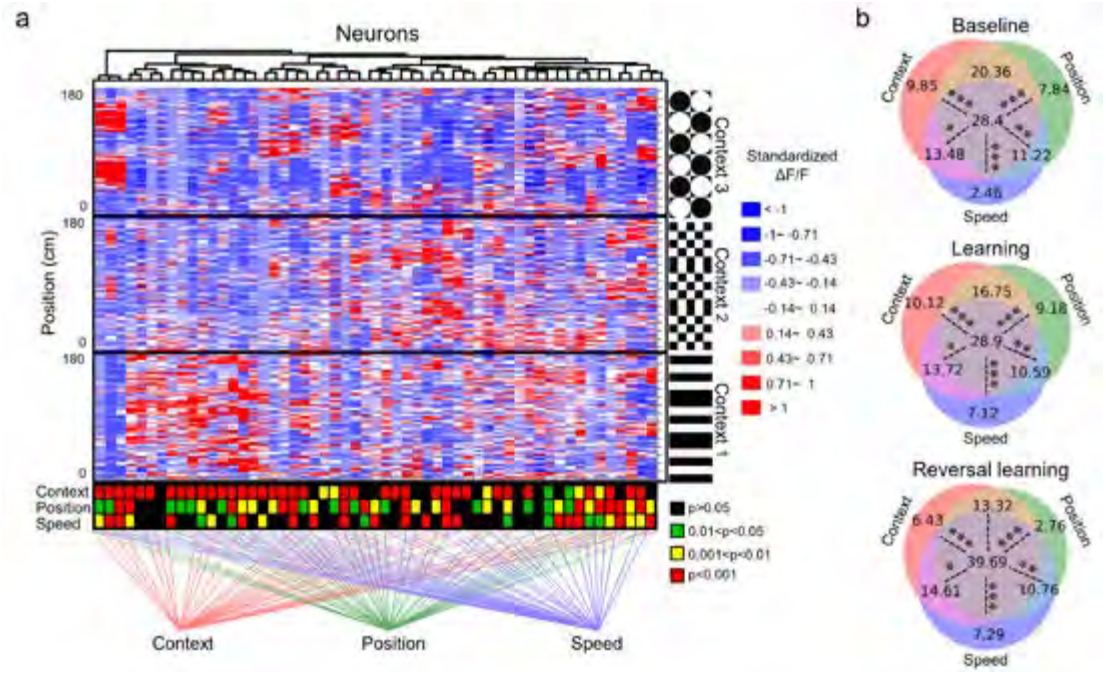
The retrosplenial cortex (RSC) is known for diverse functional inputs and is engaged by various sensory, spatial, and associative learning tasks. We examine how multiple functional aspects are integrated on the single-cell level in RSC and how encoding of task-related parameters changes across learning. Using a visuospatial context discrimination paradigm and two-photon calcium imaging in behaving mice, we found a large proportion of dysgranular RSC neurons encode multiple task-related dimensions while forming context-reward value associations across learning. During reversal learning requiring increased cognitive flexibility, we found an increased proportion of multidimensional encoding neurons that showed higher decoding accuracy for behaviorally relevant context-value associations. Chemogenetic inactivation of RSC led to decreased behavioral context discrimination during learning phases in which context-value associations were formed, while recall of previously formed associations remained intact. RSC inactivation resulted in a persistent positive behavioral bias in valuing contexts, indicating a role for the RSC in context-value updating.



Functionally distinct hippocampal rhythms and circuits underly location-specific motor, appetitive and aversive learning

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The hippocampus plays a fundamental role in learning and memory formation. Despite being extensively studied, the exact mechanisms of how motor and sensory experiences are encoded in hippocampal neuronal activity patterns, and their subsequent retrieval during memory-guided behaviour, still remain elusive. Here, we aim to uncover the hippocampal neuronal circuits and rhythms underlying location-specific motor, appetitive and aversive learning, as well as how they depend on the medial septum (MS) inputs.

We are using a combination of methods, including multi-channel local field potential (LFP) recordings, 2 Photon Calcium Imaging and optogenetics in head-fixed animals running on a treadmill and being engaged in three different location-specific learning tasks. Simultaneously, we are performing detailed behavioural monitoring of body, face movements and pupil dilation and subsequently use deep-learning algorithms in order to relate the identified behavioural modules with the recorded LFP and Calcium Imaging data.

By monitoring LFP, cellular activity and behaviour over several consecutive days during the learning tasks, we show that different hippocampal oscillations, single cell activity, as well as behavioural modules underly motor, appetitive and aversive learning. Furthermore, we are showing their interaction during the course of learning. Finally, we demonstrate that MS inhibition has a variable effect on hippocampal circuits, dependent on the behavioural state and the learning phase the animal is involved in.

These results provide novel knowledge of how different hippocampal networks and rhythms are recruited during learning and memory, as well as how they depend on MS activity.

Glutamatergic septal inputs to VTA modulate movement onset and speed in a state-dependent manner

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The ventral tegmental area (VTA) has been classically studied in relation to reward and addiction. However, different lines of research are now converging in supporting the role of VTA in locomotion: VTA neurons are active at movement onset, send inputs to the dorsal striatum in an acceleration-dependent pattern, and increase locomotion when activated (Engelhard et al., 2019; Howe & Dombeck, 2016; Jing et al., 2019). Besides, locomotion onset and speed are controlled by the VGluT2+ population of the Medial Septum and Diagonal Band of Broca (MSDB). When optogenetically stimulated, these neurons initiate locomotion and modulate the animal's speed in a frequency-dependent manner (Fuhrmann et al., 2015). While the MSDB has no direct projections to any motor-related area, we have evidence of MSDB VGluT2+ projections to the VTA. This suggests that MSDB-VTA interaction may be crucial for locomotion execution and may link movement to reward-related circuits.

We used slice electrophysiology, in vivo fiberphotometry, pharmacology, and optogenetics to investigate the role of MSDB VGluT2+ projections to VTA during locomotion and in a location-specific reward learning task. The animals were tested both head fixed on a linear treadmill and freely moving in an open field. An unsupervised machine learning algorithm (VAME - Luxem et al., 2020) allowed us to extract the animal's pose dynamics and quantify the effect of the network manipulation on the animal's behaviour.

Optogenetic activation of VGluT2+ MSDB projections in VTA was sufficient to induce locomotion onset and to control the speed in a frequency-dependent manner. These findings were supported by the increase in calcium signal of the VGluT2+ MSDB axons in VTA when the animals spontaneously moved. In vitro patch-clamp data confirmed the existence of a monosynaptic glutamatergic connection and highlighted how VGluT2+ MSDB inputs preferentially target VGluT2+ VTA neurons. Finally, in vivo optogenetic stimulation of VTA VGluT2+ neurons reliably induced locomotion, comparable to the effect of MSDB VGluT2+ cells stimulation. To exclude the possibility of a back-propagating signal from the axon terminals to the MSDB somata, the optogenetic experiment was also performed after lidocaine injection in the MSDB. Stimulation of MSDB glutamatergic terminals in VTA was sufficient to induce locomotion despite MSDB silencing. These results supported the hypothesis of the VTA as a downstream area in the locomotor pathway initiated by the glutamatergic septal neurons. Interestingly, the efficacy of the stimulation altered when a reward-learning task was introduced. Moreover, in freely moving conditions the animals increased not only the overall travelled distance but also the number of rearing episodes, classically associated with exploratory behaviour.

Taken together, these data provide a novel insight into MSDB-VTA interaction. Ongoing experiments are pointing to a specific role of this glutamatergic pathway in exploratory-driven locomotion.

Symposium

S17: Genetic and environmental aspects in chronic pain

S17-1 Small fiber pathology in genetic pain disorders – news from translational research
Nurcan Üçeyler

S17-2 Epigenetic control of functional plasticity in inflammatory pain.
Daniela Mauceri

S17-3 Multimodal analysis of structural plasticity of the prefrontal cortex and changes in behaviour in a mouse model of chronic pain
Jennifer John, Amrita Das Gupta, Livia Asan, Carlo Beretta, Ariel Iporre, Claudia Falfan-Melgoza, Felix Hörner, Wolfgang Weber-Fahr, Thomas Kuner, Johannes Knabbe

S17-4 Cognitive pain modulation and the human spinal cord
Falk Eippert

S17-5 Daily-life environmental, genetic and neural regulatory influences underlying bidirectional interactions between chronic pain and depression
Heike Tost

Small fiber pathology in genetic pain disorders – news from translational research

Nurcan Üçeyler[1]

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Small fiber neuropathies (SFN) are caused by A-delta and C nerve fiber impairment and typically lead to acral burning pain and dysesthesias accompanied by peripheral nerve fiber degeneration and/or sensitization. The underlying etiologies are manifold, while the pathomechanisms leading to peripheral denervation, neuronal sensitization, and pain are incompletely understood. With the improvement and extension of genetic diagnostics, hereditary SFN are increasingly recognized. Although rare, genetic SFN bear the immense potential to learn about basic mechanisms of nociception and peripheral neurodegeneration that can be transferred to other pain syndromes. In this presentation, examples of genetic SFN will be discussed. One is Fabry disease (FD): an X-linked lysosomal storage disorder that is caused by mutations in the gene encoding alpha-galactosidase A (GLA) leading to the accumulation of globotriaosylceramide (Gb3) in diverse tissues. FD is associated with mainly triggerable episodic pain and provides an opportunity to study the pathophysiology of SFN in a genetically caused pain syndrome. Current concepts on the potential link between lysosomal Gb3 deposition and sensory neuron dysfunction will be summarized providing novel data collected in GLA knockout mice as a model of FD. Animal data will be complemented by findings obtained in in vitro experiments with personalized sensory neurons, generated from patient-derived fibroblasts via induced pluripotent stem cells (iPSC). Further, clinical examples of patients carrying variations in voltage-gated sodium channels will be given and translational data obtained in iPSC derived sensory neurons will be presented and discussed. The potential impact of these new findings on diagnostics and treatment of patients with small fiber pathology will be discussed providing the audience exciting new insights on a novel and dynamic research field.

Epigenetic control of functional plasticity in inflammatory pain.

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Nociception is essential for our well-being. Chronic pain, on the other hand, is a debilitating condition affecting a significant percentage of the worldwide population. Persistent pain is sustained by plasticity-dependent maladaptive changes of the relevant circuits, involves alterations in gene transcription and is at times therapeutically poorly managed. Epigenetic processes have been implicated in pain chronicity, but the mechanisms linking nociceptive activity to epigenetic-regulated transcription, identity of the target genes, and the functional links to pathological pain sensitivity are not clear.

Here, we characterized the impact of nociceptive activity on two classes of epigenetic regulators, DNA methyltransferases and histone deacetylases (HDAC4), in a mouse model of persistent inflammatory pain. Specifically, we found that long-lasting inflammatory pain triggers the expression of Dnmt3a2, a synaptic activity-regulated de novo DNA methyltransferase, and results in the nuclear export and thus inactivation of HDAC4 in spinal cord dorsal horn neurons. In vivo manipulation of these epigenetic regulators revealed their critical role in the regulation of pain-relevant genes and establishment of mechanical hypersensitivity.

Using next generation RNA-sequencing analysis, we further identified an inflammatory pain-dependent HDAC4-regulated gene program in the spinal dorsal horn including well-known as well as completely novel mediators of sensitization which we functionally tested in vivo with gain of function and loss of function experiments.

Our results identify Dnmt3a2 and HDAC4 as key epigenetic regulators of chronic inflammatory pain whose activity affects gene transcription and hypersensitivity and as possible targets for pain therapies.

Multimodal analysis of structural plasticity of the prefrontal cortex and changes in behaviour in a mouse model of chronic pain

Jennifer John[1], Amrita Das Gupta[1], Livia Asan[1], Carlo Beretta[1], Ariel Iporre[1], Claudia Falfan-Melgoza[2], Felix Hörner[2], Wolfgang Weber-Fahr[2], Thomas Kuner[1], Johannes Knabbe[1,3]

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Pain is a basic and life-supporting perceptions of every organism. It functions as an immediate warning system and is crucial for preventing greater damage. However, if acute pain outlasts the healing of the original damage, it may become chronic. It is currently the leading cause of disability and disease burden globally and occurs in connection with many illnesses and even promotes further comorbidities such as depression and anxiety. Persistent chronic pain is shown to be accompanied by a reversible decrease of grey matter volume in pain-associated areas of the brain, which can also directly be transferred to rodent models of chronic pain.

The first aim of this study is the longitudinal characterizations of chronic pain-associated volume changes of cortical grey matter and their cellular basis by using correlative magnetic resonance imaging (MRI) in combination with in vivo 2-photon fluorescence imaging in mice. The second aim is the investigation of a possible correlation between cellular and behavioural changes after induction of chronic pain in mice.

For the longitudinal analysis of all cells in the brain, transgenic mice expressing Histone-GFP in every nucleus were used for the study. Nuclear morphology is distinct for each cell type, allowing to indirectly infer changes in cellular composition and spatial arrangement of cortical cells.

Chronic cranial window implantation was performed on 8 to 10 weeks old mice and four weeks later, microscopic imaging using 2-photon microscopy and macroscopic imaging by MRI were carried out. Furthermore, Von-Frey and Cold Plate (behaviour tests) were performed. In the MRI structural images were acquired using T2-weighted RARE sequences and Diffusion Tensor Imaging. Voxel-Based Morphometry (VBM) calculations were performed on the MRI data for estimating volume changes longitudinally. For 2-photon imaging, two large 3D stacks were imaged on each hemisphere in the anterior cingulate cortex and neighbouring areas. Cell counting and nuclear shape analysis were achieved using machine-learning based algorithms for automated image segmentation. The neuropathic pain model of spared nerve injury (SNI) and its corresponding Sham surgery were induced after the baseline measurements. Behavioural tests and imaging were repeated at 1 week and 12 weeks after the surgery. Two further behavioural tests allowing the measurement of the emotional aspect of chronic pain were performed 8 weeks after the SNI/ sham surgeries, the Open Field and Place Escape Avoidance Paradigm tests.

Preliminary data analysis demonstrate that SNI mice may exhibit an increased depressive behaviour, which also plays an important role in the treatment of chronic pain in humans. First results from the fully automated analysis of the imaging data show that the total cell count, nuclear volume and the nearest neighbour distance between cells change in SNI compared to Sham mice, especially in the first week after SNI. Further analyses correlating behavioural tests with MRI data will complete these results.

We present a new technique to correlate MRI and cellular changes of cortical grey matter using a chronic

pain model in mice. Fully automated analysis will make it possible to use this approach in different disease models in the future. For the study of chronic pain, the results may give a new insight into the transition from acute to chronic pain, possibly leading to novel strategies preventing this transformation in human patients.

Cognitive pain modulation and the human spinal cord

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The spinal cord is the first station of nociceptive processing in the central nervous system and also plays a substantial role in chronic forms of pain. While there is thus a clear incentive for non-invasively investigating this structure in humans, imaging the spinal cord with functional magnetic resonance imaging (fMRI) faces several unique challenges. I will start out by briefly describing approaches that aim to overcome these challenges and will then focus on studies that investigate how cognitive factors such as expectations shape nociceptive processing in the human spinal cord.

Daily-life environmental, genetic and neural regulatory influences underlying bidirectional interactions between chronic pain and depression

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Major depression and chronic pain are major contributors to the global burden of disease and are frequently comorbid, but the shared and distinct psychological and neural mechanisms are still poorly understood. This talk will provide first neuroimaging and ambulatory assessment data of the second funding period of SFB1158, which interrogates, among others, the genetic and daily-life environmental influences impacting psychological well-being, perceived pain and neural regulatory capacity in patients with major depression and chronic widespread pain. Special emphasis will be given to the shared and distinct signatures in these patient populations and the respective advantages and challenges of the employed multimodal data assessment and analysis strategy.

Symposium

S18: Challenges in autism: beyond species and brain regions - common mechanisms for neuronal dysfunction?

S18-1 Of flies and men: the impact of growth control on network development, function and behavior, and its link to Autism Spectrum Disorders
Peter Soba, Chun Hu, Federico Tenedini, Yan Tang, Froylan Calderon de Anda, Melanie Richter, Alexandros K Kanellopoulos, Claudia Bagni

S18-2 Sensory processing disruptions in autism – from mechanism to function
Susanne Schmid, Kaela Scott, Rajkamalpreet Mann, Dorit Moehrle, Brian Allman

S18-3 Olfactory processing in autism: behavioral and neuroimaging insights.
Valentina Parma

S18-4 Prefrontal circuits in social behavior
Ofer Yizhar

S18-5 Dopaminergic modulation of nucleus incertus to interpeduncular nucleus input – possible neuronal mechanism in control of novelty preference expression
Agata Szlaga, Patryk Sambak, Anna Blasiak

S18-6 Investigation of CDH13`s role in neurodevelopmental disorders using induced pluripotent stem cells (iPSCs)
Maria Rosaria Vitale, Johanna Eva Maria Zöller, Charline Jansch, Daniel van den Hove, Tim Vanmierlo, Georg Christoph Ziegler, Klaus-Peter Lesch

Of flies and men: the impact of growth control on network development, function and behavior, and its link to Autism Spectrum Disorders

Peter Soba[1,2], Chun Hu[2], Federico Tenedini[2], Yan Tang[2], Froylan Calderon de Anda[2], Melanie Richter[2], Alexandros K Kanellopoulos[3], Claudia Bagni[3]

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Autism Spectrum Disorders (ASDs) comprise a diverse group of neurodevelopmental disorders characterized by sensory and cognitive alterations with a strong impact on social behavior. Underlying these changes are genetic alterations that have been linked to numerous genes and copy number variations. Several of these risk genes affect neuronal and synaptic growth during development. However, it is to date largely unclear how these molecular changes in ASDs give rise to altered network connectivity, function and behavior.

During neuronal network development, neurons have to synchronize their growth and connect to appropriate partners. This process is continuously ongoing during juvenile development of a growing organism to maintain network function. To model this little understood process we use *Drosophila melanogaster* larvae, a fast growing organism featuring a functionally maintained nervous system. Using genetic approaches we have identified *Drosophila Tao*, a Ste20-like kinase, as a conserved regulator of neuronal growth. Tao kinase activity is continuously required to maintain normal dendrite growth and network connectivity. Loss of Tao results in altered network function and impaired sensory and social behavior, which can be functionally rescued by its human orthologue, the ASD risk gene TAOK2. Using genetic and genome wide approaches, we are currently elucidating the regulatory network of Tao kinase to identify novel effectors required for maintaining functional network connectivity during organismal growth. Conserved target genes of Tao likely play a pivotal role in neuronal growth control linked to ASDs and could help to connect other risk genes to a common pathway.

Sensory processing disruptions in autism – from mechanism to function

Susanne Schmid[1], Kaela Scott[1], Rajkamalpreet Mann[1], Dorit Moehrle[1], Brian Allman[1]

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Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder that can be caused by a multitude of genetic and/or environmental impacts on the developing brain. It's etiology and the neuronal changes underlying this complex disorder are not well understood. Like most psychiatric disorders, it is difficult to model most ASD core symptoms in an animal model that would allow for invasive approaches to study underlying causes, as these are often intrinsically human in nature and involve higher order cortical processing and language, not possible to observe in common animal models.

Changes in sensory processing have been acknowledged as a core ASD symptom with the recent DSM-5. We argue that studying sensory processing changes associated with ASD provide a unique window of opportunity to decipher common neuronal changes on the cellular and molecular level and the respective impact on neurocircuitry that underlie these sensory processing disruptions: sensory pathways and signalling in these pathways are well described, the neuronal substrates are relatively easily accessible, and, most importantly, sensory processing pathways are highly conserved across species. Nevertheless, sensory pathways are presumably affected by genetic or environmental impact in the same way as other areas of the brain and can serve therefore as a template to study disease mechanisms and to test potential therapeutics.

We used a transgenic rat model, lacking a functional Cnatnap2 gene, to study auditory processing in the brainstem and auditory cortex, as well as auditory evoked behaviours. Cntnap2 mutations are highly associated with ASD and a lack-of-function of Cntnap2 leads to a developmental disorder with core symptoms of ASD. Cntnap2 KO rats show an altered maturation of the auditory brainstem as assessed by auditory brainstem responses (ABRs) and by brainstem-dependent acoustic startle responses. They are hyper-reactive to sound, and show disruptions in sensorimotor gating, mirroring the findings in children with ASD. On the cortical level, they seem to perceive sound in a similar way as wild-type littermates, but are more sensitive to sound, as shown by a novel sound avoidance task. Electrophysiological recordings in vivo show that despite mature ABRs in adulthood, cortical auditory function is altered across circuit (cortical AEP), local (MU), and cellular recordings, a response profile that is characterized by immaturity (longer/slower responses) and hyper-excitability, which, again, parallel those reported in ASD. Finally, patch-clamp recordings confirm hyper-excitability on the single neuron level, but also a larger half-width of action potentials impacting the ability of repeated firing and implicating voltage-gated potassium channels as potential underlying cause.

In summary, our studies of auditory processing in a rat model for ASD allows for behavioural as well as electrophysiological and pharmaceutical approaches geared to decipher changes in synaptic and cellular function underlying sensory ASD symptoms. Future experiments will determine to what extent pharmaceutical approaches that reverse these auditory changes can ameliorate other ASD core symptoms.

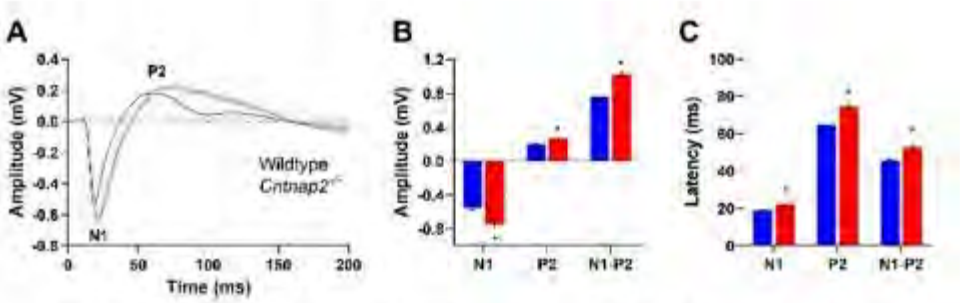

Figure 3: *Cntnap2*$^{-/-}$ cortical auditory evoked potentials reflect an immature profile. (A) Averaged cortical auditory evoked potential (CAEP) waveforms from wildtype (n = 180 waveforms) and *Cntnap2*$^{-/-}$ (n = 161 waveforms) rats in response to a 90 dB SPL noise burst. Solid line and shaded region denote mean ± standard error. (B) Increased amplitudes and (C) prolonged latencies of the N1 and P2 potentials, representing activity from the auditory nerve and lateral lemniscus terminating at the inferior colliculus respectively, reflect an immature response profile. Data represented as mean ± standard error. * $p < 0.05$.

Olfactory processing in autism: behavioral and neuroimaging insights.

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Odor stimuli are naturally volatile and dynamic and constitute a test-bed for the volatility of sensory environment often overestimated by adults with autism spectrum disorder (Lawson et al., 2017). At the behavioral level, overestimating the change in sensory environment should correspond to longer times and reduced accuracy in discrimination tasks. At the neural level, this behavior may be grounded in the functioning of areas known to preferentially encode features (e.g., quality) and sensitive to repeated exposures (e.g., piriform cortex, Li et al., 2008). We assessed these hypotheses by asking to 20 adults with autism (8F) and 14 (8F) typical controls to participate to a discrimination task and an fMRI a cross-adaptation task (as in Li et al., 2010). Participants smelled 40 pairs of odorants that could share the same perceptual quality (e.g., they were both minty/floral) or the same chemical group (e.g., they were both ketones/alcohols) to decide whether the two odorants were different.
As expected, individuals with ASD are slower than TD in discriminating the same quality, and as slow as in discriminating different qualities. Discriminating same/different chemical group takes longer in ASD. The accuracy for the different odor quality in the ASD group is reduced compared to the TD group. Our findings offer novel insights into the behavioral and physiological mechanisms underlying sensory volatility in ASD and will be contextualized in the current literature of the relationship between smell and ASD.

Prefrontal circuits in social behavior

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Mammalian social behaviors are orchestrated by a wide array of neural circuits. While some social behaviors are driven by subcortical circuits, and are considered to be highly conserved and hard-wired, others require dynamic and context-dependent modulation that integrates current state, past experience and goal-driven action selection. These cognitive social processes are known to be dependent on the integrity of the prefrontal cortex. However, the computations performed by prefrontal circuits in support of complex social functions are still largely unknown. I will present our recent work on the representation of social information in the mouse prefrontal cortex, and discuss the impact of autism-associated genetic changes on prefrontal social representations and on social behavior.

Dopaminergic modulation of nucleus incertus to interpeduncular nucleus input – possible neuronal mechanism in control of novelty preference expression

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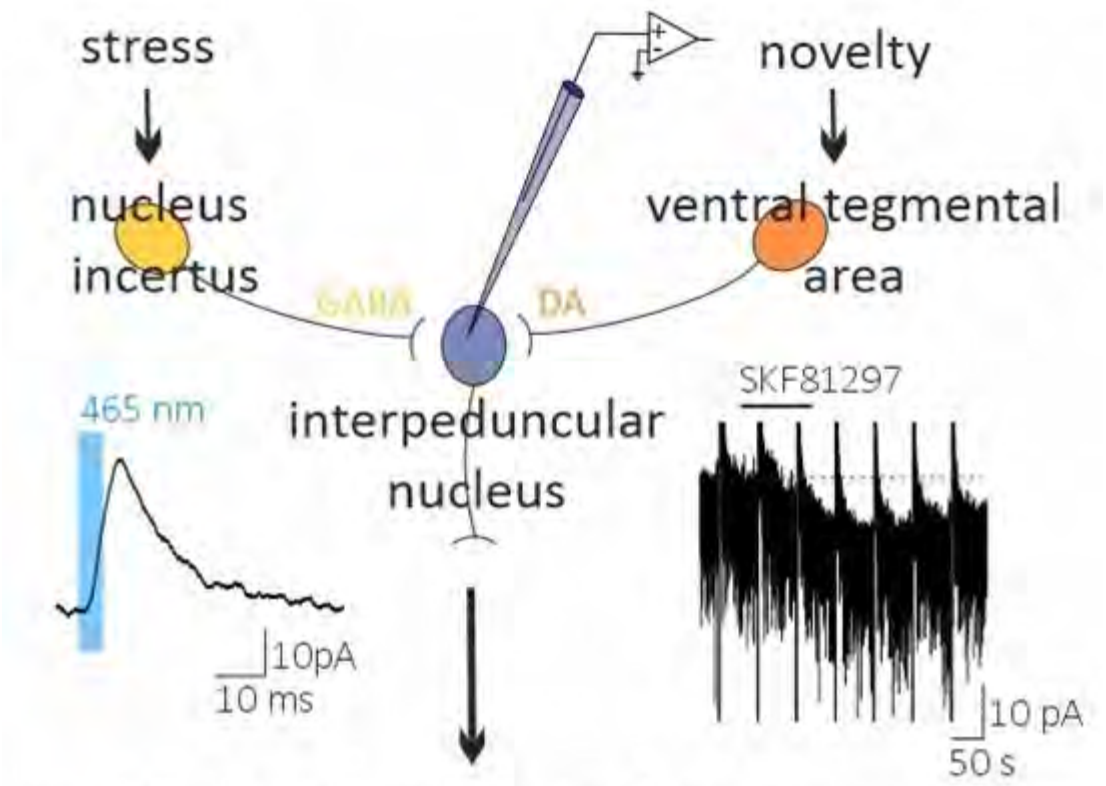
Proper discrimination between novel and familiar stimuli and adequate responses to novelty are crucial not only for proper functioning but also survival. Importantly, many neuropsychiatric disorders (such as anxiety, autism, schizophrenia and attention deficit hyperactivity disorders (ADHD)) manifest in atypical reaction to novelty. One of the predisposing factor for the development of novelty response-related deficiencies is stress. Nucleus incertus (NI) is a highly stress-sensitive brainstem structure also involved in arousal, locomotion and memory formation. NI is reciprocally connected with midbrain interpeduncular nucleus (IPN) which was shown to play a role in novelty/familiarity recognition and behavioural inhibition. At the same time, neurotransmitter dopamine (DA) has an established role in motivation-related processes and preference expression, and it was shown that ventral tegmental area dopaminergic neurons increase their activity upon exposure to novel stimuli, which then lead to dopamine release.

The aim of our studies was to investigate the functional connectivity between NI and IPN, as well as possible interactions of NI originating innervation and dopaminergic transmission, via D1 receptor, at the level of single IPN neuron. In order to test whether IPN neurons converge signals about stress and arousal (NI originating) with information about novelty (through DA/D1R system) whole-cell patch-clamp recordings of IPN neurons activity were combined with optogenetic stimulation of NI-originating fibres and bath application of D1R agonist.

Upon optogenetic stimulation of NI-originating fibres mostly inhibitory light-evoked postsynaptic currents were observed in the IPN. Moreover, 63% of recorded IPN neurons exhibited increase in inward current after D1R agonist (SKF81297, 10uM) application, suggesting than they belong to ‘novelty pathway’. Among recorded neurons sensitive to optogenetic stimulation of NI-originating fibres, 61.5% was, and at the same time, sensitive to D1R activation, showing that the same IPN neurons are sensitive to both novelty-related dopamine signalling and stress-related signals from the brainstem. Notably, analysis of the shape of light evoked postsynaptic currents revealed, that D1R agonist application lead to decrease in their amplitude. This electrophysiological data suggests a possible role of nucleus incertus – interpeduncular nucleus – ventral tegmental area neuronal loop in control of novelty preference.



modulation of novelty preference expression

Investigation of CDH13`s role in neurodevelopmental disorders using induced pluripotent stem cells (iPSCs)

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Cadherin-13 (CDH13) is a cell adhesion molecule, which regulates a wide range of cellular processes in brain development and plasticity. CDH13 is involved in cell migration, neurite outgrowth, and axon guidance. CDH13 variation is associated with neurodevelopmental and psychiatric disorders in numerous genome-wide association, copy-number variant, and whole-exome sequencing studies. Rare deletions at the CDH13 locus are linked to autism spectrum disorders, indicating clinical relevance of loss-of-function mutations. Moreover, studies observed associations with attention-deficit/hyperactivity disorder, substance use/dependence and depression.

We therefore developed a corresponding human iPSC-based in vitro system. Dermal fibroblasts were isolated from a 46-year-old healthy female and reprogrammed into induced pluripotent stem cells (iPSCs) with Sendai virus, containing the Yamanaka factors to produce transgene-free human iPSCs. To generate gene dose-dependent CDH13-deficient isogenic cell lines CRISPR/Cas9 was used. We obtained a heterozygous CDH13 knockout ($CDH13^{+/-}$) and a CDH13 null mutant ($CDH13^{-/-}$) iPSC line. All three lines show expression of pluripotency-associated markers, the ability to differentiate into cells of the three germ layers in vitro, and display a normal female karyotype.

Isogenic iPSC lines with a gene dose-dependent deficiency of CDH13 will facilitate investigation of CDH13's role in cellular processes, neuronal function and organoid network activity.

Symposium

S19: Same, same but different – Emergence of individuality in the nervous system

S19-1 The origins of individuality in the *Drosophila* visual system
Gerit Arne Linneweber

S19-2 Individualisation of behaviour in isogenic mice
Jadna Bogado-Lopes, Anna N. Grzyb, Gerd Kempermann

S19-3 Consistent behavioral differences in clonal fish
David Bierbach

S19-3 Consistent behavioral differences in clonal fish
, Kate Laskowski, Jolle W. Jolles, Carolina Doran, Max Wolf

S19-4 Associative learning in cockroaches: individuality and neural correlates
Cansu Arican, Martin Paul Nawrot

S19-5 Individual consistency in the cognitive abilities of honey bees across elemental and non-elemental learning paradigms
Valerie Finke, Martin Giurfa, Ricarda Scheiner, Aurore Avarguès-Weber

S19-6 Social basis of individual variability and drug vulnerability.
Sophie Fayad, Lauren Reynolds, Nicolas Torquet, Robin Justo, Fabio Marti, Philippe Faure

The origins of individuality in the *Drosophila* visual system

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Individual variability is highly abundant amongst all living beings. The primary source of phenotypical variation is genetic variation, but stochastic and epigenetic mechanisms can also have an important impact. Individual variability is by no means limited to morphological differences, as variability can also be found in neuronal circuits and in behaviour. Currently, it is not clear to what extent circuit variability contributes to behavioural variability in individual animals. To study the relationship between variability in neuronal circuits and behavioural variation, we have chosen the *Drosophila* visual circuitry as a model system. The fly is an ideal model to answer these questions, as we can analyse complex behavioural traits in individually identifiable groups of neurons. To test whether phenotypic variation is the primary source for stable behavioural traits in our model system, we are systematically investigating the visual circuitry of the Dorsal Cluster Neurons (DCN). Anatomical variation in this small circuit has major impact on a complex optomotor task. The anatomical variation manifests itself in individual differences in left-right symmetry. We have shown that like the individual differences in the circuitry, the flies show innate temporally stable behavioural variation in the optomotor task. We do not only find the entire range of strong to weak performing flies, but similarly we find strong temporally stable left-right asymmetries in the behaviour. Importantly, we find a strong correlation between DCN asymmetries and optomotor performance. Animals with more asymmetry show better optomotor performance than animals with more symmetric brains. Silencing DCNs abolishes correlations between anatomy and behaviour, whereas inducing DCN asymmetry suffices to improve object responses. Based on this work we are currently investigating the entire circuitry to understand if circuit variability is a general nervous system property and where ist is coming from. Furthermore, we study the importance of circuit variability also in non-visual behavioural paradigms.

Individualisation of behaviour in isogenic mice

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The interaction of individuals with the environment leads to the development of distinct behavioural patterns. Previously, we showed that isogenic mice kept in an enriched environment (ENR) established divergent and stable social and exploratory trajectories. Remarkably, the amount of exploratory activity, measured as roaming entropy (RE), correlated positively with adult hippocampal neurogenesis, a cellular plasticity mechanism in the hippocampus. Herein we investigate how an ENR influences uniqueness in inbred mice, when genetic variability is not playing a role, and what possible brain alterations explain individual behaviour in our 'individuality' model. Female mice (n = 40) were housed for 3-months in a novel large ENR enclosure, consisting of 70 connected cages equipped with radio antennae, where their behaviour was longitudinally tracked. Cyclin D2-ko mice, with suppressed adult neurogenesis, lack the increased behavioural variability observed in their wild-type littermates. Their cognitive performance was evaluated in the Morris Water Maze task (MWM) and AHN levels assessed using BrdU. We confirmed that the number of $BrdU^+$ cells correlates with RE in the wild-type animals, and cyclin D2-ko mice have impaired performance in the reversal phase of MWM. Whereas wild-type animals developed stable exploratory trajectories, the behaviour of cyclinD2-ko mice was more random. Together, these results suggest that adult neurogenesis may have a crucial role at the individualisation of brain-related phenotypes.

Consistent behavioral differences in clonal fish

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Consistent behavioral differences are thought to be caused by differences in genes and/or environmental conditions. Therefore, if these sources of variation are removed, individuals are predicted to develop similar phenotypes lacking repeatable individual variation. We use tightly controlled ontogenetic experiments with a clonal fish, the Amazon molly (Poecilia formosa), to test whether near-identical rearing conditions can dampen individual differences in behavior. In sharp contrast to our predictions, we find substantial individual variation in behavior among genetically identical individuals isolated directly after birth into highly standardized environments and that this variation does not develop over the ontogeny but is present at day 1 of the fish's life. In contrast to the current research paradigm, which focuses on genes and/or environmental drivers, our findings suggest that individuality might be an inevitable and potentially unpredictable outcome of the very early embryonic development.

Associative learning in cockroaches: individuality and neural correlates

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Learning experiments in insects have predominantly been conducted in the honeybee (Menzel, 2012) and in the fruit fly (McGuire et al., 2005). Both animal models have their strong advantages, *Drosophila melanogaster* lends itself to genetic manipulations (Davis, 1993) and detailed connectome studies (Takemura et al., 2017). Bees on the other hand show complex forms of learning in a broad repertoire of olfactory and visual classical and operant learning paradigms (Avargues-Weber and Giurfa, 2013). However, both have also several disadvantages when it comes to the investigation of neural correlates of memory formation and recall. Bees and flies have dense and compact brains, which makes electrophysiological recordings difficult and recordings under natural free-flying conditions are particularly challenging.
Here, we introduce the cockroach as model species to study individual learning behavior and its underlying neuronal basis with the prospect of investigations in freely moving animals. We have established experimental protocols for classical and operant conditioning in the cockroach and could confirm individual learning abilities with consistent performance during training and test (Arican et al., 2020). Our results in both paradigms are in line with findings in honeybee experiments (Pamir et al., 2014) and thus in two evolutionary distinct insect groups (hymenoptera and blattodea). We therefore hypothesize that individuality is a general phenomenon that should be observable in all model species, specifically and withstanding older hypotheses(Quinn et al., 1974) in *Drosophila melanogaster*.
In order to analyze neural correlates of individual learning behavior we target a population of mushroom body output neurons (MBON). Thus far, MBONs have been shown to encode the valence of conditioned stimuli in honeybees (Strube-Bloss et al., 2011, 2016) and fruit flies (Aso et al., 2014). We established *in vivo* extracellular single-unit recordings in the cockroach, adapting the methods in Strube-Bloss et al. (2011). In a first experimental protocol we analyze olfactory responses in naïve animals at the level of MBONs for ten different odors in permanent air stream. Additionally, we use whole-mount staining for visualization of electrode tracks and exact recording position adapting methods in Brill et al. (2013). Future experiments will address MBON responses during memory acquisition and memory recall.

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Individual consistency in the cognitive abilities of honey bees across elemental and non-elemental learning paradigms

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Since the first discovery that the individual performance of humans in a variety of cognitive tasks is positively correlated, the study of general intelligence or g factor has been extensively studied in humans. There is now increasing evidence for the existence of a general intelligence in a wide range of vertebrate species and factors underlying those individual differences in cognitive ability. However, the study of correlated performances in cognitive abilities of individuals and consistent individual differences has been mainly ignored in invertebrate species. The honey bee is a powerful model organism for the study of various elemental and non-elemental forms of learning and cognition. Thereby a lot of studies reported that honeybees exhibit appreciable variation in their performance in several learning tasks. However, it remained unclear if this variation in learning performance is due to different extrinsic factors or rather represents an intrinsic characteristic of an individual honey bee. In the present study it was examined if honeybees show consistent individual differences in their learning ability over time and across learning tasks of different complexity and cognitive requirements. In a first experiment the bees were subjected to elemental visual discrimination tasks for three consecutive days. The results show that the individual bees' performance remained stable over time. In a second experiment we tested the bees in an elemental discrimination task and a non-elemental concept learning task. Again, the performances of individual bees in both tasks were positively correlated. In a third experiment bees were tested in a reversal learning and negative patterning protocol, two forms of non-elemental learning. The reversal learning consists of an elemental learning phase in which one stimulus is associated with a reward (A+) and a second stimulus is not rewarded (B-) and a non-elemental reversal learning phase where the reward contingencies of the learning phase are reversed. The learning phase assesses the bees' discrimination ability, and the reversal learning phase evaluates cognitive flexibility. In the negative patterning paradigm two single stimuli are rewarded (C+ and D+) while their compound is not reinforced (CD-), it represents a form of pure non-elemental learning and assesses the bees´ ability for configural processing. The results show that the bees´ performance was positively correlated across both phases of reversal learning as well as across the learning phase and negative patterning. However, when comparing the two non-elemental paradigms, i.e. the reversal learning phase and negative patterning there was neither a correlation nor a trade-off in the individual performances. Demonstrating correlated cognitive performances of individual honey bees challenge the classical view of invertebrates being merely "reflex machines" which only stereotypically respond to stimuli. Furthermore, such findings stretch the importance of considering individual differences as important factor accounting for variability in the cognitive abilities of invertebrates. However, our results point against a "general factor" in the cognitive ability of individual honey bees and rather support the view of independent cognitive modules within the bee brain that underlie the success in certain cognitive abilities but not in others.

Social basis of individual variability and drug vulnerability.

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Consistent individual differences in behaviors represent a ubiquitous feature in animal populations. These behavioral differences among individuals define personality and have been linked to vulnerability to drug. Indeed, the susceptibility to develop drug addiction differs substantially between individuals and some traits, such as impulsivity, exploration or novelty seeking, have been shown to represent a predictive factor for the addictive properties of drugs. In that context, we aim at understanding the impact of social environment on individual behaviors and vulnerability to nicotine. Our lab has developed « Souris City », a semi-naturalistic environment designed to study the behavior of mice living in social groups. Combining a social homecage with an individual test zone (T-maze), this system allows us to study correlations between decision making strategies and other behaviors observable within the environment for each individual, while manipulating the social context. Considering the key role of the dopaminergic system in decision making and addiction, we also study the neurophysiological correlates of the behaviors observed, using electrophysiological recordings of spontaneous activity and nicotine-evoked response of dopaminergic neurons from the Ventral Tegmental Area. Finally, we are able to study the impact of nicotine on those correlations by introducing it into the environment, either via voluntary consumption, or via chronic implantation if osmotic pumps. This combinatorial approach allows to address key questions on individual personality and vulnerability to pathologies.

Symposium

S20: Store-operated calcium entry in neurons and glia

S20-1 The role of store-mediated calcium signals for in vivo sensor/effector functions of microglia
Olga Garaschuk

S20-2 TRPC channels regulate Ca2+-signaling and short-term plasticity of fast glutamatergic synapses
Yvonne Schwarz

S20-3 Orai1 channels amplify glutamate-evoked calcium signals in dendritic spines to regulate synaptic plasticity and cognitive functions.
Murali Prakriya

S20-4 Short novel STIM1B uncovers a mechanism of synaptic enhancement
Barbara Anne Niemeyer, Girish Ramesh, Lukas Jarzembowski, Yvonne Schwarz, Maik Konrad, Vanessa Poth, Gertrud Schwaer, Anna A. Lauer, Marcus O.W. Grimm, Dalia Alansary, Dieter Bruns

S20-5 TrpA1 activation dampens neuroinflammation via MHC class II modulation on astrocytes
Samantha Schmaul, Nicholas Hanuscheck, Frauke Zipp, Stefan Bittner

S20-6 Traumatic brain injury induced switch of α subunits in L-type voltage-gated calcium channels of somatostatin-positive interneurons stabilizes early cortical hyperexcitability in mice.
Natascha Ihbe, Florie Le Prieult, Qi Wang, Ute Distler, Malte Sielaff, Stefan Tenzer, Serge Thal, Thomas Mittmann

The role of store-mediated calcium signals for in vivo sensor/effector functions of microglia

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Microglia, the innate immune cells of the brain, are increasingly recognized as an important player both in the context of physiological brain function and brain pathology. As many non-excitable cells, microglia utilize changes in the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) to sense the relevant alterations in the brain parenchyma and to fulfill their executive functions. The latter include modifications in microglial morphology and gene expression profile, directed process movement and cell migration, phagocytosis, release of cytokines and other inflammation markers, etc.

We used in vivo two-photon Ca^{2+} imaging to monitor microglial Ca^{2+} signaling in the brains of healthy young adult mice, during aging and under conditions of peripheral inflammation as well as amyloid-induced neuroinflammation. Under homeostatic in vivo conditions, microglial Ca^{2+} signaling was infrequent and often localized to microglial processes. However, large somatic changes in $[Ca^{2+}]_i$ were readily observed in response to cell damage in the microglial microenvironment, in cells dwelling in the immediate vicinity of amyloid β plaques or during the early phase of lipopolysaccharide (LPS)-induced peripheral inflammation. These Ca^{2+} transients were mediated by Ca^{2+} release from the intracellular Ca^{2+} stores, likely caused by the activation of metabotropic P2Y receptors. Besides, the LPS-induced Ca^{2+} transients required the activation of the NACHT-, LRR- and pyrin (PYD)-domain-containing protein 3 (NLRP3) inflammasome, responsible for Ca^{2+}-dependent release of interleukin 1β from microglia. The number of cells showing somatic Ca^{2+} transients increased with aging in a gender-specific way. Surprisingly, microglia of middle-aged (9-11 months old) mice showed the highest frequency and the longest duration/area under the curve of the somatic Ca^{2+} transients, indicative of the hyperreactive, alerted state of middle-aged microglia. Microglial Ca^{2+} transients, recorded in aged (18-21 months old) mice, were characterized by low frequency and small duration/area under the curve, thus differing dramatically from that seen in middle-aged animals and likely reflecting the dysfunctional state of microglia. Noteworthy, long- but even short-term caloric restriction (30% reduction of ad libitum food intake) was able to shift the properties of microglial Ca^{2+} transients to a functional state seen in the younger age group.

Together, the described above data firmly identify microglial Ca^{2+} signaling as a central mechanism, linking the sensor and effector functions of microglia and show that treatments alleviating the dysfunction in microglial Ca^{2+} signaling positively impact executive functions of these key immune cells of the brain.

TRPC channels regulate Ca2+-signaling and short-term plasticity of fast glutamatergic synapses

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Transient receptor potential (TRP) proteins form Ca2+-permeable, non-selective cation channels, but their role in neuronal Ca2+ homeostasis is elusive. In the present paper, we show that TRPC channels potently regulate synaptic plasticity by changing the presynaptic Ca2+-homeostasis of hippocampal neurons. Specifically, loss of TRPC1/C4/C5 channels decreases basal evoked secretion, the pool size of readily-releasable vesicles and accelerates synaptic depression during high frequency stimulation (HFS). In contrast, primary TRPC5 channel-expressing neurons, identified by a novel TRPC5- GFP knockin mouse line, show strong short-term enhancement (STE) of synaptic signaling during HFS indicating a key role of TRPC5 in short-term plasticity. Lentiviral expression of either TRPC1 or TRPC5 turns classic synaptic depression of wild-type neurons into STE, demonstrating that TRPCs are instrumental in regulating synaptic plasticity. Presynaptic Ca2+ imaging shows that TRPC activity strongly boosts synaptic Ca2+ dynamics, showing that TRPC channels provide an additional presynaptic Ca2+ entry pathway, which efficiently regulates synaptic strength and plasticity.

Orai1 channels amplify glutamate-evoked calcium signals in dendritic spines to regulate synaptic plasticity and cognitive functions.

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Store-operated Orai1 calcium channels function as highly Ca^{2+}-selective ion channels and are broadly expressed in many tissues including the central nervous system but their contributions to cognitive processing are largely unknown. Here we report that many measures of synaptic, cellular, and behavioral models of learning are markedly attenuated in mice lacking Orai1 in forebrain excitatory neurons. Results with focal glutamate-uncaging in hippocampal neurons support an essential role of Orai1 channels in amplifying NMDA receptor-induced dendritic Ca^{2+} transients that drive activity-dependent spine morphogenesis and long-term potentiation at Schaffer collateral–CA1 synapses. Consistent with these signaling roles, mice lacking Orai1 in pyramidal neurons (but not interneurons) exhibit striking deficits in working and associative memory tasks. These findings identify Orai1 channels as essential regulators of dendritic spine Ca^{2+} signaling, synaptic plasticity, and cognition.

Short novel STIM1B uncovers a mechanism of synaptic enhancement

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Store-operated Ca2+-entry (SOCE) regulates basal and receptor-triggered Ca2+ signaling with STIM proteins sensing the endoplasmic reticulum (ER) Ca2+ content and triggering Ca2+ entry by gating Orai channels. Although crucial for immune cells, STIM1's role in neuronal Ca2+ homeostasis is controversial. Here, we characterize a novel splice variant, STIM1B, with exclusive neuronal expression, protein content surpassing conventional STIM1 in cerebellum and significant abundance in hippocampus. STIM1B results in a truncated protein with slower kinetics of ER-PM cluster formation and ICRAC as well as reduced inactivation. In primary wild-type neurons, Stim1B is targeted by its spliced-in domain B to presynaptic sites where it converts classic synaptic depression into Ca2+- and Orai-dependent short-term synaptic enhancement (STE) at high frequency stimulation (HFS). In conjunction with altered STIM1 splicing in human Alzheimer disease, our findings highlight STIM1 splicing as an important regulator of neuronal calcium homeostasis and synaptic plasticity.

TrpA1 activation dampens neuroinflammation via MHC class II modulation on astrocytes

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Multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE) are autoimmune diseases characterised by chronic autoimmune inflammation, demyelination and axonal loss. Disease progression in MS is driven both by adaptive and innate immune responses within the central nervous system (CNS) compartment, as well as by bi-directional communication of immune cells with endogenous CNS cells. This thesis focused on the interplay between astrocytes and CNS-infiltrating Th17 cells, especially during neuroinflammatory conditions. Using two-photon imaging of organotypic hippocampal slice cultures (OHSC), we were able to identify a subset of Th17 cells that contact astrocytes and interact with them for an extended period of time. This astrocyte-contacting subpopulation showed characteristics that differ from the overall population of Th17 cells. They were slower than the average Th17 cells, they display a lower displacement rate and their meandering index is decreased. We could show by blocking of MHC class II in the OHSCs as well by the addition of CNS-autoantigen-unspecific Th17 cells that this process was antigen-dependent and that blockade of this process changed the properties of the astrocyte-contacting TH17 subset. In a next step, we showed that astrocytic antigen presentation was not restricted to the in vitro situation but could be observed in vivo as well. Additionally, the expression was regulated in a disease-state dependent manner during EAE. An astrocytic-specific deletion of MHC class II significantly weakened EAE disease progression and led to a milder course. The cation channel TrpA1 has been recently implicated as astrocytic sensor of the local microenvironment. Specifically, TrpA1 was described to be involved in neurogenic inflammation and demyelination, both prototypical conditions in MS and EAE. Indeed, TrpA1 was present on astrocytes and was regulated by distinct autoimmune-inflammatory stimuli on the mRNA level. Importantly, absence of TrpA1 led to an upregulation of astrocytic MHC class II expression, underlining the important role of this ion channel for perpetuating CNS-specific immune responses. In line with this, deletion of TrpA1 during EAE exacerbated the disease course significantly. Overall, it could be shown that TrpA1 expression on astrocytes regulated MHC class II expression, thereby modulating T cell responses and disease symptoms in an animal model of neuroinflammation.

Traumatic brain injury induced switch of α subunits in L-type voltage-gated calcium channels of somatostatin-positive interneurons stabilizes early cortical hyperexcitability in mice.

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Traumatic brain injury (TBI) is one of the leading causes for mortality in industrialized countries. Even though advances in intensive care units led to a reduced mortality, patients still suffer from severe physical and cognitive outcomes (Werner and Engelhard, 2007).
Focal cortical injuries lead to early disturbances spreading on the undamaged hemisphere shortly after induction of the insult. This phenomenon, referred to as transhemispheric diaschisis, is suggested to be mediated by an imbalance in strength of glutamatergic excitatory vs. GABAergic inhibitory neurotransmission with an impairment of GABAergic inhibition (Imbrosci et al, 2014, LePrieult et al., 2017). To better understand the outcome of this diaschisis, it is needed to substantially investigate the time course of neuronal functional alterations in the contralateral hemisphere shortly after TBI. Here we hypothesized that TBI-induced alterations in the proteome of subpopulations of cortical GABAergic interneurons could stabilize early cortical disturbances.
To test this, we used a GAD67-GFP (Glutamate decarboxylase 67 – green fluorescent protein) knock-in mouse model to isolate GFP+ GABAergic interneurons from the cortex contralateral to an unilateral controlled cortical impact (CCI) to the primary motor and somatosensory cortex at postnatal day 19-21 under anesthesia in vivo. Interneurons were isolated after a survival time of 72 h. To obtain single GFP+ interneurons we used fluorescence activated cell sorting (FACS) and further analyzed single cell suspensions of 50.000 cells by mass spectrometry (MS). GFP+ interneurons from sham-operated mice that did not receive an impact, but the same anesthesia and a scalp incision served as controls.
GABAergic interneurons derived from the undamaged, contralateral cortex reacted in a diverse manner to the ipsilateral cortical injury disclosing dynamic processes over the selected time window. We specifically detected an overrepresentation of proteins linked to cellular components related to neuron projection including dopaminergic and glutamatergic synapses. Interestingly, the proteomic data showed a TBI-induced putative reciprocal regulation of α subunits of pore-forming L-type voltage-gated calcium channel (VGCCs), represented by an expression of CaV1.3 and simultaneous ablation of CaV1.2. To test whether this switch in the α subunits of VGCCs after 72h post-TBI supports the recovery of our recently observed early (24h after TBI) contralateral hyperexcitability, we performed extracellular recordings in acute brain slices on microelectrode arrays (MEA) in the presence of isradipine, a known CaV1.3-selektive antagonist at concentrations of 100 nM. Interestingly, isradipine indeed stabilized the extracellular cortical network activity back to the level of controls at 72 h post-TBI.
In addition, by whole-cell patch-clamp recordings from single cells we disclosed the GABAergic subpopulation of somatostatin-positive (SST) interneurons as a functional carrier of the VGCC α subunit switch. F/I-curves recorded from SST interneurons at 72h after TBI revealed an isradipine sensitive increase in excitability through the expression of CaV1.3. These data indicate that a TBI-induced expression of the

CaV1.3 α subunit of VGCCs in SST interneurons supports the adaptive processes in the contralateral network during the early phase after TBI.

Symposium

S21: The impact of the immune system on psychiatric disorders (DGPPN Symposium)

S21-1 Microglia phenotypes in animal models of psychiatric symptoms
Susanne Wolf, Dilansu Güneykaya, Daniele Mattei, Bilge Ugursu, Helmut Kettenmann

S21-2 Immuno-metabolic pathways in major depression
Stefan Gold

S21-3 Polysialic acid mimetics to target extrasynaptic NMDA receptors and rescue learning and memory in mouse models of neurodegenerative diseases
Shaobo Jia, Hristo Varbanov, Stoyan Stoyanov, Weilun Sun, Hauke Thiesler, Herbert Hildebrandt, Rita Gerardy-Schahn, Oleg Senkov, Alexander Dityatev

S21-4 Neuronal differentiation in different cell lines and oral stem cells as a cell source for dopaminergic neurons
Theresia Schlothauer, Alice Drobny, Philipp Arnold, Stefan Rose-John, Friederike Zunke

S21-5 Anti-NMDA receptor encephalitis, autoimmunity, and psychosis
Johanna Schöner

S21-6 Anti-Inflammatory Minocycline for Treatment-Refractory Depression: Implications for Retinoid Signaling
Julian Hellmann-Regen, Vera Clemens, Isabella Heuser

Microglia phenotypes in animal models of psychiatric symptoms

Susanne Wolf[1], Dilansu Güneykaya[2], Daniele Mattei[3], Bilge Ugursu[1], Helmut Kettenmann[4]

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Microglia are involved in the onset and progression of psychiatric disorders. We here focus on two animal models of psychiatric endophenotypes. The maternal immune activation (MIA) model is used to mimic symptoms of schizophrenia and the Neuroligin-4 (NL4)model to investigate symptoms related to Autism spectrum disorders (ASD).

MIA during pregnancy has been linked to an increased risk of developing psychiatric pathologies in later life. This link may be bridged by a defective microglial phenotype in the offspring induced by MIA, as microglia have key roles in the development and maintenance of neuronal signaling in the central nervous system. The beneficial effects of the immunomodulatory treatment with minocycline on schizophrenic patients are consistent with this hypothesis. Using the MIA mouse model, we found an altered microglial transcriptome and phagocytic function in the adult offspring accompanied by behavioral abnormalities. The changes in microglial phagocytosis on a functional and transcriptional level were similar to those observed in a mouse model of Alzheimer's disease hinting to a related microglial phenotype in neurodegenerative and psychiatric disorders. Minocycline treatment of adult MIA offspring reverted completely the transcriptional, functional and behavioral deficits, highlighting the potential benefits of therapeutic targeting of microglia in psychiatric disorders.

NL4 mutations are the most common genetic abnormalities associated with (ASD). Loss-of-function NL4 mutation in mice causes ASD related impairments in both, the synaptic and behavioral phenotype. Since microglia closely regulate synaptic development and are implicated as key players in ASD development and progression, we here studied microglial properties in the NL4 knock out mouse model. Also here, we see impairments in microglia function such as phagocytosis paralleled by changes in the transcriptome in the microglia derived from NL4 male brains.

Immuno-metabolic pathways in major depression

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Major depressive disorder (MDD) and cardiometabolic disorders are highly prevalent diseases. A strong bidirectional epidemiological link has been established between obesity and MDD, with both conditions increasing the risk of developing the other by approximately 50%. An increasing body of evidence from experimental and clinical approaches indicates that MDD and metabolic disorders might be mediated by shared (patho)biological pathways. For example, animal studies suggest that impaired metabolic function in the immune system may be sufficient to induce depression-like behavior. In addition, inflammatory mechanisms have been implicated in comorbid depression and obesity in humans, as genetic risk factors overlap between these disorders. This evidence has led to the proposal of a putative "immune-metabolic" subtype of depression. In this presentation, I will review the evidence for a convergence of biological pathways in metabolic disorders and depression on a systemic, cellular, and molecular level. In a series of studies, we have demonstrated overlapping cellular immune signatures of obesity and depressive symptoms. Moreover - even after controlling for body mass index and manifest metabolic disorders - patients with major depression show evidence for significant dyslipidemia and an altered metabolome. On a cellular level, T cells obtained from MDD patients exhibited reduced respiratory and glycolytic capacity. Gene expression analysis suggested metabolic reprogramming with a shift towards fatty acid oxidation. These results indicate immune dysregulation and mitochondrial dysfunction in MDD, with potential implications for treatment. Based on these observations, we have launched a clinical trial to investigate the potential of repurposing drugs such as statins as adjunct treatments in comorbid obesity and depression.

Polysialic acid mimetics to target extrasynaptic NMDA receptors and rescue learning and memory in mouse models of neurodegenerative diseases

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[Introduction] Neural cell adhesion molecule (NCAM) is a member of the immunoglobulin superfamily of adhesion molecules, which promotes cell-cell and cell-to-extracellular matrix (ECM) adhesion. The extracellular domain of NCAM can be modified by long homopolymers of the acidic nonasugar sialic acid (polysialic acid, polySia) which facilitates synaptic plasticity and learning. Our previous studies revealed that enzymatic removal of polySia as well as genetic ablation of NCAM or the polySia-synthesizing enzyme ST8SIA4 reduces long-term potentiation (LTP) in slices prepared from the hippocampal CA1 area and the medial prefrontal cortex (mPFC) [1, 2]. This impaired LTP could be pharmacologically rescued by: 1) the extrasynaptic GluN2B-NMDA receptor antagonist Ro 25-6981, 2) the synaptic NMDA receptor agonist D-cycloserine, or 3) by increasing the extracellular glycine level with the glycine-transporter 1 inhibitor sarcosine [2].

[Aims] Interrogate if an intranasally (i.n.) administrated polySia fragment containing 12 sialic-acid residues (DP12, 1 µg/ml) can abrogate the defects in PFC-dependent learning. To address this question we used four experimental paradigms: (i) rescue after acute enzymatic removal of polySia, (ii) rescue after genetic ablation of ST8SIA4, (iii) rescue in mice virally overexpressing human Tau[R406W] mutant and (iv) in the 5xFAD model of Alzheimer's disease.

[Methods] Thy1-GFP mice (n=3+3) were intranasally treated with 1,2-diamino-4,5-methylenedioxybenzene(DMB)-tagged disialic acid (DMB-DP2) and polySia (DMB-DP12, 10 mg/kg each) and observed under a two-photon microscope. Wildtype and polySia deficient St8sia4-/- mice (WT/KO, n=10+10) were treated with DMB-DP2/DMB-DP12 (2 mg/kg, i.n.) and then assessed with novel object recognition test (NORT) and a recency test. C57BL/6J mice (n=12) were treated with sarcosine or vehicle before and after enzymatic removal of polySia by stereotactic injection of polySia-specific endoNF (2 µg/µl, 500 nl per hemisphere) into mPFC (AP:+1.9; ML:±0.5; DV:2.25/1.5 mm). Injected mice received DP12 and free sialic acid (DP1; 1 mg/kg, i.n.) and were evaluated by NORT. To induce a tauopathy, AAV-GFP-Tau[R406W] and AAV-GFP were injected to mPFC (AP:+1.7; ML:±0.3; DV:-2.2 mm; 500 nl per hemisphere) in C57BL/6J mice (n=10+10). DP12 and DP1 (1 mg/kg, i.n.) were applied 1 month after virus injection 30 min before the recency test. 12-month-old 5xFAD mice (n=11) were used to evaluate the effect of 0.5 mg/kg DP12. Paired t-test was applied to compare exploring time near objects in the same trial. Discrimination ratio between genotypes undergoing different treatments was assessed by 2-way-RM ANOVA followed by

Holm-Sidak post-hoc test.

[Results] Two-photon imaging demonstrated that DMB-labelled DP2 and DP12 could pass the BBB, the fluorescent signals increased 0.5–3h after intranasal delivery. St8sia4$^{-/-}$ mice showed less discrimination towards novel objects (P=0.022) and recent objects (P=0.043). I.p. administration of sarcosine or intranasal delivery of DMB-DP12 restored cognitive function in St8sia4$^{-/-}$ mice in NORT (P=0.008, 0.005, respectively). DMB-DP12 had a tendency to improve the performance in the recency test (P=0.093). EndoNF treated animals showed impaired performance in NORT (P=0.015). Administration of DP12 at day 5 after endoNF injection in contrast to the treatment with free sialic acid (DP1) normalized object discrimination (unpaired t-test, P<0.05). AAV-GFP-Tau[R406W] impaired performance in the recency test (P < 0.001) and there was a significant Tau x DP12 interaction (P < 0.001). Finally, DP12 also restored cognitive function in the recency test in 5xFAD mice (P < 0.001).

[Conclusions] DP12 successfully restored cognitive deficits in a diverse set of animal models, highlighting the therapeutic potential of polySia mimetics to target extrasynaptic NMDAR signaling in neurodegenerative diseases.

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Neuronal differentiation in different cell lines and oral stem cells as a cell source for dopaminergic neurons

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Neurodegenerative disorders are characterized by neuronal cell death with often unknown molecular reasons. To better develop and test treatment strategies, it could be a promising beginning to differentiate dopaminergic neurons from a human cell source with easy access. Further, it is important to better understand intracellular mechanisms that are important for cell homeostasis and the balance of cell survival and cell death.
In this study, we have evaluated the potential of gingival stem cells (GMSCs) as a cell source for dopaminergic neurons. For this we used an establishes differentiation protocol for human induced pluripotent stem cells (Kriks et al. 2012).
Further, we have looked into the role of the metalloproteinase (ADAM) 17 during neuronal differentiation and homeostasis (Rose-John, Zunke, 2017). For this we used an established differentiation protocol of the human neuroblastoma SH-SY5Y cell line (Xicoy et al. 2017). To study the impact of ADAM17 on intracellular processes during neuronal differentiation, we used a CRISPR-Cas9 knockout approach. Our data suggests, that neuronal differentiation was less efficient, when ADAM17 was absent, which indicates an important role of the protease in neuronal homeostasis.
Our data suggest GMSCs as a promising cell source for developing dopaminergic neurons in vitro. Further our data indicate that ADAM17 seems to have an impact on neuronal differentiation and homeostasis and therefore a promising lead in understanding the cause of neurodegenerative disorders.

Anti-NMDA receptor encephalitis, autoimmunity, and psychosis

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Autoimmune encephalitides with psychotic symptoms, such as N-methyl-D-aspartate receptor (NMDAR) encephalitis, have been identified as a rare but treatable differential diagnosis for psychotic disorders. However, psychiatric diagnoses are still mainly based on clinical ICD-10/DSM-5 criteria and inflammatory markers and specific antineuronal antibodies are often not reliably detected in routine diagnostics. In this talk, epidmiological data, as well as the clinical symptoms of autoimmune encephalitides are presented; moreover, „red flags“ and diagnostic strategies are identified in order to facilitate a rapid diagnosis and initiation of immunotherapy and to avoid devastating long-term consequences for affected individuals. Finally, preliminary data of the "REPOSE" study, a Berlin register study on endogene and exogene psychosis, are shown.

Anti-Inflammatory Minocycline for Treatment-Refractory Depression: Implications for Retinoid Signaling

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The main objective of this study was to test the antidepressant (AD) efficacy of the pleiotropic and anti-inflammatory tetracycline minocycline as an adjunct to an antidepressant standard treatment in patients suffering from treatment-resistant major depressive disorder (TRD). It has widely been demonstrated that patients with TRD exhibit an altered inflammatory status with increased peripheral cytokine levels that may eventually result in chronic, sub-threshold pro-inflammatory activation of microglia in the CNS. The latter may represent one specific neurobiological correlate of depressive symptoms in TRD. Multiple lines of evidence suggest this process to be targetable by anti-inflammatory minocycline. In the MINO-TRD trial, which was conduced as a nationwide, multi-center randomized placebo-controlled trial, 200 mg of oral minocycline daily were administered as add-on to AD standard treatment for a total period of 6 weeks with a 6 month follow-up period to a total number of n = 128 patients suffering from TRD. Eligibility to participate in the trial was not based on a priori peripheral inflammatory markers. Instead, a large panel of putative predictive and therapeutic biomarkers, including parameters of retinoid homeostasis, was assessed throughout the trial. Additionally, a patient-specific cell model system based on monocyte-derived macrophages was used as an ex vivo model for patient-specific microglia-like cells. These cells were used in parallel to the clinical intervention and provide a precision medicine approach for the targeted discovery of cell-based biomarkers.

Symposium

S22: MultiSenses – MultiScales: Deciphering neural processing in multisensory integration

S22-1 Evolution of olfactory systems with a multi-scale approach: genes, networks and behaviours
Lucia Prieto Godino

S22-2 Integrating multimodal proprioceptive feedback - influence of load on movement signal processing in the insect leg muscle control system
Corinna Gebehart, Ansgar Büschges

S22-3 The thalamus that speaks to the cortex: spontaneous activity in the developing brain
Guillermina López Bendito

S22-4 Neural microcircuits underlying multisensory integration in the mouse striatum
Gilad Silberberg

S22-5 Neocortical activity of mice performing a multisensory accumulation of evidence task
Gerion Rouven Nabbefeld, Anna Ostenrath, Severin Graff, Alexander Bexter, Simon Musall, Björn M. Kampa

S22-6 Multisensory processing of self-motion information in primates
Frank Bremmer

Evolution of olfactory systems with a multi-scale approach: genes, networks and behaviours

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Sensory systems encode the world around us to guide context-dependent appropriate behaviours that are often species-specific. This must involve evolutionary changes in the way that sensory systems extract environmental features and/or in the downstream sensory-motor transformations implemented. However, we still know little about how evolution shapes neural circuits. We address these fundamental questions using as models the olfactory systems of different fly species, some of which are vectors for devastating diseases. We employ a multidisciplinary approach, including field work, the development of genetic tools across species, fast volumetric calcium imaging, single cell transcriptomics and comparative connectomics. I will discuss the progress we have made in our efforts to understand how evolution tinkers neural circuits as animals adapt to different environments.

Integrating multimodal proprioceptive feedback - influence of load on movement signal processing in the insect leg muscle control system

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Skilled motor control of body posture and movement strongly depends on proprioceptive feedback from the limbs. In legged animals, sense organs located on or within the limbs provide information about limb and joint posture and movement (muscle spindles (vertebrates); chordotonal organs (insects)), and load (Golgi tendon organs (vertebrates); campaniform sensilla (insects)). These proprioceptive signals are processed by local premotor networks in the spinal or ventral nerve cord, respectively, and used to generate the appropriate motor output such as corrective movements for posture maintenance, resistance or assistance reflexes. This requires the integration of proprioceptive signals from multiple sources into a single perceptual framework. In this study, we asked how the processing of different proprioceptive signals affect each other in the generation of the final motor output.

We combined intracellular sharp electrode recordings of sensory afferents, nonspiking interneurons (NSIs), and motor neurons (MNs) with extracellular nerve recordings and mechanical load and movement stimuli to investigate the effects of load signaling on movement feedback processing in the control loop of the stick insect femur-tibia joint.

Proprioceptive signals from the femoral chordotonal organ (fCO) and tibial campaniform sensilla (tiCS) induce reflex activation of tibial MNs. We tested the effect of combined load and movement stimuli on the gain of MN responses to altered movement (fCO) stimulus parameters (position / velocity). Simultaneous stimulation decreased the gain of positional feedback in the slow extensor tibiae MN (SETi), whereas the opposite was observed in the fast MN (FETi). In contrast, the gain of the effect of movement velocity signals in SETi and FETi was not consistently changed, while an increase was observed in the common inhibitor 1 MN (CI_1). Thus, the gain of proprioceptive movement feedback in tibial MNs was altered by load signals in a parameter- and neuron-specific way.

Both load and movement signals are integrated by a common network of local premotor NSIs which drive or inhibit the initially investigated extensor tibiae MNs. Concurrent load and movement stimulation resulted in nonlinear summation of the two signals in individually identifiable NSIs.

We then investigated where signals from tiCS and the fCO interact. We found interaction at the earliest neuronal stage, via presynaptic afferent inhibition. Activation of tiCS depolarized fCO afferents and reduced the amplitude of coinciding fCO action potentials. Presynaptic inhibition was instrumental for the observed influence of load on the processing of movement feedback, since its pharmacological removal by bath application of picrotoxin abolished the observed influence of load on movement responses in tibial MNs.

We conclude that fCO movement signal processing in the local premotor network is under the control of load feedback from tiCS. This provides a mechanism that could explain how the nervous system implements context-specificity in its computations at a local level, e.g. to alter signal processing and motor output between swing and stance phase during walking. Future experiments will focus on the behavioral effects of the control of movement gain via load feedback and on a more detailed understanding of presynaptic afferent interactions.

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The thalamus that speaks to the cortex: spontaneous activity in the developing brain

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Our research team runs several related projects studying the cellular and molecular mechanisms involved in the development of axonal connections in the brain. In particular, our aim is to uncover the principles underlying thalamocortical axonal wiring, maintenance and ultimately the rewiring of connections, through an integrated and innovative experimental programme. The development of the thalamocortical wiring requires a precise topographical sorting of its connections. Each thalamic nucleus receives specific sensory information from the environment and projects topographically to its corresponding cortical. A second level of organization is achieved within each area, where thalamocortical connections display an intra-areal topographical organization, allowing the generation of accurate spatial representations within each cortical area. Therefore, the level of organization and specificity of the thalamocortical projections is much more complex than other projection systems in the CNS. The central hypothesis of our laboratory is that thalamocortical wiring influences and maintains the functional architecture of the brain. We also believe that rewiring and plasticity events can be triggered by activity-dependent mechanisms in the thalamus. Here in this talk, I will present our recent data on the activity-dependent mechanisms involved in thalamocortical guidance and cortical development. I will also present data on the role of this activity in the thalamus in promoting neuroplastic cortical changes following sensory deprivation. Within these projects we are using several experimental programmes, these include: optical imaging, manipulation of gene expression in vivo, cell and molecular biology, biochemistry, cell culture, sensory deprivation paradigms and electrophysiology.

Neural microcircuits underlying multisensory integration in the mouse striatum

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The basal ganglia are traditionally studied with focus on their motor functions, however they receive sensory inputs from the entire neocortical sheet, including primary sensory areas. Our aim is to elucidate the functional properties of cortical and striatal neural circuits underlying sensory and motor processes. I will present in-vivo work demonstrating bilateral and multimodal sensory integration by individual striatal neurons in the healthy and dopamine-depleted striatum. I will also present recent data from our lab pertaining to the synaptic organization of the striatal microcircuitry, focusing on the connectivity between the different types of striatal interneurons and afferent pathways.

Neocortical activity of mice performing a multisensory accumulation of evidence task

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Decades of research have focused on investigating the principles by which our brain represents and processes the information of our individual senses. Yet, we consciously perceive our environment not as separated sensory spaces, but rather as multisensory percepts. The mechanisms by which our brain integrates this multisensory information are still poorly understood. To study how visual and tactile perception are integrated on the neuronal circuit level, we have developed a multisensory two-alternative forced choice task (2AFC) in head-fixed mice. Water-deprived mice are traversing a virtual corridor during which they are presented with visual, tactile or combined visuotactile cues. At the end of the corridor the animals must indicate the side with the higher cue frequency to receive a water reward. We show that mice can learn this task in all three stimulus conditions (visual, tactile & visuotactile). We find that mice display a higher performance in the multisensory condition. This effect is stronger in more difficult discrimination conditions in line with the “inverse-effectiveness” principle of multisensory integration. To investigate the neural basis of this effect we are measuring cortex-wide activity using widefield imaging in transgenic mice expressing the fluorescence Ca^{2+}-indicator GCaMP6s throughout neocortex. We aim to dissect the circuitry underlying the sensory integration and behavior using videography of the animals to account for the contribution of motor-related activity. Consistent with the increase in discrimination performance of multisensory cues, these stimuli also evoke higher neuronal activity already in primary sensory areas. Further studying the changes in cortical activity during the learning process of this task allowed us to determine the role of association areas such as posterior parietal cortex (PPC) and prefrontal cortex (PFC) on multisensory integration in a behaviorally relevant context. These results provide a deeper insight into the processes of multisensory integration in a natural cue accumulation task.

Multisensory processing of self-motion information in primates

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Neurophysiological studies in the macaque monkey, i.e., the prime animal model for human sensorimotor processing, have identified two cortical areas being involved in the encoding of self-motion information: areas MST and VIP. Neurons in both areas are truly multisensory, and functional equivalents of both areas have been identified in humans. In this talk, I will review part of our recent work on multisensory self-motion processing.

In a behavioral study, we investigated the interplay between visual and tactile flow while human observers were engaged in a distance reproduction task (Churan et al., 2017). We found that adding congruent tactile information to the visual information significantly improves the precision of the actively reproduced distances. Up- or downscaling of the tactile flow, which went unnoticed by the participants, induced under- or overshoot of travelled distances. Both results are in line with neural responses in primate area VIP.

In a second study, we asked if and how the processing of visually simulated self-motion and associated auditory stimuli is modulated by self-controlled action (predictive coding) (Krala et al., 2019). Participants had to solve a path integration task. Blood oxygen level–dependent (BOLD) activation in this active condition was compared with BOLD activation during a passive replay condition with the exact same sensory stimulus. We found supramodal BOLD suppression in parietal and frontal regions and modality-specific enhancement in early sensory areas.

In a third study, we aimed to determine the role of eye movements for heading encoding (Bremmer et al., 2017). We recorded neural activity from monkey areas MST and VIP during presentation of self-motion stimuli and concurrent reflexive eye movements. A heading-decoder performed veridically during slow eye movements. During fast eye movements (saccades), however, the decoder erroneously reported compression of heading toward straight ahead. A behavioral experiment in humans showed that perceived heading is perisaccadically compressed toward the direction of gaze. Response properties of primate areas MST and VIP are consistent with being the substrate of this newly described visual illusion.

In a fourth and final study, we used TMS in human observers to selectively disturb self-motion processing in human area MST (hMST) during a heading task (Schmitt et al., 2020). As predicted by our decoding model, which was based on monkey neurophysiological data, TMS over area hMST, but not over a control-area, increased response variance of perceived heading as compared with no-TMS stimulation trials. As hypothesized, this effect was strongest for contraversive self-motion. These data provide a first causal evidence for a critical role of hMST in visually guided navigation.

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Symposium

S23: Principles of decision-making across species

S23-1 The brain as a central regulator of nutrition: how needs turn into wants
Carlos Ribeiro

S23-2 Retracted

S23-3 Neural circuitry underlying sensorimotor decisions
Maxime Lehman, Sandra Autran, Tihana Jovanic

S23-4 Prosocial choice in rats depends on social context
Cristina Márquez

S23-5 Identifying neural circuits mediating the exploration-exploitation trade-off in *Drosophila melanogaster*
Dennis Goldschmidt, Ibrahim Tastekin, Daniel Münch, Hannah Haberkern, Célia Baltazar, Lúcia Serra, Ann M. Hermundstad, Vivek Jayaraman, Gerald M. Rubin, Carlos Ribeiro

S23-6 Neural correlates of decision between actions in a cortico-basal ganglia network
David Thura, Paul Cisek

The brain as a central regulator of nutrition: how needs turn into wants

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Nutritional decisions are key determinants of health, wellbeing and aging. We want to understand how animals decide what to eat and how these decisions affect the fitness of the animal. To achieve a mechanistic, integrated, whole-animal understanding of nutritional decision-making we work at the interface of behavior, metabolism, microbiome, and physiology in the adult *Drosophila melanogaster*. I will discuss how the powerful combination of neurogenetics, automated, quantitative behavioral analyses, nutritional and microbial manipulations, and activity imaging approaches is allowing us to achieve a mechanistic understanding of how neuronal circuits implement proto-cognitive behaviors and regulate them according to internal states. Adapting nutrient specific appetites to the needs of the animal allows these to optimize important traits such as aging and reproduction.

Neural circuitry for stimulus selection in the zebrafish visual system

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When navigating the environment, animals need to prioritize responses to the most relevant stimuli. Although a theoretical framework for selective visual attention exists, its circuit implementation has remained obscure. Here, we investigated how larval zebrafish select between simultaneously presented visual stimuli. We found that a mix of winner-take-all (WTA) and averaging strategies best simulates behavioral responses. We identified two circuits, whose activity patterns predict the relative saliencies of competing visual objects. Stimuli presented to only one eye are selected by WTA computation in the inner retina. Binocularly presented stimuli, on the other hand, are processed by reciprocal, bilateral connections between nucleus isthmi (NI) and tectum. This interhemispheric computation leads to either WTA or averaging responses. Optogenetic stimulation and laser ablation of NI neurons disrupt stimulus selection and behavioral action selection. Thus, depending on the relative locations of competing stimuli, a combination of retinotectal and isthmotectal circuits enable selective visual attention.

Neural circuitry underlying sensorimotor decisions

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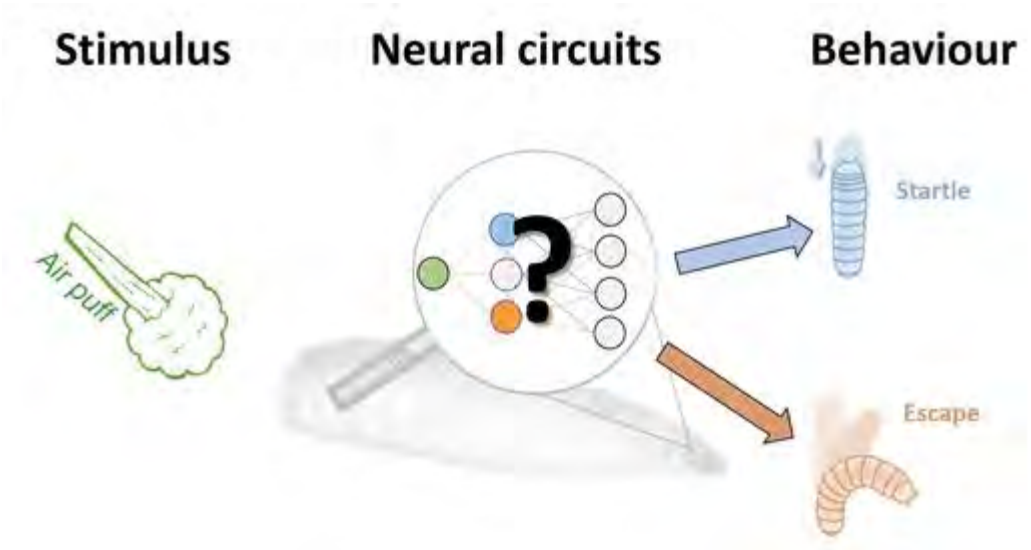
From finding nearby food to escaping a predator, animals must respond to sensory cues with appropriate motor actions in order to survive. The neural circuit mechanisms underlying the transformation of sensory information into appropriate motor output remain incompletely understood. It is for instance unclear how one action is selected among several possible responses to a single stimulus. In addition, how the selected action is specified by motor circuits depending on stimulus characteristics is not known either at the cellular and synaptic level. To understand these mechanisms better, it would be important to map the entire neural circuitry from the sensory to the motor side in a model organism allowing functional testing of such circuits. Thanks to recent progress in connectomics, allowing the mapping of entire neural circuits in electron microscopy (EM) with synaptic resolution and to the genetic toolbox for cell-specific neuronal manipulations and monitoring, Drosophila larva presents itself as such a model. We will here present the progress we made on the investigation of the neural circuitry underlying larval responses to an aversive mechanosensory stimulus, the air puff. Drosophila larvae typically respond with either startle or escape behaviours to the air-puff (Jovanic et al., 2016). Startle responses are for example Hunching (head-retraction), and Stopping. Escape responses include bending (i.e. head casting and turning) and crawling. The selection of one action implies the inhibition of other, mutually exclusive, actions. Competitive interactions between the neural modules underlying startle and escape behaviors could thus exist in order to enable the selection between the two. Using cell-specific inactivation and optogenetic activation of neuronal activity in behaving larvae combined with high-speed video tracking and automated behavioral detection (Masson et al., 2020), we determined the role of interneurons at different stages of the sensorimotor pathways and found they are differentially involved in startle and escape behaviors. Some of these neurons promote one response and repress another suggesting they are involved in competitive interactions. Using EM-based reconstruction of neurons and of their synaptic connections, we are now mapping the sensorimotor pathway underlying the startle (hunch) response with synaptic resolution and investigating where and how it could interact with the escape pathway. Neurons involved in competitive interactions should make connections with the two pathways, which would allow them to promote escape behaviors and repress startle behaviors for example. Determining where competitive interactions take place in the nervous system and what the involved neural circuit mechanisms are in a model system where we can trace all connections at synaptic resolution across the entire nervous system and manipulate single neurons may bring insights into the neural circuit mechanisms of sensorimotor decisions in general.

References:

T. Jovanic et al., Cell. 167, 858-870.e19 (2016)
J.-B. Masson et al., PLOS Genet. 16, e1008589 (2020)


Stimulus
Neural circuits
Behaviour
Air puff
?
Startle
Escape

Prosocial choice in rats depends on social context

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How we take decisions in social contexts is a fundamental aspect of our daily lives, however, the underlying mechanisms are only starting to be addressed. We previously showed that rats display prosocial behaviours by providing food to conspecifics in foraging contexts. In this talk, we will focus on the mechanisms by which social context mediates this type of decision-making, with special interest on social status, gender and familiarity. Interestingly, we found that social hierarchy modulates social distance during the decision process, strongly affecting the emergence of prosociality in rats.

We evaluated prosocial tendencies of different pairs of animals using our previously developed behavioural paradigm, where a rat (decision maker) can choose either to be selfish (rewarding only itself) or prosocial (providing food to itself and its partner). We compared pairs of animals interacting with cage-mates or unrelated conspecifics, to study the effect of familiarity, and evaluated whether females would differ to males in their prosociality. Moreover, we questioned whether social hierarchy would affect prosocial tendencies. For this, we identified the stablished social status of cage-mates and ask whether dominant and submissive animals would differ in their levels of prosocial behaviour. The reach at the behavioural mechanism, we used custom-made tracking software that enable automated and unsupervised quantification of social interactions with high spatial and subsecond temporal resolution.

Quantitative analysis of social interactions prior to decision revealed an interesting pattern of differential approach/avoidance behaviour by submissive animals which was related to decision outcomes. Our results pave the way to explore the neural circuits that make animals more prone to help others, and how are they differently instantiated according to social status.

Identifying neural circuits mediating the exploration-exploitation trade-off in *Drosophila melanogaster*

Dennis Goldschmidt[1], Ibrahim Tastekin[1], Daniel Münch[1], Hannah Haberkern[2], Célia Baltazar[1], Lúcia Serra[1], Ann M. Hermundstad[2], Vivek Jayaraman[2], Gerald M. Rubin[2], Carlos Ribeiro[1]

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In order to optimize nutrient homeostasis, animals must adapt complex foraging decisions according to nutrient availability and needs. When a foraging animal finds food, it has to decide whether to stop and engage in feeding, or continue searching for better options. These decisions are not only driven by external sensory cues, but also by internal states that reflect its physiological needs. However, the neurons and the circuit logic that underlies the dynamic computations for balancing exploration and exploitation during foraging and how they are shaped by internal states remain largely uncharacterized.

Here, we present a high-throughput experimental setup for studying nutrient foraging choices and exploration in *Drosophila melanogaster* and identifying the neuronal circuits mediating the underlying computations. We use image-based tracking to extract and analyze the movements of individual flies in an arena filled with different spatially distributed food spots, which enables us to detect specific behavioral patterns related to feeding and foraging decisions. Using this system, we tested a total of more than 15000 nutrient-deprived flies to compare the behavioral effects of silencing more than 500 different genetically targeted sparse populations of neurons using the GAL4/UAS system.

We identify multiple lines labeling different neuronal populations affecting different aspects of nutrient-specific exploitative behaviors. Strikingly, several of these labeled neurons in the central complex. When silenced the labeled neurons seem to balance the drive to explore and exploit in our nutrient foraging paradigm. More specifically, we find that these neurons are heterogeneous in function and that they modulate the probability of stopping at a food spot while altering the drive to explore. We will present our current efforts to identify the mechanisms by which these neurons mediate this trade-off.

Our results reveal a neural substrate involved in complex ethologically relevant foraging decisions, a key step towards a mechanistic explanation of cognitive functions required to compute the exploration-exploitation trade-off in order to achieve nutrient homeostasis.

Neural correlates of decision between actions in a cortico-basal ganglia network

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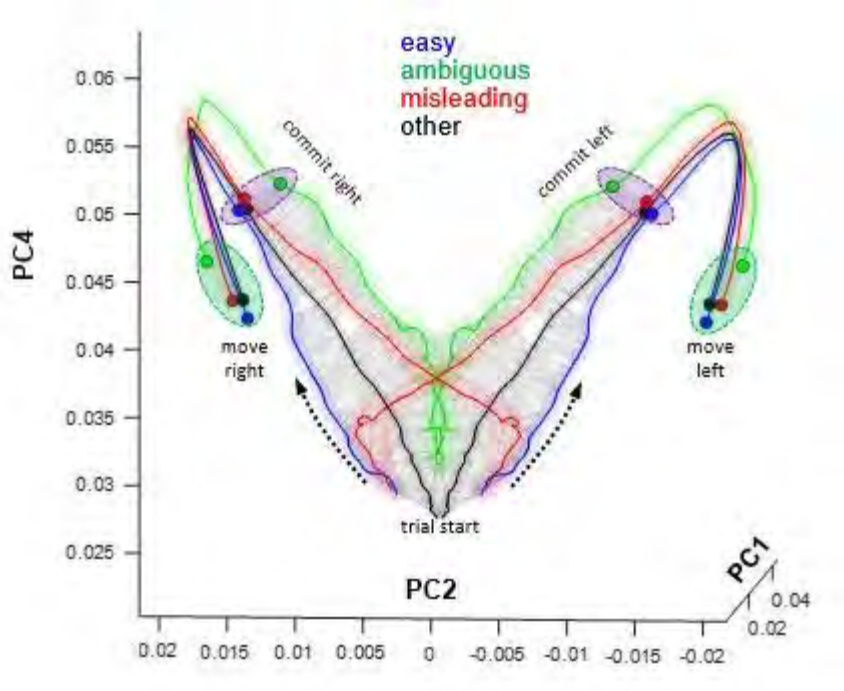
Although decision-making has traditionally been assumed to be distinct from movement execution, for many years a growing body of data has reported neural correlates of decision variables in many regions associated with motor control, casting doubt on that assumption.
In this presentation, I'll show evidence that decision-making and action execution are two modes of a unified dynamical system implemented in large distributed populations of neurons. We recorded neurons in the dorsal premotor cortex, the primary motor cortex, the dorsolateral prefrontal cortex and in the external and internal segments of the globus pallidus of two monkeys trained to perform a probabilistic reach decision task in which sensory evidence evolves within each trial. With single-neuron and "state-space" analyses, I'll show how these areas share labor during the decision process and how activity patterns are compatible with a dynamical attractor model in which cortical activity reflects a biased competition between actions, gradually amplified by an urgency signal from the basal ganglia.



Symposium

S24: Hypothalamic neuron-glial network in obesity and type 2 diabetes

S24-1 Brain Bile acid-TGR5 signaling protects from obesity
Daniela Cota

S24-2 Role of astrocytic $GABA_B$ receptors on γ-hydroxybutyric acid induced absence seizures
Davide Gobbo, Anja Scheller, Frank Kirchhoff

S24-3 Neurocircuits of food sensory perception
Sophie Steculorum

S24-4 Developing 2-D and 3-D models to disentangle the interplay between mitochondrial vulnerability and inflammation in Parkinson's disease
Helena Winterberg, Philippe Ravassard, Olga Corti, Flora Magno, Jana Heneine, Benjamin Galet, Noemi Asfogo, Julie Smeyers, Morwena Latouche, Aurore Tourville, Patrick P. Michel

S24-5 Obesity-associated hyperleptinemia alters the gliovascular interface of the hypothalamus to promote hypertension
Tim Gruber, Chenchen Pan, Raian Eduardo Contreras, Tobias Wiedemann, Don Morgan , Alicja Skowronski, Francisco Ruiz-Ojeda, Melanie Huber, Timo Dirk Müller, Siegfried Ussar, Paul Pfluger, Ali Ertürk, Charles LeDuc, Kamal Rahmouni, Miriam Granado, Tamas Horvath, Matthias Tschöp, Cristina Garcia-Caceres

S24-6 Hypothalamic neuron-glial network in obesity and type 2 diabetes.
Chun-Xia Yi

Brain Bile acid-TGR5 signaling protects from obesity

Daniela Cota[1]

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Previous studies have shown that bile acids are critical regulator of metabolic responses in peripheral organs, by particularly involving the activation of the bile acid membrane Takeda-G-protein-receptor-5 (TGR5). Data that we have generated support the existence of a hypothalamic bile acids-TGR5 signaling system, which is particularly relevant to contrast diet-induced obesity. Pharmacological stimulation of central TGR5 reduces food intake and body weight, while decreasing adiposity and improving insulin sensitivity in diet-induced obese mice. These metabolic improvements are due to central TGR5 recruitment of the sympathetic nervous system, which ultimately increases energy expenditure, thermogenesis and lipolysis in adipose tissue. Conversely, AAV-mediated downregulation of hypothalamic TGR5 favors obesity in lean mice and worsens obesity in already obese animals. Investigations currently ongoing will help define the cellular and molecular mechanisms involved, thus providing further evidence supporting a beneficial role for bile acids in obesity and type 2 diabetes.

Role of astrocytic $GABA_B$ receptors on γ-hydroxybutyric acid induced absence seizures

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Absence seizures are non-convulsive epileptic events characterized by brief losses of consciousness, unresponsiveness to stimuli and are commonly observed in pediatric or juvenile epilepsies. The weak $GABA_B$ receptor agonist γ-hydroxybutyric acid (GHB) mimics generalized spike and slow wave discharges (SWDs) characterizing absence epilepsy and is therefore used as a pharmacological model of absence seizures. Given the role of astroglia in modulating and sustaining neuronal synaptic activity and their involvement in many different pathological scenarios, we investigated the role of astrocytic $GABA_B$ receptor in the genesis and progression of GHB-induced absence seizures. To this aim, we took advantage of the CreERT2-LoxP system to induce time-controlled astrocyte-specific gene deletion of the GABAB1 subunit resulting in lack of functional $GABA_B$ receptors in astrocytes. *Ex vivo* GHB administration in presence of the voltage-gated Na^+ channel blocker tetrodotoxin induced longer intracellular Ca^{2+} signals with higher amplitudes in astrocytes expressing the genetically encoded Ca^{2+} indicator GCaMP3 and imaged by two-photon laser-scanning microscopy (2P-LSM). Mice lacking functional astrocytic $GABA_B$ receptors showed unaltered Ca^{2+} signals compared to baseline. *In vivo* GHB induced highly synchronous Ca^{2+} waves in cortical astrocytes immediately after intravenous injection in control animals. On the contrary, in conditional knock-out animals Ca^{2+} signals after GHB injection were lower in amplitude compared to sham-treated animals. Moreover, loss of astrocytic $GABA_B$ receptors resulted in the alteration of GHB-induced dose-response assessed through *in vivo* telemetric electroencephalographical recording of brain activity and behavioural video monitoring. GHB-induced alterations lasted shorter independently of the administered dose. Taken together, these results suggest a role of astrocytic $GABA_B$ receptors in the mechanisms underlying GHB-induced absence seizures. This makes this receptor a promising target for the treatment of absence epilepsy, which is still effective in only half of the patients.

Neurocircuits of food sensory perception

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Food sensory perception has recently emerged as a potent regulator of specialized feeding circuits by rapidly reversing the neuronal activity state of key feeding-regulatory neurons. Exposure to food cues inhibits neurons promoting feeding and activates the ones decreasing it. However, the resulting behavioral outputs and the underlying neuronal principles remain poorly understood. We investigated the feeding-regulatory role of projections from the olfactory bulb to hypothalamic and non-hypothalamic regions. I will discuss our results uncovering sensory pathways that integrate food odors to control food intake.

Developing 2-D and 3-D models to disentangle the interplay between mitochondrial vulnerability and inflammation in Parkinson's disease

Helena Winterberg[1,2], Philippe Ravassard[2], Olga Corti[2], Flora Magno[2], Jana Heneine[2], Benjamin Galet[2], Noemi Asfogo[2], Julie Smeyers[2], Morwena Latouche[2], Aurore Tourville[2], Patrick P. Michel[2]

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Neuroinflammation and mitochondrial dysfunction play an increasingly important role in the susceptibility and progression of Parkinson's disease (PD), emerging as promising targets for the development of therapies aimed at halting neurodegeneration. Two major regulators of mitochondrial quality control and mitophagy, PINK1 and Parkin, are associated with early onset familial PD. Recent observations of our team have highlighted a hyperresponsiveness of microglial cells from Parkin-deficient mice *(Park2-/-)* and macrophages from patients with *PARK2*/Parkin mutations to activators of the NLRP3 inflammasome, suggesting an involvement of this proinflammatory pathway in this familial form of PD.

To date, there has been little research on the interplay between neuronal and microglia cells in the context of *PINK1* and *PARK2*/Parkin mutations. Our objective is to explore whether and how the hyperactivation of the NLRP3 inflammasome pathway in a Parkin-deficient context affects the dopaminergic neurons that degenerate in PD, taking advantage of 2-D and 3-D culture mesencephalic models. Different types of primary mouse 2-D cultures are being explored: in a first paradigm, conditioned medium from microglia activated in vitro by specific proinflammatory stimuli known to induce NLRP3 inflammasome-dependent responses is transferred to purely neuronal E13/14 mesencephalic cultures. Our preliminary experiments demonstrated that priming of microglia with the bacterial inflammogen LPS and treatment with 2'(3')-O-(4-benzoylbenzoyl)-adenosine-5'-triphosphate (BzATP), a ligand of the P2X7 ion channel and activator the NLRP3 inflammasome pathway, leads to the expected release of the inflammasome-dependent cytokine IL-1ß. As a complementary strategy, we are studying the direct impact of microglial cells layered on top of the mesencephalic neurons, following addition of the NLRP3 inflammasome activators to the co-culture. In our first experiments, LPS and BzATP did not have direct deleterious effects when applied to neurons cultured alone. In current work, we are investigating the functional integrity and survival of dopaminergic neurons incubated with microglia-conditioned medium or co-cultured with microglia isolated from *Park2-/-* or wildtype mice.

In parallel, we are developing a 3-D model to study neuro-immune interactions in a context similar to the human brain. We have adapted a robust protocol for the generation of brain organoids with midbrain identity (hMO). These organoids showed a progressive increase in the expression of dopaminergic markers, including the rate-limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase (TH), and the FOXA2 transcription factor. In ongoing work, human microglia-like cells differentiated from peripheral blood-derived monocytes have been incorporated into 30 day-old hMOs, and we are investigating their long-term

integration, and their impact on dopamine neuron maturation and organization within the hMO.
As a perspective, these studies will be extended to integrate into hMO microglia-like cells from PD patients carrying *PARK2* gene mutations. Altogether, these approaches should provide novel insight into the complex interplay between neuroinflammation and mitochondrial vulnerability in *PARK2*-linked PD.

Obesity-associated hyperleptinemia alters the gliovascular interface of the hypothalamus to promote hypertension

Tim Gruber[1], Chenchen Pan[2], Raian Eduardo Contreras[3], Tobias Wiedemann[4], Don Morgan [5], Alicja Skowronski[6], Francisco Ruiz-Ojeda[7], Melanie Huber[1], Timo Dirk Müller[8], Siegfried Ussar[7], Paul Pfluger[3], Ali Ertürk[2], Charles LeDuc[6], Kamal Rahmouni[5], Miriam Granado[9], Tamas Horvath[10], Matthias Tschöp[1], Cristina Garcia-Caceres[1]

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Vascular pathologies represent hallmarks of the metabolic syndrome comprising deteriorations in certain vulnerable microcirculations as well as chronic elevations in blood pressure. Here we report that obesity-associated increases in serum leptin trigger the select expansion of the micro-angioarchitecture in pre-autonomic brain centers implicated in systemic hemodynamic regulation. By using a series of cell- and region-specific loss- and gain-of-function models we show that this pathophysiological process depends on hypothalamic astroglial HIF1α-VEGF (Hypoxia-inducible factor 1α-Vascular endothelial growth factor) signaling, which is tuned by astroglial leptin signaling. Importantly, several distinct models of HIF1α-VEGF pathway disruption in astrocytes are not only protected from diet-induced hypothalamic angiopathy, but also do not develop the sympathetic hyperactivity or arterial hypertension typically resulting from chronic exposure to hypercaloric environments. In summary, we here report the discovery that hyperleptinemia promotes obesity-induced hypertension via a HIF1α-VEGF signaling cascade in hypothalamic astrocytes, establishing a novel mechanistic link that connects hypothalamic micro-angioarchitecture with control over systemic blood pressure.

Hypothalamic neuron-glial network in obesity and type 2 diabetes.

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Brain microglia are long-surviving and self-renewing innate immune cells that are crucial for scavenging cell debris and pathogens to maintain brain tissue homeostasis. The hypothalamus contains highly heterogeneous and condensed populations of neurons in different regions. The mediobasal hypothalamus (MBH) functions as an important “brain window” for sensing blood-borne substances. It is reasonable that this region constantly produces cell debris and metabolic waste during different metabolic states. In order to keep a healthy and clean microenvironment for the hypothalamic neurons to function, the microglial activity in the MBH needs to match the high demands for immune surveillances and debris phagocytosing/clearances. This was demonstrated by the fact that microglia in the MBH showed a significantly higher reactivity than in other hypothalamic regions when experimental animals were exposed to high-fat high-sugar diet (HFHS), and that this occurs rapidly after receiving the HFHS diets. Unlike the peripheral macrophages that can be defined into a pro-inflammatory (M1) and an anti-inflammatory/pro-resolving (M2) phenotype, the microglial phenotypes are way more complex following their varied non-macrophage-like functions in the brain. The diet-induced reactive microglia in the MBH not only express more M1 phenotype-associated pro-inflammatory cytokines such as interleukin 1 beta and tumor necrosis factor (TNF), but also more lipids’ uptake gate-keeper lipoprotein lipase (LPL) and less hexokinase 2, indicating an increase of fatty-acid oxidation and OXPHOS and less glycolysis which belong to the M2 phenotype. This illustrates that in the HFHS diet-activated microglia, their immunometabolism is reprogrammed differently from the canonical switching process between peripheral M1 and M2 macrophages. In the obesogenic environments, TNF is one of the major cytokines produced by the reactive microglia. TNF acts on neighboring hypothalamic pro-opiomelanocortin (POMC) neurons and induces mitochondrial stress, which might be one of the major causes of diet-induced POMC neural dysfunction. This POMC neural loss is not only observed in the diet-induced obese animals, but also in postmortem hypothalamic tissue of type 2 diabetic patients. The reactive microglia in the MBH upon HFHS diet is not only characterized by increased cytokine production, but also impaired phagocytic capacity, as shown by a downregulation of phagocytic indicator CD68 expression in HFHS diet-fed rats. In microglia, besides governing lipid uptake for fueling, the LPL-gated phospholipid production is also crucial for phagolysosome formation and turnover. Thus, HFHS diet might impair microglial phagocytosis in the MBH, whereas a compensatory mechanism drives more LPL expression and phospholipids production in order to maintain the phagocytic capacity. Not surprisingly, in HFHS diet-fed mice that had lack of LPL in microglia, there was a further down-regulation of CD68, and worsened phagocytic capacity, ultimately associated with lesser POMC neurons and more vulnerability to diet-induced metabolic disorders. This suggests that lacking a sufficient microglial phagocytosis has a detrimental effect on POMC neural survival upon diet challenge. As in HFHS diet-activated microglia, the increased pro-inflammatory cytokines oppose phagocytic capacity, targeting microglial pathway in treating obesity and type 2 diabetes needs to tackle both inflammatory and phagocytic pathways.

Symposium

S25: Optical imaging to assess the plasticity function of sleep

S25-1 How sleep balances cortical circuit activity
Niels Niethard, Jan Born

S25-2 Experience-dependent modulation of cortical dendritic activity across wake and sleep states
Julie Seibt, Johanna Sigl-Glöckner, Naoya Takahashi, Kiran GR Kumar, Matthew E Larkum

S25-3 REM sleep promotes experience-dependent synaptic reorganization in the mouse cortex
Wen-Biao Gan

S25-4 Optical probing of sleep circuits and functions
Antoine Adamantidis

S25-5 Selective sensory gates for sleep regulation in Drosophila
Davide Raccuglia, Raquel Suárez-Grimalt, David Owald

How sleep balances cortical circuit activity

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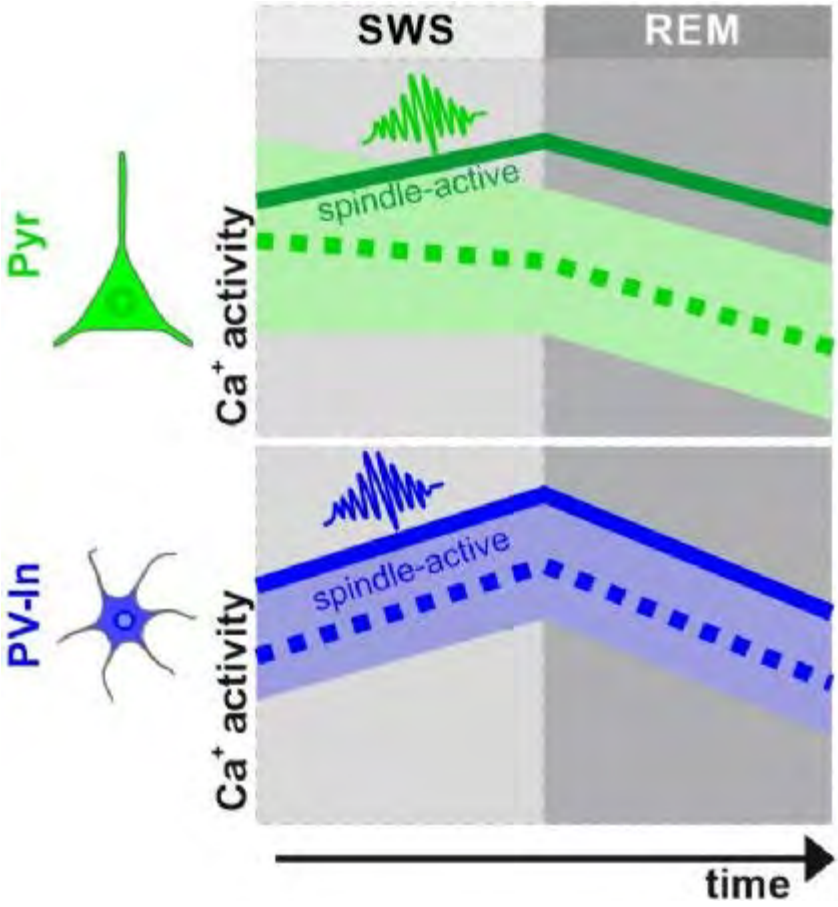
Sleep concurrently contributes to homeostatic down-regulation and mnemonic synaptic up-regulation in cortical networks. Here we used two-photon imaging of calcium activity to examine how these seemingly opposing functions are established. Pyramidal (Pyr) cells representing the majority of excitatory cells, decreased activity during both epochs of slow-wave sleep (SWS) and rapid-eye-movement (REM) sleep. During SWS, but not REM epochs, activity of parvalbumin-positive interneurons (PV-In) simultaneously increased, suggesting that down-regulation of Pyr activity during SWS reflects increased somatic inhibition. Contrasting with the general down-regulation of Pyr, a subpopulation of Pyr cells showing highest activity during sleep spindles, up-regulated activity during SWS epochs, consistent with an involvement of these cells in mnemonic processing. During succeeding REM epochs these spindle-active Pyr cells showed a profound and persistent decrease in activity, in parallel with decreasing PV-In activity. This pattern suggests that REM sleep generally produces synaptic down-scaling, even capturing spindle-active neurons participating in mnemonic processing.


SWS
REM
Pyr
spindle-active
Ca+ activity
PV-In
spindle-active
Ca+ activity
time

Experience-dependent modulation of cortical dendritic activity across wake and sleep states

Julie Seibt[1], Johanna Sigl-Glöckner[2], Naoya Takahashi[3], Kiran GR Kumar[1], Matthew E Larkum[4]

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During sleep, structural and functional changes of dendrites have been proposed to serve as the substrate of memory storage in the cortex. Apical dendrites of layer 5 neurons (L5 dendrites) integrate countless synaptic inputs and, owing to their intrinsic properties, can express localized synaptic plasticity. While high resolution optical approaches have recently provided unprecedented insight into the role of dendritic activity in plasticity, only few studies have leveraged these methods to investigate the role and influence of brain state (i.e. wake and sleep) in dendritic activity regulation.

Using a combination of fiber optic and two-photon imaging, we have previously shown that population calcium (Ca^{2+}) activity of L5 apical shaft is increased and synchronized during non-rapid eye movement (NREM) sleep period rich in spindle oscillations (Seibt et al. Nat. Commun. 2017). This work provided a novel link between the previously observed increase in spindles after learning and the requirement for dendritic (re)activation during NREM sleep for memory consolidation. However, this study did not explore the influence of experience, which has been shown to elicit changes in dendritic spine structure, involving specifically rapid-eye-movement (REM) sleep (Li et al., Nat. Neurosci., 2017; Zhou et al., Nat Commun., 2020).

In this new study, we aim to gain a better understanding of how sleep stages, neuronal oscillations and experience interact to modulate Ca^{2+} activity in apical tuft dendrites. Compared to the apical shaft, the apical tuft of dendrites are where spines are located and thus where structural plasticity takes place. We used two-photon Ca^{2+} imaging of L5 apical tuft dendrites in the somatosensory cortex across active wake [AW], quiet wake [QW], NREM and REM sleep. Because we previously found that during sleep, somatic and dendritic activity show independent regulation, we also investigated experience-dependent Ca^{2+} activity changes in L5 cell bodies for comparison. Given the pivotal role of precisely timed dendritic inhibition in plasticity, and their regulation by sleep states (Niethard et al., Curr. Biol., 2016), we also imaged somatostatin (SST) interneurons that are known to target apical dendrites. To assess the influence of experience, we use acute (3 hours) exposure to an enriched environment (EE), a paradigm that has been shown to induce robust cortical plasticity in the somatosensory cortex during sleep. L5 dendrites, L5 cell bodies and SST neurons (in layers 2/3) were imaged in different mice (4/group, total of 12 mice) and Ca^{2+} activity was measured during a baseline period and after three hours of EE exposure the next day.

REM sleep promotes experience-dependent synaptic reorganization in the mouse cortex

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The functions and underlying mechanisms of rapid eye movement (REM) sleep remain unclear. We show that motor learning induces newly formed postsynaptic dendritic spines of layer 5 pyramidal neurons in the mouse motor cortex. Subsequent REM sleep strengthens and maintains a fraction of these newly formed spines. On the other hand, auditory-cued fear conditioning (FC) or monocular deprivation (MD) causes the elimination of existing dendritic spines in the frontal association or visual cortex respectively. REM sleep facilitates such spine elimination induced by FC and MD. In addition, we show that dendritic calcium spikes increase substantially during REM sleep in various cortical regions. The blockade of these calcium spikes prevents the strengthening of new spines induced by motor learning, as well as the elimination of existing spines induced by FC and MD. Together, these findings suggest that REM sleep plays an important role in promoting experience-dependent reorganization (either formation or elimination) of synaptic connections in diverse cortical regions via dendritic calcium spike-dependent mechanisms.

Optical probing of sleep circuits and functions

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The activity of multiple brain circuits is strongly modulated during sleep states. Some of these are implicated in the temporal control of the sleep-wake cycle, while others support sleep-dependent functions including memory consolidation. In this lecture, I will summarize our recent work investigating the role of REM sleep in stabilizing hypothalamic control of food-related behaviours and its implication for the maintenance of innate behaviour.

Selective sensory gates for sleep regulation in Drosophila

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Sleep disruption is a hallmark of insomnia, severely diminishes sleep quality and is linked to depression and deficits in attention and learning. To maintain sleep, neural networks in our brains create selective sensory “gates” that block sensory information while we sleep. Since sleep is a phenomenon widely conserved throughout the animal kingdom, it is likely that all animals share similar strategies to filter sensory information. However, the fundamental neurophysiological principles and neural interactions behind the creation of selective sensory gates for sleep regulation are essentially unknown.

During sleep in mammals, reptiles and even in Zebrafish, neural networks undergo large-scale synchronizations of slow-wave oscillatory activity. Interestingly, cognitive impairments in sleep deprived but awake mammals have been linked to local synchronization of slow-wave oscillations (SWO) in specific neural networks, indicating a role for SWO in shutting down sensory processing in specific neural networks and signaling the need for sleep. I recently lead a study, in which we used genetically encoded voltage indicators (GEVIs) to discover that even in the evolutionary distant fruit fly Drosophila melanogaster electrical SWO play an important role in the regulation of sleep drive and sensory gating. We found that sleep drive mediating R5 neurons synchronize their electrical patterns to generate network-specific SWO that facilitate consolidated sleep phases. Our findings therefore suggest that SWO might be an evolutionary optimized strategy to block sensory information and provide consolidated sleep. However, the underlying neural interactions and neurophysiological principles that could explain how SWO create selective sensory gates remain unclear.

In Drosophila, the less complex brain, unprecedented genetic accessibility and the sleep-related physiological analogies of the neural networks involved in sleep offer a unique opportunity to study and understand the essential components needed to construct selective sensory gates. I here focus on a sleep-regulating recurrent circuitry composed of R5 neurons, Helicon cells and the dorsal fan shaped body to investigate how synaptic interactions between these neurons integrate circadian and homeostatic sleep drive to synchronize neural activity patterns and provide the neurophysiological framework to construct sensory gates.

Symposium

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S26-3 The ancestry of neurosecretory vesicles
Ronja Alica Angelika Göhde, Benjamin Naumann, Pawel Burkhardt

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Synaptic vesicle cycling defects in epilepsy

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Genes coding for synaptic proteins are emerging as a class of genes mutated in a spectrum of epilepsy diseases, ranging from mild epilepsy to severe epileptic encephalopathy with or without comorbid pathologies as intellectual disability or autism. Among synaptic genes mutated in genetic epilepsies are regulators of synaptic vesicle (SV) cycling, but how defective SV trafficking results in hyperexcitability and epileptogenesis is far from being elucidated. By modelling pathogenic mutations for the synaptic genes TBC1D24, ATP6V1A and DMXL2 in in vitro murine and human-derived neurons, as well as in in vivo mouse models, we revealed complex roles of these synaptic proteins in regulating neuronal development, synapse formation together with SV cycling and neurotransmission. Interestingly the above-mentioned genes resulted also involved in the maintenance of intracellular pH homeostasis and regulation of autophagy process, which are known to play fundamental roles at the synapse in neurotransmitter loading and SV protein degradation. Distinct functions of these genes in developing and mature neurons and their relation with the spectrum of epileptic phenotype associated with human mutations is discussed.

A relation between synaptic vesicle recycling and vesicle ageing

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Synaptic transmission, which relies on the precise release of neurotransmitters from a handful of organelles at any one time, could be severely disrupted by aged and damaged vesicles. This suggests that neurons should have mechanisms in place that either block aged vesicle from release, or promote the release of young vesicles. At the same time, synaptic vesicles can be broadly separated into (i) an active recycling pool, which includes the readily releasable vesicles that are docked at the release sites, and (ii) an inactive reserve pool that participates little in release under most stimulation conditions. It is therefore tempting to hypothesize that the reserve pool represents an aged vesicle population. This leads to related questions, as how are vesicles targeted for degradation and/or for inactivation? How long do they recycle in neurons, before being targeted for degradation?

We set out to answer these questions in hippocampal cultured neurons, by studying the age of the proteins used by synaptic vesicles during recycling. We relied on a large number of fluorescence imaging assays, along with non-optical nanoSIMS imaging. We found that the recycling vesicle proteins were metabolically younger than those of non-recycling reserve vesicles. Vesicle proteins such as Synaptotagmin 1, VGAT and VAMP2/Synaptobrevin 2 were preferentially used in their young age, and were removed from functional reactions after ~12-24 hours. They participated in roughly ~200 vesicle recycling events during their activity period. The vesicles then became contaminated with the plasma membrane protein SNAP25, which inhibited their priming, and rendered the old vesicles less able to fuse than young vesicles. The old vesicles remained in the synapse 1–2 days before their eventual degradation, which may have been in part triggered by the SNAP25 contamination. Before degradation, the old vesicles spent their time in an inactive vesicle pool that has the hallmarks of the reserve pool.

This suggests that a mechanism based on SNAP25 and related proteins separates old and young vesicles, and ensures that neurons use preferentially the latter in neurotransmitter release.

The ancestry of neurosecretory vesicles

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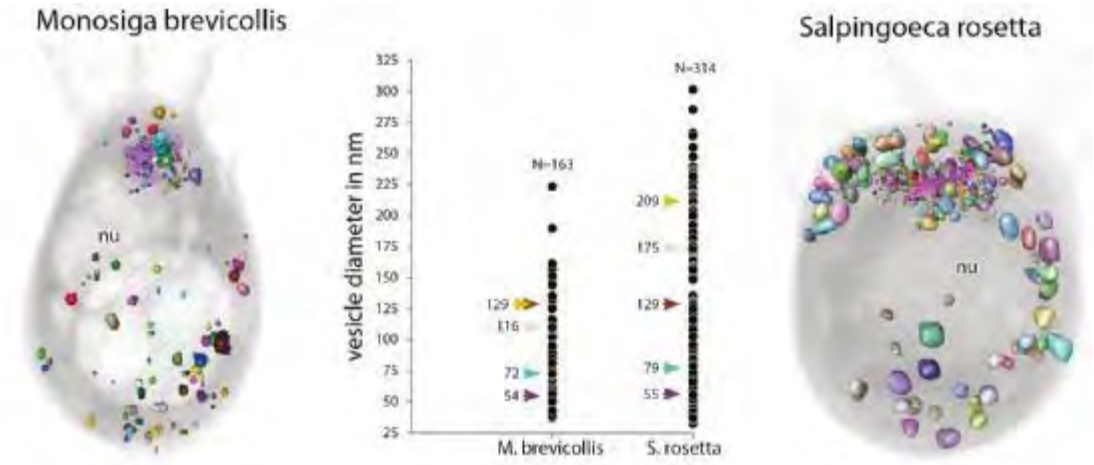
Neurosecretory vesicles mediate the release of neurotransmitters at presynaptic nerve endings and are therefore crucial for animal cell-signalling. Although neurons exhibit a great diversity in terms of their anatomy and function in animals, the protein composition of neurosecretory vesicles is remarkably similar in bilaterians. This characteristic hints towards a common evolutionary origin. Homologues of many key neurosecretory vesicle proteins are even present in organisms outside of the animal kingdom and consequently seem to predate not only neurons, but also the origin of the first animals. However, the molecular composition of neurosecretory vesicles in non-bilaterian animals and their closest unicellular relatives remains elusive, making it extremely difficult to draw conclusions about their evolutionary origin. Our comparative analysis of a set of 28 core neurosecretory vesicle proteins in a total of 13 different species demonstrated that the majority of the assessed proteins is present in unicellular organisms, like in choanoflagellates, the closest unicellular relatives of animals. One of the neurosecretory vesicle protein homologues present in choanoflagellates is the vesicle-localized SNARE protein synaptobrevin. We therefore chose synaptobrevin as a suitable marker for the presence of putative secretory vesicles in choanoflagellates. Immunostainings using an antibody raised against synaptobrevin indicate the presence of secretory vesicles at the apical and basal pole of the choanoflagellate *Salpingoeca rosetta*. Further examinations using 3D reconstructions revealed diverse vesicular landscapes in the choanoflagellate species *Salpingoeca rosetta* and *Monosiga brevicollis*. Strikingly, the presence of synaptobrevin and intracellular vesicles localized in close proximity to the poles in *S. rosetta* are characteristics that are also shared by synapses, which secrete the content of neurosecretory vesicles into the synaptic cleft. Our study sheds light on the ancestral molecular machinery of neurosecretory vesicles and provides a framework to understand the origin and evolution of secretory cells, synapses, and neurons.



Axonal transport and presynaptic targeting of clathrin packets.

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Clathrin has established roles in endocytosis, with clathrin-cages enclosing membrane infoldings, followed by rapid disassembly and reuse of monomers. However, in neurons, clathrin synthesized in cell-bodies is conveyed into axons and synapses via slow axonal transport; as shown by classic pulse-chase radiolabeling. What is the cargo-structure, and mechanisms underlying transport and presynaptic-targeting of clathrin? What is the precise organization at synapses? Combining live-imaging, mass-spectrometry (MS), Apex-labeled EM-tomography and super-resolution, we found that unlike dendrites where clathrin transiently assembles/disassembles as expected, axons contain stable ‘transport-packets’ that move intermittently with an anterograde bias; with actin/myosin-VI as putative tethers. Transport-packets are unrelated to endocytosis, and the overall kinetics generate a slow biased flow of axonal clathrin. Synapses have integer-numbers of clathrin-packets circumferentially abutting the synaptic-vesicle cluster, advocating a model where delivery of clathrin-packets by slow axonal transport generates a radial organization of clathrin at synapses. Our experiments reveal novel trafficking mechanisms, and an unexpected nanoscale organization of synaptic clathrin.

Regulation of synaptic vesicle recycling by APP/Aβ-derived fragments

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Amyloid beta (Aβ) is well-known for its link to the pathology of Alzheimer's disease (AD). However, Aβ is produced in large amounts also in healthy brains and an interference with its production affect normal cognition. How the physiological Aβ contributes to the brain function remains insufficiently explored. It has been proposed that physiological Aβ enhances neuroplasticity and memory formation by increasing the neurotransmitter release from presynapse. Experiments in cultured neurons and in acute brain slices demonstrated that physiological Aβ controls recycling of synaptic vesicles (SVs). Our recent work showed that regulation of SV recycling requires alpha7 nicotinic acetylcholine receptors (α7nAChRs) and involves CDK5 and calcineurin signalling. In the current work, we dissected effects of Aβ1-42 and its N- and C-terminal fragments on SV recycling. We showed that Aβ1-42 and Aβ1-16, but not Aβ17-42, increased size of the recycling pool of synaptic vesicles at glutamatergic synapses. This presynaptic effect relied on an enhancement of endogenous cholinergic signalling via α7nAChRs. Modulation of α7nAChRs by Aβ induced changes in Ca2+-dependent phosphorylation of synapsin1 and consequently, it led to a reorganization of functional SV pools so that their availability for sustained neurotransmission increased. Together, our results identify synapsin1 as a molecular target of Aβ and reveal a role of physiological Aβ in fine-tuning of cholinergic modulation of glutamatergic neurotransmission. These findings provide new perspectives for understanding of cholinergic dysfunction observed in AD.

Amyloid beta is a mediator of homeostatic synaptic plasticity

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The amyloid precursor protein (APP) and its fragments produced by proteolytic processing have been extensively studied during the last two decades because of their involvement in Alzheimer's disease. Studies employing mice deficient for APP have uncovered impairments in long term potentiation (LTP), i.e., Hebbian plasticity. These impairments can be rescued by application of APPsα, the major product of the non-amyloidogenic processing pathway of APP. In this study, we investigated the relevance of APP and its cleavage products in homeostatic synaptic plasticity. Entorhino-hippocampal tissue cultures were prepared from APP-deficient mice and single-cell patch-clamp recordings of dentate gyrus granule cells were performed. This revealed that APP-deficient granule cells are unable to homeostatically adjust their excitatory synaptic inputs after tetrodotoxin (TTX) -induced activity blockade, while the structural and functional properties of the APP-deficient granule cells were not significantly different compared to wild type. Addition of APPsα to the tissue cultures, using different molecular, viral, and genetic approaches, did not rescue the ability of APP-deficient granule cells to express homeostatic synaptic plasticity, indicating that processing of APP through the non-amyloidogenic pathway promotes Hebbian but not homeostatic synaptic plasticity. We then tested the relevance of the amyloidogenic processing pathway, i.e. Aβ, in homeostatic synaptic plasticity. Indeed, co-application of TTX and Aβ in APP-deficient tissue cultures rescued the ability of dentate granule cells to homeostatically adjust their excitatory synapses. Furthermore, inhibiting the production of Aβ with β- and γ- secretase inhibitors blocked the homeostatic response to the TTX treatment in wild type tissue cultures. This effect of Aβ seemed to depend on interactions with intracellular Ca^{2+}-regulating and sensing molecules, in particular the actin-associated protein synaptopodin, which is a marker and essential component of the Ca^{2+}-storing spine apparatus organelle. Together, these results demonstrate that Aβ is a mediator of homeostatic synaptic plasticity thus providing evidence for a physiological function of Aβ in synaptic plasticity. Supported by DFG.

Symposium

S27: Sound processing, adaptation, and perception in the auditory system - From midbrain to cortical networks

S27-1 Auditory contrast adaptation: from neural circuits to perception
Andrew J. King, Michael Lohse, Victoria M Bajo, Ben DB Willmore

S27-2 A neuronal correlate for predictive coding? Emergence of deviance detection along the auditory neuroaxis and beyond
Manuel S. Malmierca, Guillermo V. Carbajal, Lorena Casado-Roman, David Pérez-González

S27-3 Modulation of spatial tuning of auditory cortex neurons during active sensing
Michael Pecka, Diana Amaro, Dardo N. Ferreiro, Benedikt Grothe

S27-4 Enabling good spatial hearing after early hearing loss:
The role of precise interaural timing
Nicole Rosskothen-Kuhl, Alexa N Buck, Theresa A Preyer, Sarah Buchholz, Jan W Schnupp

S27-5 Effect of hearing experience on interarual time difference sensitivity and the implications for behaviour
Alexa Nadezhda Buck, Nicole Rosskothen-Kuhl, Jan Schnupp

S27-6 Auditory corticocollicular neurons: tuning, tonotopy and complex sound processing
Tatjana Schmitt, Simon L Wadle, Jan J Hirtz

Auditory contrast adaptation: from neural circuits to perception

Andrew J. King[1], Michael Lohse[1], Victoria M Bajo[1], Ben DB Willmore[1]

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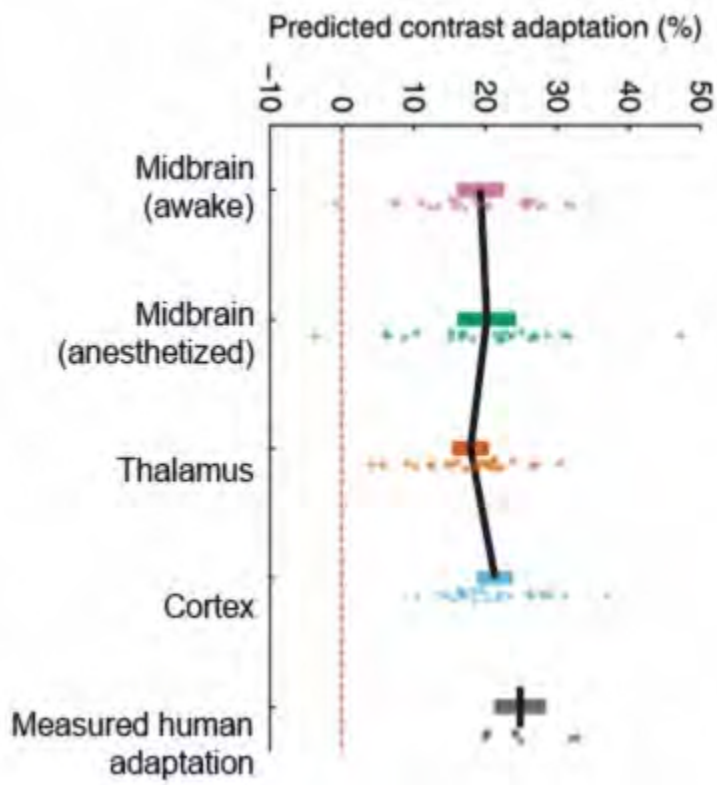
Auditory neurons cope with constantly changing acoustic environments by adapting their responses to match the statistics of the sounds that are heard. For example, the dynamic range – the range of stimulus values encoded by a neuron by a change in its firing rate – can shift to compensate for changes in mean stimulus level, thereby maintaining maximum sensitivity over the most commonly encountered values. Similarly, neurons can scale their response gain to compensate for changes in stimulus contrast – the variance in the sound level distribution – which helps to generate noise-tolerant representations of sounds in the brain. Although adaptation to sound statistics has been observed throughout the auditory system, contrast gain control is thought to be primarily a property of neural circuits in the auditory cortex and recent studies have investigated the role of inhibitory interneurons in this process (Cooke et al., 2020, J Neurophysiology 123:1536-1551). Surprisingly, we found that neurons in the auditory thalamus and midbrain of mice show equally robust contrast gain control, and that this is implemented independently of cortical activity (Lohse et al., 2020, Nature Communications 11:324). However, adaptation time constants become longer at later stages of the processing hierarchy, resulting in progressively more stable representations. We also found that auditory discrimination thresholds in human listeners compensate for changes in contrast, and that the strength of this perceptual adaptation can be predicted from the contrast gain control exhibited by neurons at both subcortical and cortical levels in mice (Figure 1). Our results therefore show that contrast adaptation is a robust property of neurons in both the subcortical and cortical auditory system and accounts for the short-term adaptability of human perceptual judgments. Furthermore, these findings have implications for modelling the auditory system since incorporating subcortical adaptation improves the capacity of standard spectrotemporal models to describe the response properties of auditory cortical neurons.


Predicted contrast adaptation (%)
−10
0
10
20
30
40
50
Midbrain (awake)
Midbrain (anesthetized)
Thalamus
Cortex
Measured human adaptation

A neuronal correlate for predictive coding? Emergence of deviance detection along the auditory neuroaxis and beyond

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Stimulus-specific adaptation (SSA) is the reduction in the responses to a common sound relative to the same sound when rare. It was originally described in the primary auditory cortex (AC) as the neuronal correlate of the mismatch negativity (MMN), an important component of the auditory event-related potentials that is elicited by changes in the auditory environment. However, the relationship between SSA and the MMN is still a subject of debate. The MMN is a mid-late potential (~150-200 ms in humans), and its neural sources have been located mainly within non-primary auditory cortex in humans and animal models. Moreover, SSA is also present as early as in the auditory midbrain and thalamus (IC and MGB).
In this talk, I will show our recent findings on recordings from single neurons in the IC, MGB, AC and beyond of anaesthetized rats and awake mouse to an oddball paradigm similar to that used for MMN studies. Our data demonstrate that most neurons in the non-lemnical divisions of the auditory brain show strong SSA and that there is a hierarchical emergence of prediction error signals along the central auditory system. Recordings from prefrontal cortex show that neurons exhibit the highest degree of prediction error along the auditory hierarchy. We have also observed that acetylcholine and dopamine play a role in shaping the precision of prediction erros by differently affecting the response to the standard or deviant tones sounds only in IC or AC, respectively.
Taken together our results unify three coexisting views of perceptual deviance detection at different levels of description: neuronal physiology, cognitive neuroscience and the theoretical predictive coding framework.

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Modulation of spatial tuning of auditory cortex neurons during active sensing

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Information about the position of auditory objects is vital to navigate natural scenes. Moreover, attributing appropriate behavioral relevance to distinct objects is essential for survival. Auditory neurons compute information about the position of a sound source via head-angle specific differences of sound features between the ears. From brainstem to primary auditory cortex (A1), a predominance of broad neuronal tuning to ipsi- or contralateral head-to-source angles has been reported1,2,3. Accordingly, spatial tuning is assumed egocentric. Nevertheless, most studies on spatial tuning to date were conducted in either anesthetized or head-fixed and passively listening animals, while restricting or omitting active engagement and thus lack fundamental aspects of real-life localization behavior that may crucially alter the nature of spatial coding:

First, A1 activity is strongly modulated by the behavioral context4,5,6. Task engagement improves signal detection7 and sharpens spatial tuning8. Second, perceived self-motion can reveal novel neuronal representations9. Third, free exploration constantly alters the head-to-source angles and thus fundamentally impacts the egocentric representation of sound source positions10. However, the nature of auditory spatial coding under realistic listening conditions during active localization of a behaviorally meaningful sound source remains undetermined.

We used the Sensory Island Task (SIT) paradigm11 and trained Mongolian gerbils to report whenever a sound presentation of a pulsed harmonic stack switched from a “background” loudspeaker to a “target” loudspeaker (separated by 180° in a circular arena). The source change was triggered whenever gerbils entered a specific target area in the arena (the “island”), whose position was randomized across trials. The animals were trained to freely explore the arena in search of the island and report the detection of the change by remaining within the island to receive a food reward. The animals were implanted with custom-made tetrodes to allow chronic recordings of action potentials from neurons in A1 and assessing their spatial tuning.

Remarkably, the spatial tuning of a large fraction of the neurons was sensitive to the task-specific identity of the sound source, as their tuning differed for the two loudspeakers. These neurons were either only significantly tuned to one of the two sound sources or exhibited significantly different tuning angle for the two sources (difference > 90°). Notably, in a sizable fraction of neurons that classified as spatially un-tuned to either loudspeaker, the response magnitude differed significantly between the two loudspeakers, indicative of source angle independent identity coding. Use of an artificial neural network model revealed that these diverse activity patterns incorporated significant information not only for the head-to-source angle but also in identifying the sound source irrespective of the head-to-source angle. Thus, the observed neuronal response patterns gave rise to an interlaced egocentric and allocentric population code of active sound source localization during active sensing. Together, our findings may provide a potential mechanism for the selective tracking of a specific sound source in the environment during self-motion.

Enabling good spatial hearing after early hearing loss: The role of precise interaural timing

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The central question of this talk is how we can improve spatial hearing in early deafened patients. In particular, we seek to understand why such patients are often insensitive to ITDs even if they are fitted with bilateral cochlear implants (CIs) at a young age. It is widely suspected that this issue arises at least in part from lack of sensory input during early development (the “critical period hypothesis”), we suspect the reason lies in the missing synchronization of today´s speech processors. In fact, current CI processors are unable to synchronize the timing of their stimulus pulses binaurally, resulting in inconsistent and therefore uninformative delivery of ITD cues to the electrically stimulated auditory system. This might prevent binaural CI recipients from learning to use ITDs normally even if there was no strong critical period causing permanent deficits by the time the devices are fitted.

We decided to investigate the critical period hypothesis by testing early deafened, adult cochlear implanted rats using a new behavioral setup. These rats experienced a period of profound auditory deprivation throughout their early development, but they were nevertheless able to learn to perform ITD lateralization tasks with very high sensitivity exhibiting thresholds of around 50 µs, no worse than their normally hearing litter mates. Good ITD sensitivity can therefore be developed even in the absence of early sensory input if the bilateral CIs provide useful ITD cues from right after implantation. In electrophysiological experiments we could furthermore show that IC neurons of the hearing inexperienced system are highly ITD sensitive from immediately after implantation. Based on this result, we propose that, at least in rats, there is no “strong” critical period for the development of ITD sensitivity, and we now investigate to what extent varying, uninformative presentation of ITDs interferes with the development of ITD sensitivity in hearing inexperienced CI rats.

Our behavioral studies in neonatally deafened, adult CI-supplied rats showed that ITDs were only used for sound lateralization if the animals were presented with informative ITDs from the beginning of stimulation. In contrast, when animals were exposed to stimulation with varying, uninformative ITDs, mimicking the stimulation which patients fitted with asynchronous CIs would experience, the animals did not learn to use ITD cues for lateralization.
Overall, our results suggest that ITD sensitivity appears to be little affected by the absence of early auditory experience in very early life but may be perturbed by uninformative ITD cues provided by unsynchronised CI stimulation after binaural CIs are fitted.

Effect of hearing experience on interarual time difference sensitivity and the implications for behaviour

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While cochlear implants (CIs) can restore substantial amounts of functional hearing in deaf patients, binaural hearing, and in particular, the perception of interaural time differences (ITDs) with current CIs is notoriously poor, especially if they suffer from early hearing loss. One popular hypothesis posits that a lack of early binaural experience during a developmental period may be a principal cause for the poor ITD perception in pre-lingually deaf CI users, and previous electrophysiological studies on neonatally deaf CI-supplied animals have reported reduced ITD sensitivity. However, we recently demonstrated that neonatally deafened CI rats can quickly learn to discriminate microsecond ITDs under optimised stimulation conditions. Here, we characterised ITD sensitivity and tuning of neurons in the inferior colliculus (IC) under bilateral CI stimulation in neonatally deafened (n= 4) and hearing experienced (n=4) rats.

Both groups showed comparably large proportions of ITD sensitive mulit-units in the IC (Deaf: 82.46%, Hearing: 84.81%), and the strength of ITD tuning, quantified as mutual information between response and stimulus ITD, was independent of hearing experience.

Importantly the shapes of tuning curves did differ substantially with respect to hearing experience. We observed three main classes of tuning curves overall: contralateral, central, or ipsilateral peaks. We found that, while the vast majority of multi-units for hearing experienced rats showed almost exclusively a contralateral and ipsilateral tuning. In contrast multi-units of neonatally deafened rats showed central tuning in 50% of the units and only half the number of contralaterally tuned units compared to hearing experienced animals. In addition, we related these tuning curve shapes within the ITD physiological range to a potential behavioural mechanism we have shown that although tuning curve shapes may vary with hearing experience the neonatally deafened animals still show significant amounts of lateralisation in their neural responses. This should, at least in theory, be enough to allow them to at least learn to use ITD cues in a behavioural task.

These results indicate that, at least in rats, substantial amounts of highly precise, “innate” ITD sensitivity can be found even in the absence of hearing experience. Furthermore, while tuning curve shapes appear to be strongly influenced by auditory experience the neural lateralisation ability suggests that, even with altered tuning shapes, behavioural ITD localization should be possible which implies that in fact there is no ITD developmental critical period.

Auditory corticocollicular neurons: tuning, tonotopy and complex sound processing

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The auditory cortex (AC) modulates the activity of upstream pathways in the auditory brainstem via descending (corticofugal) projections. This feedback system plays an important role in the plasticity of the auditory system by shaping response properties of neurons in many subcortical nuclei. Around half of layer 5 (L5) neurons are corticofugal, of which the majority projects to the inferior colliculus (IC). This pathway is involved in processing of complex sounds, auditory-related learning and defense behavior. Partly due to their location in deep cortical layers (500 – 800 µm), population activity patterns of corticofugal neurons within neuronal ensembles of the AC remain poorly understood. We employed two-photon imaging to record the activity of hundreds of L5 neurons simultaneously in anesthetized mice (postnatal day 50-70). To focus on corticocollicular neurons, we injected rAAV2-retro (Tervo et al. 2016. A Designer AAV Variant Permits Efficient Retrograde Access to Projection Neurons. Neuron. 92: 372–382) into the mouse IC. Using this approach, we expressed GCaMP7f exclusively in corticocollicular neurons and presented different acoustic stimulation paradigms to investigate tuning, tonotopy and neuronal ensemble activity. In addition, we mapped the AC using widefield imaging to distinguish between different subfields containing corticocollicular neurons. In a further set of experiments, we expressed GCaMP7f in AC, either exclusively in pyramidal neurons or indiscriminately of neuron subtype, to investigate tuning and tonotopy or complex sound processing, respectively. By additionally injecting rAAV2-retro-tdTomato into the IC we could image activity of L5 neurons in general, but were also able to identify the subpopulation of corticocollicular neurons and analyze their specific response properties. We could show that approximately a quarter of L5 pyramidal neurons exhibit a clear frequency tuning. These neurons displayed either single- or double-peak tuning, with the majority being single-peaked with a bandwidth of 0.7 octaves.
Corticocollicular neurons do not differ from other pyramidal neurons regarding their tuning properties.
Further investigation of corticocollicular neuronal ensemble activity during different sound stimulation protocols will contribute to the understanding of the physiology and function of the auditory corticofugal system.

Symposium

S28: FAIR data management and data sharing in neuroscience

S28-1 Let’s be FAIR about FAIR: Where are we and where can we go
Maryann E. Martone

S28-2 OpenScope: a shared observatory for neuroscience
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S28-4 Approaches for improving rigor and efficiency in sharing complex neurophysiological data
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S28-6 Educating and training for a FAIR future
Adina Svenja Wagner

Let’s be FAIR about FAIR: Where are we and where can we go

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Since the FAIR data principles were introduced in 2016, they have become the defacto aspirational standard for scientific data. Major funders and scientific projects in Europe and the US have embraced making data Findable, Accessible, Interoperable and Reusable. Who wouldn’t want to be FAIR? The question now is how? While many are familiar with the meaning of the acronym, fewer are as familiar with the 15 specific recommendations of the FAIR principles, which lay out guidelines for achieving FAIR. These recommendations lay out both specific recommendations, e.g., all data should be accessible via a persistent identifier, and also general recommendations such as data being adhering to community standards and being accompanied by a “plurality of relevant attributes.” Thus, FAIR delegates a certain amount of interpretation to individual research communities. Thus, although FAIR is rightly concerned with technical infrastructure and requirements, interpretation and implementation also require human and community infrastructure if they are to be successful. In this presentation, I will introduce the FAIR principles and consider the current state of FAIR in neuroscience from both the technical and human infrastructure side. I will introduce programs the International Neuroinformatics Coordinating Facility's efforts to develop programs in support of open and FAIR neuroscience. I will describe efforts underway in several large US-based projects to achieve FAIR including the BRAIN Initiative Cell Census Network (BICCN), Stimulating Peripheral Activity to Relieve Conditions (SPARC), the Open Data Commons for Spinal Cord Injury and the ReproNIM project for reproducible neuroimaging.

OpenScope: a shared observatory for neuroscience

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Shared astronomy observatories have resulted in major insights about the physical universe that could not have been achieved by individual researchers. Despite the unquestionable success of this shared model in the field of astronomy, such shared observatories have been missing in the field of neuroscience. OpenScope [1] is the first neuroscience observatory that is exploring whether this shared observatory model can also lead to major findings in the field of systems neuroscience.

The OpenScope project is based on the Allen Brain Observatory [2] located at the Allen Institute for Brain Science [3]. The Allen Brain Observatory is a two-photon calcium imaging platform that allows standardized high-throughput in vivo experiments to study neuronal activity in the mouse visual cortex. The Allen Brain Observatory has already produced very large and open datasets of visually evoked activity across different cortical areas and layers. Now, in OpenScope, researchers external to the Allen Institute can propose projects that are run on this experimental platform by optical imaging experts at the Allen Institute. The resulting data of those experiments is made freely available to the researchers and at a later stage also to the rest of the community. This shared observatory model of OpenScope thus allows researchers from across the world and from different neuroscience disciplines, e.g. computational neuroscience, to test hypotheses and ideas and thereby has the potential to reveal fundamental insights about how the brain works.

Together with Lucy Palmer (University of Melbourne), Richard Naud (University of Ottawa), and Daniel Millman and Saskia de Vries (Allen Institute for Brain Science), we are currently running such a collaborative project on the OpenScope observatory. Our project aims at investigating the role of feedback from higher visual areas onto sensory processing in the primary visual cortex in the mouse. The data collection by the OpenScope team has been finished and we are currently in the analysis phase of the project.

In this talk, I will present OpenScope and introduce the concept behind the first shared observatory for neuroscience. Furthermore, by showing examples from our own project, I will provide insights into this novel model of conducting systems neuroscience research.

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Exploring high-level human cognition with studyforrest.org

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The studyforrest.org project aims to foster collaborative open science projects investigating the brain's behavior and neural representations under quasi natural stimulation conditions. After the initial data acquisition in 2013 and the first release in 2014, it has grown into a versatile and unique resource with multiple follow-up data releases, more than two dozen published articles, and more than a dozen published studies employing this dataset that were conducted by independent groups (so far).
This talk will briefly summarize the goals of the project and present conclusions on the benefits and disappointments of this open science effort, as they appear five years after its start. In particular, the talk will introduce a data management software solution, DataLad (http://datalad.org) that was developed based on lessons learned from the studyforrest.org project. The talk argues that this tool provides unique features that benefit any research effort (large or small, collaborative or not) that needs to break the boundaries of time, individual labs, research domains or field of expertise.

Approaches for improving rigor and efficiency in sharing complex neurophysiological data

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Sharing complex neurophysiological data, e.g. in a collaboration or in a publication, in such a way that they can be understood and used is a challenge. Issues to be solved are to define adequate metadata representations to describe the experiment, find standardized formats to store the data, or to capture the details of the data preprocessing workflows. For most of these challenges easy or generic solutions do not yet exist that could be used “out of the box”. Where solutions do exist, the knowledge and expertise on how to employ them in the context of a particular experiment is often scattered in the community. In consequence, the implementation of a research data management strategy in an experimental laboratory is often a balance act between ensuring a certain level of rigor, reproducibility and documentation on the one hand, and efficiency (in terms of time and personnel) of designing and implementing strategies on the other hand.

Moreover, the results of this balancing act may turn out to be frustrating: even with comparatively high investment in improving the quality of data and metadata descriptions and data curation process, the result will often be disappointing in the sense that the benefits associated with good research data management are not realized. This holds in particular when a lack of standards for data formats, structures, and common vocabulary prevents the curated dataset to be easily (e.g., automatically) used by other scientists, analyzed by existing tools, or integrated into data stores. Thus, despite the efforts, the data remain difficult to use and comprehend. Moreover, the solutions for data management developed on a project-by-project basis, while based on promising ideas, are rarely developed to a level of stability and generality required for standardization, and tend to be lost after the project’s lifetime.

To overcome this unsatisfactory situation, we here argue for the need to promote the level of standardization as a community effort. To this end, we analyze the process of data acquisition and preprocessing of electrophysiological data [1,2]. This example includes a number of aspects that tend to complicate the data curation process, such as a data acquisition process that spans over multiple years of a running experiment, or the need for collaboration across labs. We describe the steps that we required to accomplish the goal of curating data to a degree that they become sharable and publishable in sufficient detail to ensure easy and unsupervised reuse in data analysis workflows [3]. In this endeavor, we consider conceptual considerations underlying the design of the data acquisition and curation process and highlight the standardization strategies and tools (e.g., [4-7]) that can help in reducing the effort of handling the data. Some of these tools are coordinated and harmonized as part of the EBRAINS e-infrastructure for neuroscience [8], which is committed to contribute and shape a layer of interoperable compute and data services for future neuroscience. Likewise we will outline the needs where complementary standardization is still non-adequate or non-existent, and suggest how community efforts coordinated by the NFDI-Neuro consortium [9] could address, seed, and drive towards concrete solutions. Getting into a habit of treating the design of data management for new experiments early on in harmony with community standards and recommendations will give scientists the opportunity to spend more time analyzing the wealth of electrophysiological data they leverage with low-barrier collaborations, rather than dealing with data formats and data integrity.

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Limited replicability and evidence for publication bias within the memory reconsolidation field

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Research on memory reconsolidation has been booming in the last two decades, with numerous high-impact publications reporting promising amnestic interventions in rodents and humans. Such findings have inspired the formulation of theories and clinical applications for the treatment of phobias and PTSD. However, our own extensive series of failed (exact) replication attempts of reactivation-dependent amnesia for fear memories in both rats and humans suggests that such amnestic effects are not readily found and that they depend on subtle and possibly uncontrollable parameters. Those null findings, although corroborated by personal communication with experts in the field, were in stark contrast with the published literature, which contains a plethora of significant amnestic effects and hardly any negative results. This discrepancy led us to formally investigate publication bias in a preregistered study (https://osf.io/apu9t).

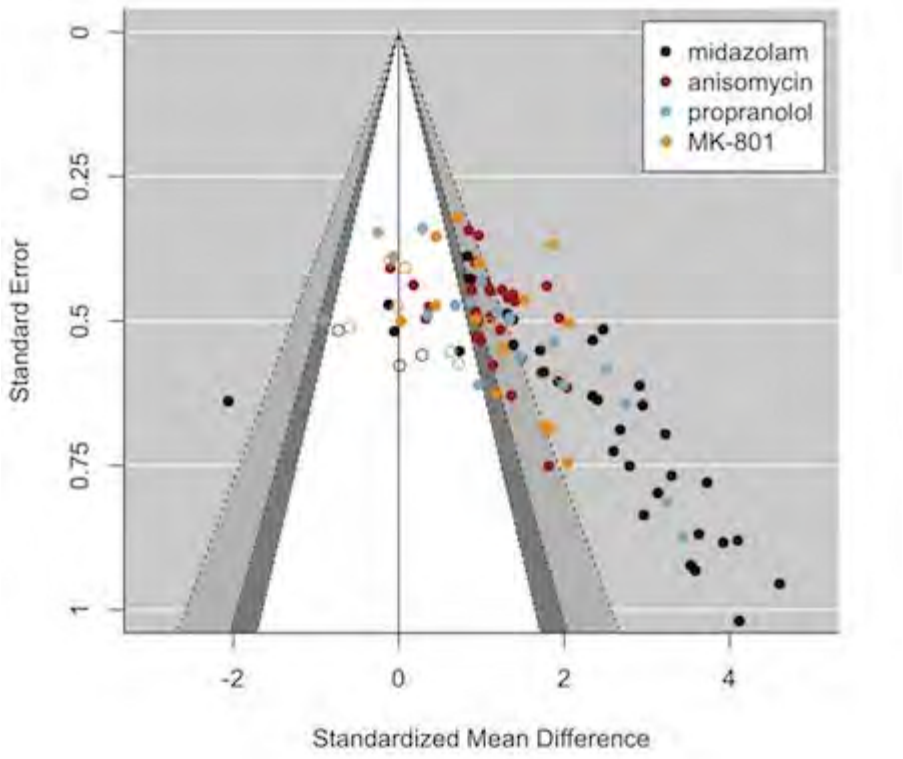
More specifically, we aimed to gauge the presence of publication bias in a well-delineated part of the reconsolidation literature. To this end, we performed a systematic review of the literature on reactivation-dependent amnesia for contextual fear memories in rodents, followed by a statistical assessment of publication bias in this sample. In addition, we contacted relevant researchers for unpublished results, which were included in our analyses.

The contour-enhanced funnel plot below includes 95 identified published studies using one of the most commonly used amnestic drugs in rodents (midazolam, anisomycin, proporanolol or MK-801, filled circles). Published studies are mainly located in the region of statistical significance (to the right of the white area) and are most densely plotted at the border of statistical significance. Those observations, in combination with the asymmetrical funnel shape that was confirmed statistically by Egger's regression ($p < .001$ when considering the moderating influence of drug and research group), suggest the presence of publication bias. In contrast to published results, studies that remained unpublished (empty circles) showed smaller, and mostly non-significant, amnestic effects. This contrast between published and unpublished results further supports the presence of publication bias. To conclude, the obtained results suggest that the current literature is biased, providing a misleading overall estimate of the size and replicability of amnestic effects.

The mixed successes of clinical studies may be in line with this conclusion, rather than reflecting translational issues. In addition, the presence of bias is not only problematic for clinical application, but can misinform researchers when deciding on how to optimally invest their resources. Finally, the outcomes of

our replication studies suggest that current mechanistic theories that are commonly used to explain reactivation-dependent amnesia, such as reconsolidation or state-dependency, are at least incomplete, as they cannot predict when amnestic effects will occur.

We propose that replication, non-biased publication of obtained results, and the adoption of open science practices are essential for the reconsolidation field (and empirical science in general) to move forward. Apart from sharing our experiences with performing exact and conceptual replication studies and investigating publication bias, I will present a set of tools that offer opportunities to increase transparency, reproducibility and credibility of research findings.



Educating and training for a FAIR future

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With a growing awareness of the role of sample size and replicable results (Button et al., 2013; Turner et al., 2018), a rise of platforms, tools, and standards that aim to facilitate data sharing and management (Wiener et al., 2016), unprecedented sample sizes (e.g., UKBiobank; Bzdok & Yeo, 2017), and increasingly complex data analyses (e.g, Glasser et al., 2013; Alfaro-Almagro et al., 2018), research data management (RDM) is essential to put open and FAIR neuroimaging research into effect. But just as FAIRness and RDM can not be an afterthought in any given scientific project, they also shouldn't be an afterthought in the training and education of current and future generations of neuroscientists. This training has to fulfill the demands of different stakeholders in science: 1) Researchers, that apply RDM in their scientific projects, 2) PIs and similar personnel with management tasks, that need to set out and justify plans for the implementation of RDM and FAIR principles, and trainers, such as librarians or data managers, that educate users on tools and practices for FAIR science (Fothergill et al., 2019, Grisham et al., 2016). Researchers of any career level and of any background need accessible tutorial-like educational content and documentation for relevant tools and concepts to apply FAIR RDM from the get go. Planners need high-level, non-technical information in order to make informed yet efficient decisions on whether a tool fulfils their needs. And trainers need reliable, open teaching material.

A user-driven alternative to scientific software documentation by software developers, "Documentation Crowdsourcing", has been successfully employed by the NumPy project (Oliphant, 2006; Pawlik et al., 2015). Extending this concept beyond documentation, we have created the DataLad handbook (handbook.datalad.org) as a free & open-source, user-driven and -focused educational instrument and resource for trainers, users, and planners for (research) data management, independent of their background and skill level (Wagner et al., 2020). Drawing from the experiences of creating more than 400 pages of educational material, with almost 40 independent contributors from around the world, and nearly 2 years of in-person and virtual teaching based on the handbook, I want to highlight the unique challenges of RDM training and as well as its opportunities for the field of neuroscience.

Symposium

S29: Odors and Metabolism - neuromodulation in sensory processing

S29-1 A novel hypothesis on the role of mammalian olfactory bulb granule cells in olfactory coding
Veronica Egger

S29-2 Olfactory control of energy metabolism
Celine Riera

S29-3 Internal state configures olfactory behavior and early sensory processing in *Drosophila* larvae
Katrin Vogt, David M. Zimmerman, Matthias Schlichting, Luis Hernandez-Nunez, Shanshan Qin, Karen Malacon, Michael Rosbash, Cengiz Pehlevan, Albert Cardona, Aravinthan D.T. Samuel

S29-4 Immune receptor signaling and the mushroom body mediate post-ingestion pathogen avoidance
Francisco Jesus Rodriguez Jimenez, Johanna M. Kobler, Irina Petcu, Ilona C. Grunwald Kadow

S29-5 Honeybees fail to discriminate floral scents in a complex learning task after consuming a neonicotinoid pesticide
Geraldine Wright

S29-6 Active smelling in the American cockroach
Antoine Hoffmann, Einat Couzin-Fuchs

A novel hypothesis on the role of mammalian olfactory bulb granule cells in olfactory coding

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Judged by their sheer number and anatomical connectivity, the granule cells of the mammalian olfactory bulb seem placed to provide strong local recurrent and lateral inhibition within and across the principal neurons, the mitral and tufted cells, and thus to substantially modulate bulbar processing. Moreover, granule cells are known to serve as a major gateway for top-down corticofugal control of bulbar activity. However, the role of granule cells in olfactory processing is surrounded by several enigmatic observations, such as the existence of reciprocal spines and the mechanisms for GABA release from them, the missing evidence for functional reciprocal connectivity, and the apparently low inhibitory drive of granule cells, both with respect to recurrent and lateral inhibition. Here, I summarize recent results with regard to GABA release from the reciprocal spines onto mitral cell lateral dendrites, based on two-photon uncaging of glutamate in acute juvenile rat brain slices, simulations and ultrastructural evidence. Most importantly we observed a cooperation between the local postsynaptic spine spike and presynaptic NMDA receptor activation, leading to a novel hypothesis on granule cell function that can resolve most of these enigmas. I predict that granule cells can provide dynamically switched, non-topographical lateral inhibition between coactive glomerular columns and thereby enable olfactory combinatorial coding at the level of the bulb.

Olfactory control of energy metabolism

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Olfactory inputs are important for hedonic evaluation of food, resulting in food choice and possible consumption. The hypothalamus is a well established center integrating internal hormonal signals originating from peripheral organs to adjust energy intake and expenditure. Whether external stimuli, such as olfactory information are processed within the hypothalamus to contribute to energy balance regulation remains unknown. Here, we employ chemogenetic manipulation of olfactory mitral cells to specifically interrogate alterations in hypothalamic activity and whole-body energy metabolism. Using mice genetically endocing the hM3D receptor, we show that acute stimulation of Tbx-21-positive neurons caused by clozapine-n-oxide (CNO) led to a rapid rise in oxygen consumption, heat production, and an increase in brown adipose tissue temperature in females, with no observed changes in activity or food intake. Analysis of thermogenesis related gene expression in brown adipose tissue (BAT) suggests that these changes are due to an increase in creatine metabolism in BAT in females with no changes observed in males. Acute CNO delivery produced c-fos early gene activation in various regions in the brain, including main olfactory bulb, amygdala and several hypothalamic nuclei. In particular, cFos activation was visible in the female dorsomedial hypothalamus, a hypothalamic nucleus playing a role in feeding, thermoregulation and circadian regulation. Taken together, these results indicate that the mammalian brain has developed dedicated neurocircuits to integrate olfactory inputs in the control of energy balance, which present different characteristics in male and female rodents

Internal state configures olfactory behavior and early sensory processing in *Drosophila* larvae

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Animals exhibit different behavioral responses to the same sensory cue depending on their internal state at a given moment. How and where in the brain are sensory inputs combined with state information to select an appropriate behavior? Here, we investigate how food deprivation affects olfactory behavior in *Drosophila* larvae. We find that certain odors repel well-fed animals but attract food-deprived animals and that feeding state flexibly alters neural processing in the first olfactory center, the antennal lobe. Hunger differentially modulates two output pathways required for opposing behavioral responses. Upon food deprivation, attraction-mediating uniglomerular projection neurons show elevated odor-evoked activity, whereas an aversion-mediating multiglomerular projection neuron receives odor-evoked inhibition. The switch between these two pathways is regulated by the lone serotonergic neuron in the antennal lobe, CSD. Our findings demonstrate how flexible behaviors can arise from state-dependent circuit dynamics in an early sensory processing center.

Immune receptor signaling and the mushroom body mediate post-ingestion pathogen avoidance

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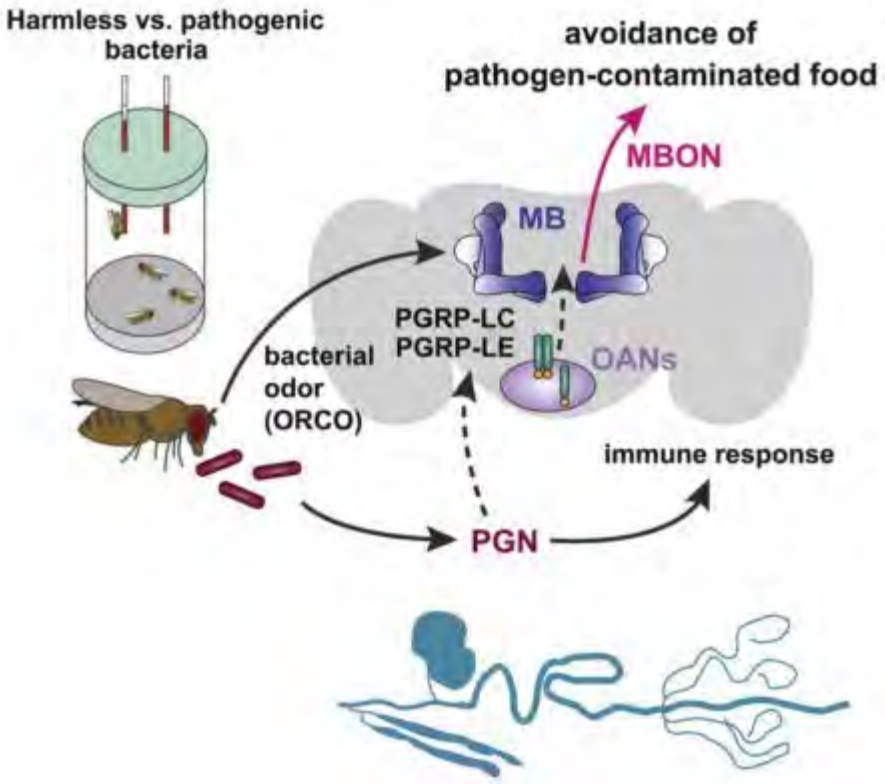
Despite the beneficial effects of bacteria on health, animals are exposed to harmful species, forcing the animals to weigh nutritional benefits against detrimental post-ingestive repercussions and adapt their behavior accordingly. Here, we elucidate how the immune system communicates with the brain, enabling avoidance of harmful food, using *Drosophila melanogaster*. Using two different fly pathogens, *Erwinia carotovora* (*Ecc15*) and *Pseudomonas entomophila* (*Pe*), mildly pathogenic and highly virulent, respectively, we analyzed preference behavior in naive flies and after ingestion of either of these pathogens. Although survival assays demonstrated the harmful effect of pathogen ingestion, naive flies preferred the odor of either pathogen to air and also the harmless mutant bacteria, indicating that these microbes are not inherently aversive to the flies. By contrast, feeding assays showed that, when given a choice between pathogenic and harmless bacteria, flies -after an initial period of indifference- shifted to a preference for the harmless strain, a behavior that lasted for several hours. Removal of the synaptic output of the mushroom body (MB), the fly's brain center for associative memory formation, forced an inability to distinguish between pathogenic and harmless strains, suggesting this to be an adaptive behavior. Interestingly, this behavior relied on the immune receptors PGRP-LC and -LE and their presence in octopaminergic neurons. We postulate a model wherein pathogen ingestion triggers PGRP signaling in octopaminergic neurons, which in turn transmit the information about the harmful food source directly or indirectly to the MB, where an appropriate behavioral output is generated.


Harmless vs. pathogenic bacteria
avoidance of pathogen-contaminated food
MBON
MB
PGRP-LC
PGRP-LE
OANs
bacterial odor (ORCO)
immune response
PGN

Honeybees fail to discriminate floral scents in a complex learning task after consuming a neonicotinoid pesticide

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Neonicotinoids are pesticides used to protect crops but with known secondary influences at sublethal doses on bees. Honeybees use their sense of smell to identify the queen and nestmates, to signal danger and to distinguish flowers during foraging. Few behavioural studies to date have examined how neonicotinoid pesticides affect the ability of bees to distinguish odours. Here, we use a differential learning task to test how neonicotinoid exposure affects learning, memory, and olfactory perception in foraging-age honeybees. Bees fed with thiamethoxam could not perform differential learning and could not distinguish odours during short and long-term memory tests. Our data indicate that thiamethoxam directly impacts the cognitive processes involved in working memory required during differential olfactory learning. Using a combination of behavioural assays, we also identified that thiamethoxam has a direct impact on the olfactory perception of similar odours. Honeybees fed with other neonicotinoids (clothianidin, imidacloprid, dinotefuran) performed the differential learning task, but at a slower rate than the control. These bees could also distinguish the odours. Our data are the first to show that neonicotinoids have compound specific effects on the ability of bees to perform a complex olfactory learning task. Deficits in decision-making caused by thiamethoxam exposure could be more harmful than other neonicotinoids, leading to inefficient foraging and a reduced ability to identify nest mates.

Active smelling in the American cockroach

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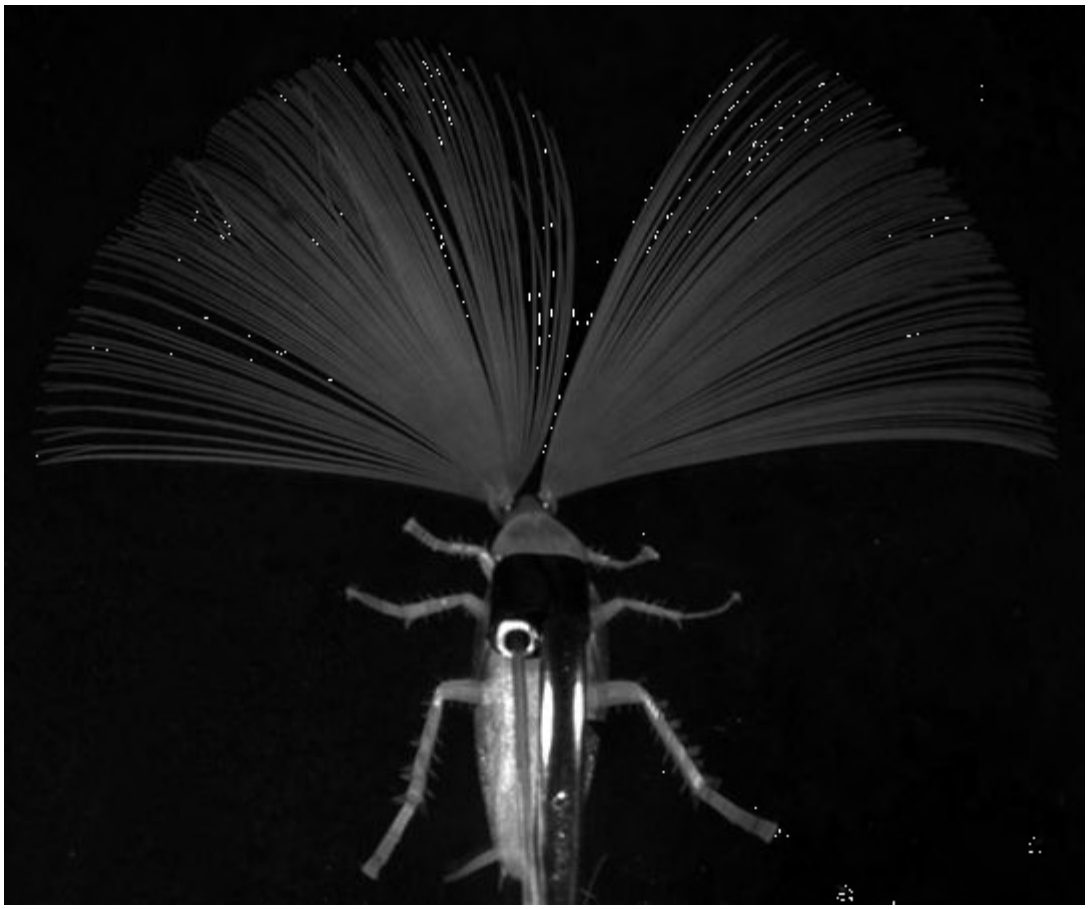
Active sensing is the process by which animals use self-generated energy (e.g., movement) to improve the sampling of a sensory modality. Specific behaviors had previously been shown to improve odor-sampling in mammals (“sniffing”), flying insects (“zig-zag” flight maneuvers in flies and moths) and aquatic crustaceans (antennule “flicking” in lobsters). Here, we employ the American cockroach with its long and highly mobile antennae to understand their role in active olfactory sensing strategies. We investigate how cockroaches adapt their movements to changing characteristics of an olfactory environment. Using behavioral wind-tunnel experiments that involve 3D tracking of the antennae, head, and legs, we manipulate spatiotemporal aspects of odor stimuli to study the relationship between an odor encounter and movement decisions. To quantify antennal movement features, we use a wavelet analysis and find that odor stimuli affect them: Upon odor encounter, we observe an increase in power for antennal sweeping frequencies above 1 Hz in azimuth and especially in elevation. This behavior can last beyond the odor offset. In addition, antennal movements alone aid in locating an odor in space: The position of antennal sweeps correlates with the location of a shifting odor, especially for an attractive odor. Thus, while similar up-wind navigation to flies/moths had previously been shown in the walking patterns of cockroaches, it seems that at the scale of their antennal movements, similar principles apply, as the oscillating antennae seek contact with an odor of interest if the latter gets lost. We are also further investigating the kinematic coupling of the antennae and thus their capacity to act as independent sensors as we observe that one antenna responds to an odor stimulus delivered to the other. Furthermore, we explore the interactions between walking behaviors and antennal movements during sensing. We see a recurring - but not always complete - behavioral sequence after odor onset: The animal stops walking, the antennae respond and finally, the animal starts walking again in a given direction. Walking pauses occur even in absence of a delivered odor stimulus, however, only in presence of our stimulus do we see a consistent increase in antennal movements during them. In conclusion, we hypothesize that antennal movements may improve odor-sensing in two ways: 1. Map odorants in space through wide movements in order to make relevant decisions for navigation, and 2. Increase odor contact occurrences through short, fast sweeps (i.e., “catch” odorants similarly to mammal “sniffing” or lobster antennule “flicking”).



Symposium

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Molecular dynamics at inhibitory synapses during iLTP

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An increasing body of evidence shows that, similarly to excitatory glutamatergic synapses, inhibitory GABAergic synapses can exhibit plasticity. This feature is expected to significantly contribute to the activity-dependent shaping of neuronal network function. The mechanisms underlying inhibitory synaptic plasticity have started to emerge. It has been demonstrated that in addition to the modulation of presynaptic mechanisms, modifications of the inhibitory postsynaptic density (iPSD) account for the potentiation or depression of GABAergic synapses. In particular, as the result of moderate activation of NMDA receptors, GABAergic synapses have been shown to express inhibitory long-term potentiation (iLTP) due to increased availability of synaptic GABAA receptors.

This talk will present the molecular mechanisms of postsynaptic iLTP from a single-molecule perspective, highlighting that the dynamics of the molecules composing the iPSD play a crucial role in the increased stabilization of GABAA receptors at postsynaptic sites. More specifically, we will discuss how i) GABAA receptor intracellular trafficking, ii) surface receptor lateral diffusion/anchoring at synaptic sites and iii) scaffold protein dynamics collectively affect the nano-organization of the inhibitory postsynaptic density and contribute to iLTP expression. We will also present new insights in the subsynaptic-domain (SSD) architecture of GABAA receptor and gephyrin, revealing the coordinated molecular reorganization of the GABAergic synapse during postsynaptic inhibitory plasticity.

Glycinergic inhibition in health and disease

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Glycine receptors (GlyRs) mainly expressed in the brainstem and spinal cord enable inhibitory neurotransmission in the central nervous system. Impairment of glycinergic inhibition results in neurological diseases such as Startle disease (hyperekplexia) or stiff person syndrome (SPS). Efficient inhibitory control in the central nervous system is based on correctly assembled and posttranslational modified GlyRs targeted to their synaptic compartments. GlyRs are localized in heteromeric configurations at the postsynaptic membrane and are anchored via the scaffold protein gephyrin to the GlyR beta-subunit. Less understood is the role of presynaptic homomeric glycine receptors regulating the release of glycine from presynaptic sites via the induction of small depolarizing currents following activation.
Our group focuses on the pathophysiology of human and murine GlyR variants leading to the neurological disease hyperekplexia. Moreover, we have identified the molecular mechanisms of autoantibodies generated against the GlyR underlying SPS or the more severe form progressive encephalomyelitis with rigidity and myoclonus (PERM).
Cell culture models using cell lines and primary neurons as well as mouse models are used to study disease mechanisms. Our data are accomplished by proteinbiochemical investigations, immunocyto- and histological analysis, behavioral readouts as well as electrophysiological characterizations.
Recessive GlyR variants exhibit disturbances in protein biogenesis and transport which were specified to impaired neuronal ER-Golgi trafficking. Consequences of altered receptor maturation are changes in receptor physiology. Recently, using a novel mouse model for startle disease, we identified an extracellular element of the receptor important for synaptic integration and inhibitory signaling.
Besides genetic receptor variants, GlyRs represent a target for autoantibodies generated in human patients with SPS. GlyR autoantibodies are however not the only autoantibodies that have been described in SPS patients. More common are GAD autoantibodies but also amphiphysin autoantibodies have been identified. In 2004 it was specified that GlyR autoantibodies are mainly of the IgG1 subclass, able to crosslink receptors and induce enhanced receptor internalization and complement activation. Recently, we have identified a common epitope of GlyR autoantibodies in the extracellular domain of the receptor. Following autoantibody binding, the generated receptor configuration shows reduced neurotransmitter potency underlying reduced inhibition in affected neuronal circuits. To ensure a causal relation between motor dysfunction and GlyR autoantibodies, we used the zebrafish model. Following injection of the GlyR autoantibodies, the zebrafish larvae generated an impaired escape behavior compatible with abnormal startle response in SPS or PERM patients.

Structural insights into gephyrin interacting with the full-length glycine receptor intracellular domains within a pentameric assembly

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Efficient signal transmission in the nervous system requires a dynamic regulation of excitatory and inhibitory neuronal activity. In spinal cord and brain stem the inhibitory signal transmission is primarily based on the activation of glycine receptors (GlyRs). Synaptic plasticity events at glycinergic synapses are regulated by GlyR diffusion which is modulated by the interaction with the major scaffolding protein of inhibitory synapses, called gephyrin. The oligomerization behavior of gephyrin and its subdomains is thought to build the basis for a highly dynamic network providing the binding sites for GlyRs at postsynaptic membranes. Synaptically localized GlyRs critically depend on the assembly into heteropentameric receptors containing β-subunits as their intracellular cytosolic domain (ICD) drives the interaction with gephyrin. However, the molecular mechanism and the structure of the postsynaptic clusters formed by the GlyR and gephyrin still remain elusive.
So far, structural studies on the complex between the receptor and gephyrin were only based on isolated GlyR peptides. Thus, these studies omit the conformation of the full-length receptor ICDs interacting with gephyrin as well as intersubunit effects within the pentameric assembly of the GlyR. Therefore, we established a soluble GlyR model system based on yeast lumazine synthase (LS). The LS exhibits spatial expansions similar to the GlyR and thus allows the incorporation of the full-length GlyR ICDs into a pentameric assembly. We incorporated the ICDs of the GlyR α- and β-subunit into the LS, which assembled into pentamers with a two to three stoichiometry upon co-expression and displayed a high-affinity interaction with gephyrin. The complex formed between the LS-GlyR-ICD chimera and the gephyrin E-domain was analyzed via single particle negative staining electron microscopy. The obtained low resolution 3D model revealed the interaction of two gephyrin E-domain dimers with one LS pentamer. Moreover, the presence of two neighboring GlyR β-ICDs was suggested, that display a less flexible conformation compared to the other GlyR ICDs and interact simultaneously with one gephyrin E-domain dimer.
In summary, the LS displays a suitable tool for structural studies of the full-length GlyR ICDs within a pentameric assembly to further characterize the interaction with gephyrin. Overall, the structure of the complex formed by gephyrin and the GlyR ICDs within the LS, might give further insights into the assembly of postsynaptic clusters at glycinergic synapses.

Quantitative SR-CLEM reveals stereotypic glycine receptor packing at native spinal cord synapses

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Precise quantitative information about the molecular architecture of synapses is essential to understanding the functional specificity and downstream signaling processes at specific populations of synapses. Inhibitory glycinergic networks in the spinal cord vary in function, however the nanoscale organization of glycine receptors (GlyRs) underlying different network specificities has not been defined. We have characterized the molecular organization and ultra-structure of glycinergic synapses in native spinal cord tissue using quantitative super-resolution correlative light and electron microscopy (SR-CLEM). We have thereby identified a stereotypic packing of GlyR complexes at inhibitory synapses in the dorsal and ventral horn, suggesting that the morphology and size of the postsynaptic density rather than differences in the molecular arrangement of the receptors determine the strength of glycinergic neurotransmission in the spinal cord.

Augmentation of endogenous neurosteroid synthesis alters experimental status epilepticus dynamics

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Abstract

Neurosteroids can modulate γ-aminobutyric acid type A receptor-mediated inhibitory currents. Recently, we discovered that the neurosteroids progesterone, 5α-dihydroprogesterone, allopregnanolone, and pregnanolone are reduced in the cerebrospinal fluid of patients with status epilepticus (SE). However, it is undetermined whether neurosteroids influence SE. For this reason, first, we evaluated whether the inhibitor of adrenocortical steroid production trilostane (50 mg/kg) could modify the levels of neurosteroids in the hippocampus and neocortex, and we found a remarkable increase in pregnenolone, progesterone, 5α-dihydroprogesterone, and allopregnanolone levels using liquid chromatography tandem mass spectrometry. Second, we characterized the dynamics of SE in the presence of the varied neurosteroidal milieu by a single intraperitoneal kainic acid (KA; 15 mg/kg) injection in trilostane-treated rats and their controls. Convulsions started in advance in the trilostane group, already appearing 90 minutes after the KA injection. In contrast to controls, convulsions prevalently developed as generalized seizures with loss of posture in the trilostane group. However, this effect was transient, and convulsions waned 2 hours before the control group. Moreover, electrocorticographic traces of convulsions were shorter in trilostane-treated rats, especially at the 180- minute ($P < .001$) and 210-minute ($P < .01$) time points. These findings indicate that endogenous neurosteroids remarkably modulate SE dynamics.

Keywords: allopregnanolone, hippocampus, kainic acid, LC-MS/MS, status epilepticus, trilostane

1. Introduction

Status epilepticus (SE) affects approximately 41 of every 100 000 adults and is difficult to treat. For this reason, up to 20% of cases are reported to involve mortality [1]. Various neurological disorders are associated with SE, including cerebrovascular diseases, trauma, intoxication, and also others, but it is unclear why SE develops only in some patients affected by the previously mentioned central nervous system (CNS) disorders. In addition, SE also develops without a clear reason in some cases. Finally, patients with epilepsy may experience one or more episodes of SE, whereas others do not. Presently, the possible factors responsible for this different propensity to develop SE are undetermined. By characterizing neurosteroid levels in cerebrospinal fluid of patients with SE, we found that progesterone, 5α-dihydroprogesterone, allopregnanolone, and pregnanolone were all significantly reduced in comparison to presumably healthy controls [2]. All the mentioned neurosteroids are known to modulate γ-aminobutyric acid (GABA) type A receptor (GABA) activity, and especially 3α-reduced neurosteroids such as

allopregnanolone and pregnanolone are potent positive modulators of $GABA_A$-mediated inhibitory currents [3]. For this reason, we hypothesized that the changes observed in CNS neurosteroid levels could be relevant for SE onset or evolution.
On the other hand, the possible role of neurosteroids in SE has been questioned by a multicenter trial based on treatment with the allopregnanolone analogue brexanolone, which failed in demonstrating beneficial effects on the course of refractory SE [4]. A possible limitation in this study was the lack of stimulation of endogenous neurosteroid production because different types of neurosteroids can interact with the $GABA_A$ receptor. Both allopregnanolone and pregnanolone are reduced in patients with SE; therefore, a more general approach to increase the overall neurosteroid CNS concentration should be followed to potentiate the effects of neurosteroids on GABAergic function.
To evaluate the possibility of inducing an overall increase of neurosteroid levels in the CNS, we considered the 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase inhibitor trilostane, which is actually used for treatment of pituitary- dependent hyperadrenocorticism, primary hyperadrenocorticism, and alopecia X in dogs [5]. A known consequence of trilostane administration is the increase in pregnenolone, allopregnanolone, and allotetrahydrocorticosterone CNS levels in castrated rodents [6]. This occurs because trilostane blocks the conversion of pregnenolone to progesterone in the adrenal cortex, inducing an increase in the peripheral synthesis of the precursor pregnenolone that, when reaching the brain, is then used to boost overall neurosteroid production. Thus, we aimed to reproduce the same phenomenon in healthy rats and to assess whether the increased availability of neurosteroids could affect the course of SE induced by kainic acid (KA) administration.

2. Materials and Methods

2.1 Animals and treatments

Forty-two adult male Sprague-Dawley rats (Charles River) of 175g to 200g body weight were used in this study. All animals were housed in a specific pathogen–free facility under a controlled environment with ad libitum access to water and food. Experiments received local approval from the Animal Welfare Body, as well from the Italian Ministry of Health (544/2020-PR). Studies were conducted in accordance with the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals. All efforts were made to im- prove welfare and to reduce the number of animals used for experiments.

2.2 Experimental design

Two different experiments were performed, the first to determine the changes in hippocampal and neocortical levels of neurosteroids in naive rats not experiencing the SE, according to a previously established protocol of trilostane administration [6], and the second to characterize the dynamics of KA-induced SE in the presence of the different neurosteroid levels determined by trilostane treatment. To this aim, trilostane (Cayman Chemical) was dissolved in

sesame oil and subcutaneously injected (50 mg/kg) 16 and 2 hours before euthanasia (n = 12, first experiment), or 16 and 2 hours before the induction of SE by KA (n = 9, second experiment). Control rats were also treated with sesame oil 16 and 2 hours before euthanasia (n = 12, first experiment), or 16 and 2 hours before the induction of SE (n = 9, second experiment). KA (Sigma-Aldrich) was dissolved in saline and intraperitoneally injected (15 mg/kg) as described previously [7].

2.3 Electrode implantation and video- electrocorticography

We implanted electrodes and obtained video-electrocorticographic (ECoG) recordings from 18 rats belonging to the previously described groups (vehicle and trilostane) and treated with KA (second experiment), starting before the induction of SE. The investigators who scored the seizures (C.L. and L.S.) were blinded to the treatment received by the animals.

2.4 Statistical analysis

Data were analyzed using Sigma Plot 11 (Systat Software). Hippocampal and neocortical neurosteroid levels in treatment groups were compared by the Mann-Whitney test. Outliers were identified using the Grubbs test and removed before analyzing video-ECoG data using a repeated measure two-way analysis of variance, considering time and treatment as the between and within factors, respectively. Then, groups were compared by post hoc Holm-Šídák test. The area under the curve of seizure duration (stage 4 + 5) was compared by the Student t test. Results are reported as median and interquartile range, or mean values and standard error of the mean, and considered significant when $P < .05$.

3. Results

First, we analyzed the impact of trilostane on brain neurosteroid levels. We found that the 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase inhibitor, injected twice before euthanasia, induced a remarkable increase in hippocampal and neocortical neurosteroid levels. Specifically, pregnenolone, progesterone, 5α-dihydroprogesterone, and allopregnanolone were respectively increased to up to 469% ($P \leq .001$ vs vehicle-treated rats, Mann-Whitney test), 592% ($P \leq .001$), 168% ($P < .05$), and 183% ($P < .01$) of control levels in the hippocampus of trilostane-treated rats; in contrast, pregnenolone sulfate and pregnanolone did not vary significantly (Table 1). Also, in the neocortex, we found similar results for almost all the evaluated neurosteroids (pregnenolone sulfate, +123%, $P = .01$ vs vehicle- treated rats; pregnenolone, +606%, $P \leq .001$; progesterone, +946%, $P \leq .001$; 5α-dihydroprogesterone, +380%, $P \leq .001$; allopregnanolone, +527%, $P \leq .001$), with the only exception being pregnanolone (Table 1).

Second, we characterized the dynamics of SE in the presence of the modified neurosteroidal milieu. Although we did not find any significant difference in the latency to SE onset (trilostane-treated rats, 46.67 ± 4.9 minutes; vehicle-treated rats, 44.16 ± 7.2 minutes) and the respective SE duration (trilostane-treated rats, 9.02 ± 0.5 hours; vehicle-treated rats, 10.83 ± 0.8 hours), we observed remarkable differences in seizures recorded in the course of SE, in both occurrence and duration.

Specifically, 90 minutes after the induction of SE, stage 4 seizures were more frequent in the trilostane group ($P < .05$ vs vehicle-treated rats, Holm-Šídák test), reaching the peak 30 minutes later (2 hours after KA administration) and maintaining this high level up to the 150-minute time point (Figure 1A). In vehicle-treated rats, stage 4 seizures increased 60 minutes later, reaching the same level as trilostane-treated rats at the 150-minute time point. However, the reduction in stage 4 seizures was more rapid in trilostane-treated rats, as shown at the 4-hour time point, at which seizures were lower in these animals compared to controls ($P < .05$).

Stage 5 seizures (Figure 1B) also presented accelerated development in the same animals, peaking 90 minutes after the KA injection ($P < .001$ vs vehicle-treated rats). Moreover, the peak observed in trilostane-treated rats doubled that of vehicle-treated rats, in which it appeared at the 3-hour time point. After their respective peaks, stage 5 seizures wane following a similar dynamic in both treatment groups.

By considering stage 4 and 5 seizures together (Figure 1C), it is possible to observe that convulsive seizures occurred in both treatment groups to the same extent, with early increases at 90-120 minutes in trilostane-treated rats (respectively, $P < .001$ and $P < .05$ vs vehicle-treated rats). The peak in the vehicle group instead occurred at the 150- minute time point. Subsequently, convulsive seizures similarly decreased in both groups, almost disappearing 7 hours after KA administration.

Then, we evaluated the duration of convulsive seizures over time. Interestingly, stage 4 seizures had a longer duration in vehicle-treated rats (Figure 1D), starting from the 150-minute time point ($P < .05$ vs trilostane-treated rats) up to the 330-minute time point ($P < .001$ vs trilostane-treated rats at 180-210 minutes; $P < .05$ at 300-330 minutes) except for the 240- to 270-minute time point. The same peak of stage 4 seizure duration observed in vehicle-treated rats was never reached in trilostane-treated animals.

Consistently, stage 5 seizures also appeared to be more durable in vehicle-treated rats compared to trilostane-treated rats 3 hours after KA administration ($P < .001$, Figure 1E). In the vehicle group, the

duration of stage 5 seizures evaluated at its peak doubled that of trilostane-treated rats. However, in the following time point duration of stage 5 seizures was comparable in both treatment groups.
When stage 4 and stage 5 convulsive seizures were averaged, the duration of convulsive seizures was significantly lower in rats treated with trilostane at the 180-minute ($P < .001$ vs vehicle-treated rats) and 210-minute ($P < .05$) time points (Figure 1F). Additionally, by considering the area under the curve for each rat of both groups, we found a remarkable 2.5-fold difference in the overall seizure duration, which was 1 666 171.11 ± 476 331.82 seconds in vehicle-treated rats versus 657 987.75 ± 155 321.00 seconds in trilostane-treated rats ($P = .038$, Student t-test).

4. Discussion

Our purpose was to describe the development of SE in the presence of altered neurosteroid levels in the brain. For this reason, first, we evaluated the effects of trilostane administration on neocortical and hippocampal neurosteroid levels. Trilostane is an inhibitor of 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase that was developed as a cancer drug for humans, then used to reduce hyperadrenocorticism in dogs. Specifically, trilostane blocks corticosterone synthesis and removes the inhibitory control of adrenocorticotropic hormone (ACTH), leading to enhanced stimulatory drive on adrenocortical activity. Thus, ACTH stimulates the conversion of cholesterol to pregnenolone and overcomes the block of 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase to restore corticosterone production and to increase the availability of the neurosteroid precursor pregnenolone [8]. A previous investigation in castrated mice showed that trilostane increases levels of pregnenolone and allopregnanolone in the CNS [9] similar to our results. In the same work, trilostane also induced a remarkable increase in allotetrahydrocorticosterone, which was not measured in our animals.
The changes in CNS neurosteroid levels we found in trilostane-treated rats resulted in remarkable effects on the dynamics of SE induced by KA. Although the latency to SE onset was not affected, trilostane-treated rats anticipated the occurrence of convulsive seizures in response to KA. The most severe stage 5 seizures were also more frequent in trilostane-treated rats, suggesting a proconvulsant effect of the increase in neurosteroid levels. However, this initial effect was not durable. Moreover, considering the seizure duration, the overall response to trilostane appeared to be more anticonvulsant than proconvulsant, because it was mainly characterized by a reduction in duration of convulsive seizures. In particular, trilostane-treated rats presented with shorter stage 4 seizures for hours.
We have no simple explanation for the changes described in trilostane-treated rats. GABAergic neurons play a complex role in establishing seizure onset and duration. By optogenetically stimulating parvalbumin interneurons in epileptic mice, Lévesque and collaborators found that the seizure rate was reduced, but the seizure probability instead increased, indicating that the enhanced GABA release from parvalbumin interneurons can paradoxically promote seizures [10]. Although we have not evaluated these phenomena in our animals, the GABAergic tone could be expected to increase in trilostane-treated animals, possibly reproducing a situation similar to that characterized by Lévesque and collaborators in their epileptic mice.
Neurosteroids are currently under investigation for their possible therapeutic effects in epilepsy and SE. Encouraging preclinical evidence for allopregnanolone and its 3β-methyl derivate ganaxolone, administered 30 minutes after SE onset, has been obtained in a mouse model based on tetramethylenedisulfotetramine injection [11]. For the same molecules, the positive findings were confirmed in the lithium-pilocarpine model of SE [12]. Interestingly, additive properties were also described by combining ganaxolone with tiagabine or midazolam [13]. This further evidence suggests that neurosteroids could be used in polytherapy to address SE. Not only allopregnanolone but also other neurosteroids could be candidates for this purpose because both androstanediol and 5α-dihydroprogesterone have displayed anticonvulsant effects [14,15]. However, the use of neurosteroids could be limited in male subjects, who have appeared to be less sensitive than female subjects to the anticonvulsant properties of at least some of them [14]. The use of trilostane to increase the availability of a variety of neurosteroids in the brain could be an alternative to the administration of a single steroid. Steroids are generally metabolized slowly and could afford longer protection than other endogenous anticonvulsant molecules, such as peptides. For instance, in ovariectomized rats, the half-life of

exogenously administered progesterone was 1.21 ± 0.21 hours [16]. Because we found a six-fold increase in progesterone levels in the brain, we hypothesized that this neurosteroid was more available during SE induction and possibly also in the period of convulsive seizures. Trilostane might have contributed to maintaining elevated brain neurosteroid levels, because its effects on the suppression of cortisol production in dogs were shown to last up to 12 hours[5]. This is because the reported half-life of trilostane is 8 hours. Although trilostane is reported to be specific for the adrenal cortex, we are not aware of any possible direct effect of this drug on the brain or, especially, whether this effect may appear because of dysfunction of the blood-brain barrier in the course of SE. This question must be addressed in future experiments aimed at establishing the time course of the changes in neurosteroid levels in cerebral tissue during SE. However, our findings suggest that the neurosteroids are able to modulate SE, providing that most of them are increased. It remains to be established whether similar effects could be observed if trilostane is administered after the onset of SE, to assess the possible clinical use of this drug in the course of SE.

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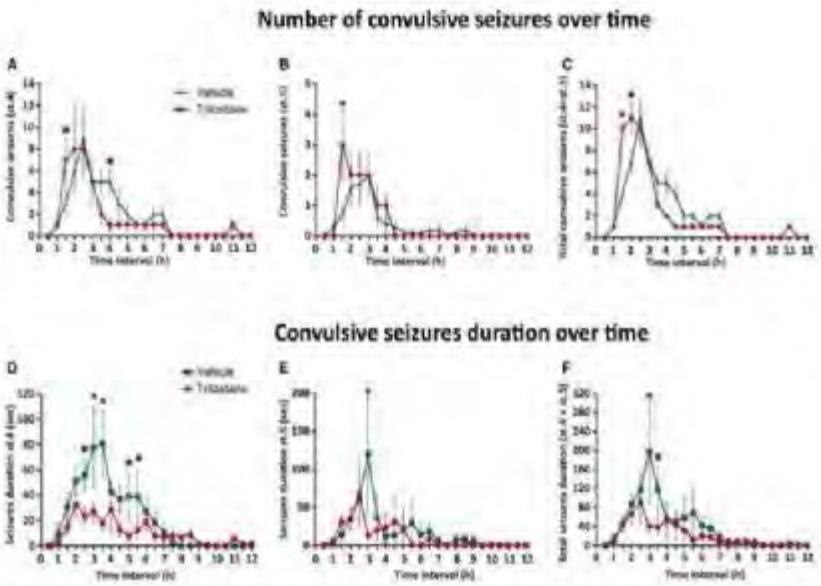

Figure 1: Occurrence and duration of video-recorded seizures during kainic acid (KA)-induced status epilepticus (SE). A-C, Seizures induced by KA were counted every 30 minutes for 12 hours. A, note that stage (st.) 4 seizures increased early (*$P < .05$ vs vehicle-treated rats, Holm-Šidák test) in the trilostane group 90 minutes after induction of SE. Conversely, 150 minutes later these seizures were remarkably reduced in the same group (*$P < .05$ vs vehicle-treated rats). B, Stage 5 seizures also developed precociously in trilostane-treated rats, peaking at the 90-minute time point (*$P < .001$ vs vehicle-treated rats, Holm-Šidák test). C, the average occurrence of stage 4 and 5 seizures was respectively reached at 90 minutes (*$P < .001$ vs vehicle-treated rats) and 120 minutes (*$P < .05$) in trilostane-treated rats. D-F, Duration of convulsive seizures over time. D, Interestingly, stage 4 seizures presented longer duration in vehicle-treated rats (150 minutes, *$P < .05$; 180 minutes, *$P < .001$; 210 minutes, *$P < .001$; 300 minutes, *$P < .05$; 330 minutes, *$P < .05$ vs trilostane-treated rats, Holm-Šidák test). E, in contrast, stage 5 seizures were longer in the vehicle-treated group only at the 180-minute time point (*$P < .001$). F, considering stage 4 and stage 5 convulsive seizures together, rats treated with trilostane presented a significant reduction in seizure duration at the 180-minute (*$P < .001$) and 210-minute (*$P < .05$) time points.

Table 1: Tissue levels of various neurosteroids in both hippocampus and neocortex of rats treated with trilostane and their respective controls

Analytes, ng/mg	Vehicle	Trilostane	Statistics
Pregnenolone sulfate, hippocampus	0.000529 (0.00043-0.00055)	0.000513 (0.00043-0.00058)	NS
Pregnenolone sulfate, neocortex	0.000195 (0.000190-0.000225)	0.000240 (0.000220-0.000305)	$P = .010$
Pregnenolone, hippocampus	0.0160 (0.014-0.022)	0.0751 (0.033-0.137)	$P \leq .001$
Pregnenolone, neocortex	0.017 (0.0164-0.0208)	0.103 (0.0422-0.145)	$P \leq .001$
Progesterone, hippocampus	0.00066 (0.000599-0.000798)	0.00391 (0.0019-0.0072)	$P \leq .001$
Progesterone, neocortex	0.00048 (0.00038-0.00073)	0.00454 (0.0019-0.0072)	$P \leq .001$
5α-Dihydroprogesterone, hippocampus	0.000411 (0.00037-0.00045)	0.000691 (0.00046-0.00078)	$P < .05$
5α-Dihydroprogesterone, neocortex	0.000175 (0.000155-0.000205)	0.000665 (0.000335-0.000828)	$P \leq .001$
Allopregnanolone, hippocampus	0.000253 (0.000169-0.000512)	0.000464 (0.000310-0.000576)	$P < .01$
Allopregnanolone, neocortex	0.000091 (0.00008-0.00013)	0.000480 (0.000245-0.000732)	$P \leq .001$
Pregnanolone, hippocampus	0.000216 (0.000175-0.000283)	0.000197 (0.000155-0.000251)	NS
Pregnanolone, neocortex	0.0000857 (0.0000763-0.0000912)	0.0000824 (0.000064-0.000101)	NS

Note: Results are presented as median and interquartile range. Values were compared by the Mann-Whitney test. Abbreviation: NS, not significant.

Bioorthogonal click-chemistry labeling of proteins at the GABAergic synapse

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Superresolution microscopy (SRM) of neuronal synapses relies on small labels that show minor interference with synaptic physiology and minimal linkage error between target epitope and photon emitting fluorophor. Recently, these requirements were achieved via genetic code expansion of membrane receptors by incorporation of unnatural amino acids that can be reacted with tetrazine-dye conjugates via strain promoted inverse electron demand Diels-Alder cycloaddition. This fast labeling reaction is biorthogonal, provides small carrier size in combination with small linkage distance, and most importantly preserves the receptor function. The talk will focus on introduction of click-chemistry labeling of unnatural amino acids incorporated in GABAergic synapse proteins.

Symposium

S31: Odor spaces: from odor molecules to behavior

Hyperbolic geometry of the olfactory space

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In this presentation I will describe both theoretical reasons and experimental evidence that natural stimuli and human perception can be mapped onto a low dimensional curved surface. This surface turns out to have a negative curvature, corresponding to a hyperbolic metric. Although this map was derived purely from the statistics of co-occurrence between mono-molecular odorants in the natural environment it revealed topography in the organization of human perception of smell. I will conclude with arguments for why hyperbolic metric can be useful for other sensory systems, and provide examples of hyperbolic geometry at different levels of biological organization, such as at the level of gene expression.

What's in a plume? Accessing the information encoded in spatio-temporal odor plumes.

Michael Schmuker[1], Andreas Schaefer[2], Brian Smith[3], Justus Verhagen[4], Jonathan Victor[5], John Crimaldi[6]

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[3] Arizona State University, Tempe, AZ, USA
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Olfaction research has undergone a transition from assuming natural olfactory stimuli to be slow and static towards recognizing that they follow complex and fast dynamics. Already decades ago, insights into plume structure (see e.g. [1-3]) suggested that odor-guided navigation in insects is based on brief, intermittent odor encounters (see [4] for a review). This insight also gave rise to theoretical considerations on how to navigate with intermittent cues [5].

Although olfactory systems in insects and vertebrates have long been shown to be capable of producing a rich spatiotemporal repertoire of activity [6,7], their response dynamics were often probed using only quasi-static stimuli. In hindsight, this is surprising since physiological experiments have shown that olfactory neurons exhibit adaptation on sub-second timescales [8], which jeopardizes reliable encoding of static stimuli.

In recent years, the field has undergone a paradigm shift, as an increasing number of labs have transitioned away from using static stimuli towards investigating responses to brief pulses and random stimuli [9-11]. Olfaction is now increasingly seen as a “wide-bandwidth temporal sense” [12,13]. At the same time, a transition has started in robot olfaction, where it has now also been discovered that temporal cues can be accessed with available sensors carry information useful for navigation such as downwind and crosswind source distance [14,15].

We are only beginning to understand the implications of this paradigm-shift on our view of olfactory circuits and the function of olfacto-motor loops. We are part of the NeuroNex: Odor to Action consortium which has formed to understand how odors lead to behavioral actions [16]. In this contribution we will review insights into the information encoded in turbulent odor plumes, shine light on how animals could access this information, and will outline what could be the key challenge for olfactory behavioral neuroscience in the coming years, which is to re-interpret the huge body of work that used static stimuli in the light of natural odor dynamics.

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Alcohol consumption makes male flies more attractive

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Male flies are highly attracted to alcohols and especially to methanol. Here we reveal an ecologically sound explanation to this phenomenon. We also describe the underlying neural substrate allowing the fly to balance between the positive and negative effects of odour-based attraction to methanol.

From odor coding to decision making in cockroach groups

Einat Couzin-Fuchs[1], Yannick Günzel[1], Jaclyn McCollum[1], Marco Paoli[1,2], Giovanni Galizia[1], Inga Petelski[1]

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Animals sense odours from their environment to inform survival-critical decisions about food, shelters and mates. In social species, each of these decisions influence, and is also influenced by, the decisions of surrounding individuals. In this talk, I will present our study on the sensory basis of individual, and collective, decision-making, using cockroach shelter selection as a model system. Collective sheltering in cockroaches demonstrates a clear interplay between the benefit of aggregation and the cost of competition. Individually, cockroaches prefer shelters associated with a food odour (vanillin), while in a group the unscented alternative is more likely to be selected. The same preference inversion occurs also when individuals are tested alone with a ‘group’ odour (an extract of the colony faeces). This indicates that olfaction plays an important role for shelter evaluation, and allows us to investigate the integration of food and social cues, influencing shelter decisions, in a single sensory centre. Using analysis of shelter choices, antennal probing and exploration in individual and groups with the corresponding representations of the olfactory environments, we study how and why this inversion occurs. Our results indicate that in parallel to the reduced vanillin preference in social contexts, there is a reduction in vanillin-sensitive projection neurons’ activity as the social odour concentration increases. I will discuss our ongoing study to establish the relations between early odour coding, individual, and collective preferences as well as investigate their ethological implications as a sensory-based mechanism to avoid recently exploited resources.

Walking fruit flies navigate complex odor plumes using stochastic decisions biased by the timing of odor encounters

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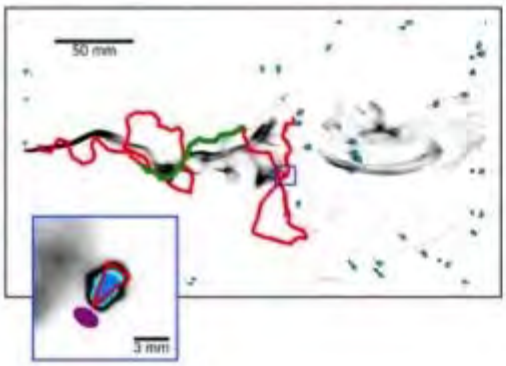
Flying insects navigate stationary odor ribbons by surging upwind when entering the plume and casting laterally or counterturning upon odor loss. Upwind turns and surges are also observed when insects enter an odorized, uniform block of laminar wind. In both cases, the odor is either spatially uniform or static in time, and the wind is unidirectional, providing a reliable directional cue about odor source location. In contrast, naturalistic plumes are intermittent and spatially variable, generated by complex air flows that break continuous odor regions into disconnected patches. Quantifying navigational strategies in these dynamic plumes has been hampered by an inability to image odors simultaneously with unrestrained animal behavior. Here, we imaged fluctuating, intermittent odor plumes simultaneously with freely-walking flies, allowing us to quantify behavior in response not just to overall whiff statistics, but in response to each individual odor whiff perceived by flies as they navigate. In contrast to more regular environments, whiffs did not trigger reflexive surging, casting, or counterturning as they do in straight odor ribbons. Instead, flies turned stochastically using stereotyped saccades whose magnitude and rate were, strikingly, uncorrelated to the frequency of plume encounters. Though turn rates were constant, turn direction was biased upwind by the timing of previously perceived whiffs. Unlike in homogeneous odor clouds or ribbons, flies did not strongly adjust walking speed following plume encounters. Rather, they used whiff timing to modulate discrete transitions between walks and stops. By fitting various parametric stochastic models to the data, we find that stop decisions are modulated by the arrival time only of the most recent whiff, while stopped flies accumulate evidence from past whiffs before initiating a walk. Pairing this with agent-based simulations, we show that this strategy of gathering information without losing position forms a critical component of effective navigation in rapidly-fluctuating plumes. Finally, in contrast to navigation in laminar environments, we find that the arrival time of whiffs fully account for navigational decisions; duration and concentration play a negligible role. These results suggest that when the timing and location of odor encounters are uncertain, animals dispense with reflexive strategies determined by the most recent whiff, and instead navigate using biased random walks shaped by an entire sequence of encounters.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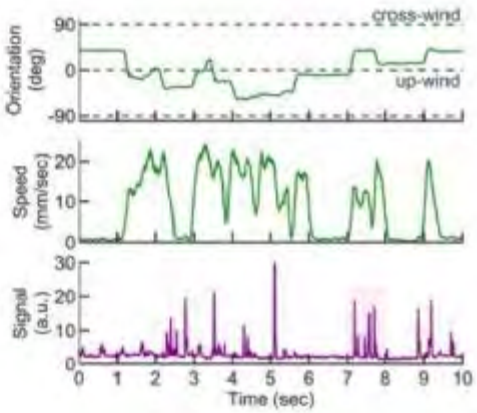
cross-wind
up-wind
Orientation (deg)
90
0
-90
Speed (mm/sec)
20
10
0
Signal (a.u.)
30
20
10
0
0 1 2 3 4 5 6 7 8 9 10
Time (sec)

Wired for Love: Evolution of Sexual Communication in *Drosophila*

Mohammed A. Khallaf[1], Markus Knaden[1], Bill S. Hansson[1]

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Wired for Love: Evolution of Sexual Communication in *Drosophila*

How animals evolve extraordinarily diverse communication systems to effectively identify mating partners is a fascinating unsolved problem, which can shed light on how sensory systems rapidly change and underlie speciation. In our work we address this question by investigating the evolution of sex pheromone communication systems in the genus *Drosophila*. This genus offers exceptional possibilities to elucidate the genetic and neural correlates linked to the evolution of sexual signaling in diverse ecological niches. Through a wide range series of whole genome sequencing, phylogenetic analyses, chemical identifications and syntheses, neuronal recordings, and behavioral experiments, we identified the olfactory sex pheromones of 99 species in the family Drosophilidae and demonstrate how these signals govern mate recognition and promote pre-mating isolation barriers. Our results not only examine probably the most important channel of communication between insect species in a completely unprecedented way, but also advance and change our understanding of the evolution of neural circuits underlying sexual communication and the reproductive isolation barriers.

Symposium

S32: Translational Aspects in Neurological Diseases: from pathophysiology to new therapeutic approache

S32-1 Inflammation in neurodegenerative diseases
Gisa Ellrichmann, Carsten Saft, Alina Blusch, Jennifer Koenig, Christiane Reick, Ralf Linker, Konstanze Winklhofer, Ralf Gold

S32-2 Synaptic pathophysiology in autoimmune encephalitis
Christian Geis

S32-3 Deciphering the molecular basis of Tauopathies
Günter U Höglinger

S32-4 Thalamic contributions to the transformation of perception into action
Melanie Wilke

S32-5 Bortezomib-induced polyneuropathy in rats: A model for generation and regeneration mechanisms in neuropathic pain
Anna-Lena Bettenhausen, Reine-Solange Sauer, Claudia Sommer, Heike L. Rittner

Inflammation in neurodegenerative diseases

Gisa Ellrichmann[1], Carsten Saft[2], Alina Blusch[3], Jennifer Koenig[3], Christiane Reick[4], Ralf Linker[5], Konstanze Winklhofer[6], Ralf Gold[2]

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Huntington's disease (HD) is an autosomal dominantly inherited neurodegenerative disease characterized by progressive motor deficits, cognitive decline and conspicuous social behavior. The transgenic mouse model R6/2 recapitulates some of these deficits and basic pathophysiological similarities of HD. Therefore, it can be used as an experimental model to design novel therapeutic approaches and to understand underlying mechanisms of this neurodegenerative disease for which there is no established treatment so far.

Immunomodulatory substances, that are already used in other diseases as e.g. relapsing remitting multiple sclerosis (fumaric acid esters, laquinimod) have been transferred into neurodegenerative in vivo and in vitro models. Additionally, regenerative potential was tested in a model of the peripheral nervous system.
Behavioral analyses as well as histological and immunohistochemical analyses in the motor cortex and striatum (neurons, medium spiny neuron's (MSN's), mutant Huntingtin) of transgenic R6/2 mice have been done. We focussed on biochemical analyses (e.g. BDNF) to better understand underlying mechanisms and pathways. Influence on oxidative stress reduced neuroinflammation and increased neuroprotection.
Our findings suggest that treatment with substances affecting the immune system could provide a potential therapy for the up-regulation or modulation of neuroprotective pathways in neurodegenerative diseases.

Synaptic pathophysiology in autoimmune encephalitis

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The discovery of autoantibodies against synaptic antigens in the central nervous system (CNS) in patients with severe neuropsychiatric disorders was a breakthrough in neurology. More than 15 target molecules have been identified to which specific autoantibodies are directed each defining a subtype of disease. These target antigens are all part of central synapses and are comprised of ionotropic and metabotrobic receptors (e.g. NMDA, Glycine and $GABA_B$ receptors) as well as adhesion and transsynaptic signaling molecules (e.g. LGI). This novel entity of CNS disorders has been termed “autoimmune encephalitis”. The underlying pathophysiology including the molecular interactions and the often detrimental impact of the antibodies on neurons, synapses, and consequently on network function are only partly understood. As a consequence, target-specific therapies are currently not available. Here, I will present recent advances of translational research in autoimmune encephalitis mediated by antibodies against NMDA and AMPA receptors and in limbic encephalitis with autoantibodies to LGI1. Antibody-mediated pathophysiology will be outlined on the level of individual synapses in-vitro and in mouse models using electrophysiological and imaging approaches and behavioral analysis. Specifically, disturbance of synaptic signaling and synaptic plasticity will be related to network dysfunction and behavioral abnormalities in-vivo. Experimental findings will be considered with respect to clinical symptoms and disease course of patients with autoimmune encephalitis.

Deciphering the molecular basis of Tauopathies

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In this presentation, I will focus on the interplay between aging, environment, and neurodegeneration, covering molecular, functional, and clinical aspects of tauopathies.

The increasing possibility of early - preclinical diagnostics is an essential instrument on which immense progress is currently being made. New technologies enable early identification of patients with increased risk via signatures and biomarkers. A better understanding of the molecular processes that precede the onset of disease allows a more targeted use of novel, but also a more targeted application of existing forms of therapy.

In particular, I will highlight recent developments in biomarker research and development of neuroprotective therapies with focus on tauopathies.

Thalamic contributions to the transformation of perception into action

Melanie Wilke[1]

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Most concepts of visually-guided behavior emphasize interactions within large-scale cortical networks. Direct connections between cortical areas are paralleled by indirect routes through higher-order thalamic nuclei such as the pulvinar, leaving the question what kind of information is contributed by this route. In my talk I will present evidence from lesion studies in monkeys and humans suggesting that the pulvinar serves as an integrator of sensory and postural information enabling spatial selection and visuomotor transformations for saccade and reach-grasp behavior. Results further suggest that it serves this function by modulating the gain in fronto-parietal cortices.

Bortezomib-induced polyneuropathy in rats: A model for generation and regeneration mechanisms in neuropathic pain

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Neuropathic pain is a common and harmful complication of chemotherapeutic drugs e.g. the proteasome inhibitor bortezomib (BTZ). It is used as first-line therapy in multiple myeloma and representative for the dose-limiting neurotoxicity affecting up to one third of patients. Previous studies have identified mitochondrial dysfunction and protein degradation damaging axons. However, these studies mainly focused on damaging processes after administration of high cumulative dosages, disregarding that already lower doses could permit changes in the endoneural microenvironment. Indeed, other (e.g. diabetic or traumatic) peripheral neuropathies are associated with impairment of the neuronal homeostasis and show alteration of sealing tight junction proteins (TJPs), or changes in pro- and anti-inflammatory cytokines.
Therefore, we assumed that in the preclinical model of BTZ-induced polyneuropathy (BIPN), the blood-nerve barrier (BNB) is leaky and essential TJPs are downregulated. The subsequent phase of two-week regeneration would restore the barrier with upregulation of involved TJPs and changes in barrier-controlling cytokines.

After approval by the government of Unterfranken, male Wistar rats (n=6) were injected with BTZ 0.2 mg/kg body weight, or solvent i.p. on day 1, 4, 8 and 11 - similar to the therapy cycles in humans. Nociceptive thresholds were determined using the Von Frey and Hargreaves test (mechanical and thermal hypersensitivity). Sciatic nerves were harvested at the time of maximal hypersensitivity on day 11, and in recovery (day 18 and 25). Evans blue albumin or sodium fluorescein incubation of the sciatic nerve tested perineural permeability; qPCR and immunohistochemistry were used to assess expression and distribution of important TJPs and cytokines. A histomorphological assessment of the nerves was performed by resin embedding and staining of myelin sheaths with methylene blue. Fluorescence intensity was measured with ImageJ, while mRNA was quantified relative to GAPDH. Analysis were performed using Two Way ANOVA and Bonferroni post-hoc test, Kruskal-Wallis or unpaired t-test.

BTZ-injections evoked a significant lowering of nociceptive reflexive thresholds starting from day 4, reaching its maximum on day 11 and recovered completely after 2 weeks. In this model, mechanical hypersensitivity was accompanied by a perineural barrier leakiness for small molecules and a decrease in claudin-1 and occludin. G-ratio values for small nerve fibres increased, indicating an imbalance in the axon-myelin ratio. During the regeneration phase, barrier function was restored, TJP expression normalized and the anti-inflammatory *Il10* increased.

Endoneural integrity highly depends on controlled paracellular exchange of ions and molecules. TJs like claudin-1 guarantee protection from potentially harmful molecules and restrict the influx into the peripheral nerve. Barrier leakiness could allow for degenerative processes in small sensory nerve fibres. Regenerative mechanisms involve the upregulation of TJPs with restoration of the BNB and the increase of the barrier fixing *Il10*. Our data support previous data that early onset of BIPN is not driven by remarkable inflammatory processes, but rather small changes in the endoneural milieu. Pharmacological support of the early recovery

processes could prevent the need of BTZ reduction and improve myeloma treatment.
Funded by the Graduate School of Life Sciences and the University of Würzburg.

Symposium

S33: Genetic and environmental factors shaping neuronal network defects and cognitive impairment

S33-1 Creating and analysing mouse models to understand cognitive deficits in Down syndrome.
Elizabeth Fisher, Pishan Chang, Daniel Bush, Phillip Muza, Perez Gonzalez Marta, Hannan Saad, Smart Trevor, Tybulewicz Victor, Walker Matthew

S33-2 Interneuron-specific changes in the hippocampal GABAergic microcircuitry in a Down syndrome mouse model
Jan Michael Schulz, Jonas-Frederic Sauer, Rocio Mayoral-Torres, Josef Bischofberger

S33-3 Impaired top-down sensory processing in a genetic mouse model of schizophrenia predisposition
Torfi Sigurdsson

S33-4 Poor decision making and sociability impairment following central serotonin depletion in Tph2 knockdown rats
Lucille Alonso, Polina Peeva, Tania Fernandez del Valle Alquicira, Narda Erdelyi, Angel Gil-Nolskog, Susann Matthes, Michael Bader, York Winter, Natalia Alenina, Marion Rivalan

S33-5 Developmental mechanisms of schizophrenia: lessons from dual-hit genetic-environmental mouse models
Ileana Hanganu-Opatz

Creating and analysing mouse models to understand cognitive deficits in Down syndrome.

Elizabeth Fisher[1], Pishan Chang[1], Daniel Bush[1], Phillip Muza[1], Perez Gonzalez Marta[1], Hannan Saad[1], Smart Trevor[1], Tybulewicz Victor[2], Walker Matthew[1]

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[2]Francis Crick Institute,[2] London, United Kingdom

Down syndrome is the most common form of intellectual disability and arises from having three copies of human chromosome 21. The syndrome has distinct – and highly variable – deficits in particular cognitive domains. To understand this genetic disorder, we have created a set of mouse models that allow us to investigate the biology of Down syndrome and to map the underlying genes. By looking into the genes that are dosage-sensitive in Down syndrome and give rise to phenotypes, we may be able to target therapies for specific aspects of the disorder. Here, we describe the human condition, the mouse modelling, and our results so far in looking at the nervous system and behaviour, and underlying genetic deficits.

Interneuron-specific changes in the hippocampal GABAergic microcircuitry in a Down syndrome mouse model

Jan Michael Schulz[1], Jonas-Frederic Sauer[2], Rocio Mayoral-Torres[1], Josef Bischofberger[1]

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Down syndrome (DS) or Trisomy 21 is the most common congenital cause of mild to moderate intellectual disability. This form of intellectual disability is characterized by disruption of both working and explicit long-term memory including hippocampus-dependent episodic memory. Previous studies in mouse models suggest that increased GABAergic inhibition is a main factor for decreased synaptic plasticity, the cellular mechanism underlying memory.

To investigate the specific changes in inhibition, we analyzed $GABA_A$ receptor-mediated synaptic transmission in pyramidal cells in the CA1 subfield of the hippocampus of the Ts65Dn DS mouse model. Targeted electrical microstimulation of layer-specific inhibitory inputs in brain slices showed that GABAergic synaptic inhibition onto dendrites of hippocampal pyramidal cells is increased. By contrast, optogenetic stimulation of parvalbumin-positive (PV) interneurons showed very similar levels of perisomatic inhibition in Ts65Dn and wildtype (wt) littermates indicating normal GABA synapse density on the perisomatic compartment in Ts65Dn mice. However, the recruitment of PV interneurons in Ts65Dn mice was strongly increased in response to stimulation of the Schaffer Collaterals. This caused increased feedforward inhibition with an inhibitory to excitatory PSC ratio of 1.4 ± 0.2 in Ts65Dn mice versus 0.6 ± 0.1 in wt littermates ($P<0.01$). Importantly, *in vivo* silicon probe recordings of optogenetically-identified PV-interneurons in Ts65Dn mice showed significantly increased activity levels in in these soma-targeting GABAergic interneurons. These results suggest that increased recruitment of PV+ interneurons and increased feedforward inhibition may be an important cause for elevated inhibition in DS. Thus, both dendritic and somatic inhibition may be increased in Ts65Dn mice albeit due to different mechanisms.

Supported by grants from the Jerome Lejeune Foundation and Eucor – The European Campus

Impaired top-down sensory processing in a genetic mouse model of schizophrenia predisposition

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Schizophrenia is a complex and devastating psychiatric disease affecting approximately 1% of the population. Although the underlying causes are not well understood, disturbances in sensory processing likely play an important role in the pathophysiology of the disease. One sensory disturbance that has been consistently reported in schizophrenia patients is an impairment in the processing of self-generated stimuli. Much of the brain's sensory input, for example the sound of our own footsteps, is directly caused by our actions and must be distinguished from externally generated sensory input. It has long been suggested that the brain solves this problem using "corollary discharge" signals that represent the expected sensory consequences of movement, and which are subtracted from the actual sensory input. Consistent with this idea, electroencephalographic measurements in human subjects have consistently found that stimuli, including sounds, which are generated by the subject's behavior elicit smaller neural responses. In schizophrenia patients, in contrast, this attenuation is reduced in magnitude, which might contribute to the hallucinations and delusions observed in the disease. However, the neuronal circuit mechanisms underlying this sensory disturbance, and how it relates to the risk factors for the disease, is not known. To examine this, we have developed an experimental paradigm for examining the processing of self-generated sounds in the auditory cortex of the mouse. In this talk, I will describe experiments using this paradigm to reveal impairments in the processing of self-generated sounds in Df16+/- mice, which model one of the largest genetic risk factors for schizophrenia, the 22q11.2 microdeletion. I will also discuss our ongoing work examining disruptions in inter-areal communication that might contribute to these impairments.

Poor decision making and sociability impairment following central serotonin depletion in Tph2 knockdown rats

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Serotonin is known to be a critical modulator of animals' socio-cognitive abilities. However, in a previous study we found that the complete depletion of central serotonin in rats via the genetic deletion of the tryptophan hydroxylase 2 (TPH2), the rate limiting enzyme of serotonin synthesis in the brain, did not impair cognitive abilities but only social behaviour.
The aim of this project was to investigate if a mild acute decrease in central serotonin would affect rats' performances in decision making, cognitive flexibility, and social recognition memory using a novel rat model of inducible serotonin depletion, the TetO-shTph2 rats. In these transgenic animals, oral doxycycline administration induces expression of shRNAs against the rat Tph2 gene which results in a decrease of 25% of brain serotonin levels.
The TetO-shTph2 rats were tested in the Rat Gambling task (RGT), the reversed-RGT, the probability discounting task, and the social recognition test. Post-mortem measures of serotonin levels were done in the dorsal raphe, prefrontal and orbitofrontal cortices by HPLC and measures of TPH2 enzymatic activity were done in the dorsal raphe by enzymatic assay.
Our results indicate that the doxycycline treated TetO-shTph2 rats were more prone to poor decision making and that individuals identified as poor decision makers within the doxycycline treated TetO-shTph2 group were more sensitive to probabilistic discounting and had poorer recognition memory. Cognitive flexibility was unaffected by acute central serotonin reduction.
The new TetO-shTph2 rats are a promising model to study the effects of mild central serotonin decrease on decision making abilities in complex and risky situations of choice and on social recognition memory in otherwise healthy individuals. Decrease in central serotonin appears to specifically augment cognitive impairments of those individuals in the population that were identified as poor decision makers.

Developmental mechanisms of schizophrenia: lessons from dual-hit genetic-environmental mouse models

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Cognitive deficits, core features of mental illness, largely result from dysfunction of limbic networks. This dysfunction emerges already during early development, before a detectable behavioral readout, yet the mechanisms and cellular elements controlling the abnormal maturation are still unknown. Here we address this open question by combining in vivo electrophysiology, optogenetics, neuroanatomy and behavioral assays during development in mice mimicking the dual genetic–environmental etiology of psychiatric disorders. We report that pyramidal neurons in superficial layers of prefrontal cortex are key elements causing disorganized oscillatory entrainment of local circuits in beta-gamma frequencies. Their abnormal firing rate and timing relate to sparser dendritic arborization and lower spine density. Administration of minocycline during the first postnatal week, potentially acting via microglial cells, rescues the neuronal deficits and restores pre-juvenile cognitive abilities. Besides prefrontal dysfunction, the hippocampal and entorhinal activity patterns as well as their drive to prefrontal cortex are profoundly impaired during early stages of development. Elucidation of the cellular substrate of developmental miswiring causing later cognitive deficits opens new perspectives for identification of neurobiological targets, amenable to therapies.

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 Neuriendocrine 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

T1: Stem cells, Neurogenesis and Gliogenesis

Sequence heterochrony in insect brain development leads to an immature form of the central complex: a fly-beetle insight

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Animal species differ greatly in their adaptations of brain structure and function. Stark differences can even occur in one individual at different life stages, as is often the case for brains of larval and adult forms of holometabolous insects. All such differences occur during development, but the evolutionary mechanisms behind them remain poorly explored. Here, we want to present our work on two holometabolous insects, *Drosophila melanogaster* and *Tribolium castaneum*, where life stage differences between larva and adult, and differences between those species, are dramatically apparent. Interestingly, the central complex, fascilitating multiple behavioural elements behind spatial orientation, is conserved between species at the adult stage, but differs strongly between larvae and adults of one species as well as between larvae of different taxa. We will present our work involving genome editing and establishing transgenic lines which allowed us to visualize cells expressing the conserved transcription factor retinal homeobox. With such comparative transgenic lines, we labelled genetic neural lineages in both *Drosophila* and *Tribolium*. This approach was essential to compare the development of homologous neural cells between species from embryo to adult. We identified a much more complex picture of different types and degrees of heterochronies involving the central complex than originally assumed. These include heterochronic shifts in developmental events in embryonic and pupal stages. Moreover, we provide, to our knowledge, the first example of sequence heterochrony in brain development, where developmental steps changed their position within the developmental event progression. We show that through this sequence heterochrony, an immature developmental stage of the central complex gains functionality in *Tribolium* larvae. This immature larval form differs strongly from its adult counterpart, meaning that *Tribolium* has a specific set of central complex neuropils for each stage of active locomotion. We believe that the *Tribolium* larval central complex can be used in the future to characterise which behaviours can be facilitated with a functional, but immature, neuropil, promising to further shed light on the function and evolution of this intriguing brain area.

AT101 as a specific therapy against glioma stem-like cells: Mechanism of action in the microenvironment of the tumor

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Glioblastoma multiforme (GBM) is the most common and most malignant brain tumor in adults. Despite the standard therapy including surgical resection and adjuvant combined radio/chemotherapy, the average survival of GBM patients is still poor with 12-15 month. Thus, there is a compelling need to develop alternative treatment strategies in order to overcome the resistance against the preferred chemotherapeutic drug temozolomide (TMZ). A promising candidate for the treatment of GBMs is AT101, the R(-) enantiomer of gossypol.

The present study investigates the effects of AT101 on two GBM cell lines (U87MG and U251MG) with a focus on the effects on tumor stem-like cells, which are often found to mediate the development of tumor recurrences. The analysis comprised cytotoxicity assays and growth analysis, as well as the investigation of changes in signaling pathways and on the gene expression level. In addition, the role of the tumor microenvironment was analyzed by stimulating native cells with stem-like cell conditioned medium. The therapeutic response towards AT101 was thereby compared between treatment with TMZ or the combination of both.

AT101 was shown to induce strong cytotoxic effects on U251MG and U87MG stem-like cells in comparison to the respective native cells, while treatment with TMZ had only moderate effects on both native and stem-like cells. Furthermore, a higher sensitivity against treatment with AT101 was observed upon incubation of native cells with stem-like cell conditioned medium, indicating the role of the tumor microenvironment, especially the stem cell niche, on the therapeutic response of GBMs. The different responses of native cells and stem-like cells to treatment with TMZ, AT101, or a combination of both were also found to be reflected by a different activation of the Erk signaling pathway. Analysis of the expression level of various cytokine and chemokine receptors revealed that especially CXCR7 was found to be consistently downregulated in both cell lines upon stimulation of native cells.

Our findings indicate that AT101 represents an alternative drug for future GBM therapy. Since the tumor stem-like cells responded strongly to treatment with AT101, this might serve as a promising approach in order to kill the remaining tumor cells after resection more efficiently than the therapy with TMZ and thus, prevent the development of tumor recurrences.

Genetic therapies – what does the future hold for neurological disorders?

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AP-2 is a heterotetrameric complex comprised of α, β, μ, and σ; subunits that link clathrin and other endocytic proteins to sites of clathrin-mediated endocytosis. Full body knockout of AP-2(μ) in mice causes embryonic lethality before day 3.5 postcoitus (Mitsunari, T. et al., 2005). In contrast, depletion of AP-2(μ) in neurons results in postnatal neurodegeneration and defective synaptic vesicle recycling (Kononenko et al., 2014, Kononenko et al., 2017). However, it does not block plasma membrane retrieval during neuronal activity, questioning the canonical function of AP-2 in neurons and suggesting that AP-2 might perform different functions in mitotic versus postmitotic cells. Using a combination of biochemical, cell biology, and live imaging approaches, we show that AP-2 controls neuronal progenitor cells (NPCs) proliferation but is not required for neuronal differentiation. In wildtype NPCs, AP-2 can be found at the centrosomes, where it colocalizes with gamma-tubulin complex protein 3 (GCP3) subunit of γ-tubulin small complex (γTuSC). Using mass spectrometry analysis, we identified GCP2, and GCP3, as novel interaction partners of AP-2 complex in neuronal cells, where the interaction between the γTuSC and AP-2 was confirmed in co-immunoprecipitation studies. Deletion of AP-2μ in NPCs leads to abnormal centrosome morphology, multinucleation, cell cycle arrest, and altered microtubule dynamics. This phenotype was not reproduced in NPCs treated with clathrin inhibitor PitStop2, suggesting that the role of AP-2 at centrosomes is independent of its function in clathrin-mediated endocytosis. Surprisingly, AP-2 was not required in NPCs committed to becoming neurons, suggesting that AP-2 is a positive regulator of proliferative symmetric cell division in neuronal cells. Despite no differences found in differentiation, AP-2 KO NPCs reveal defective migratory behavior, which results in an accumulation of doublecortin-positive cells in the lateral ventricle and causes the disorganization of cortical structure in AP-2μ KO brains. Since γTuSC comprises the part of the large γ-Tubulin organizing complex, necessary for centrosome function during the cell cycle, our data suggest that AP-2 is required in neuronal mitotic cells for centrosome assembly during proliferative symmetric cell division, while AP-2 function in postmitotic neurons additionally includes the regulation of neuronal migration.

Role of C-terminal binding protein 1 in the regulation of adult neurogenesis

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Generation of adult born neurons and their integration into functional circuit is required for normal cognition and functioning of brain. During neurogenesis, quiescent neuronal stem cells become activated, proliferate, and go through cell-fate decisions to finally survive and acquire a mature phenotype. The whole process of neurogenesis is highly dependent on various transcriptional regulators. CtBP1 is a transcriptional co-repressor and a potential regulator of adult neurogenesis. Patients with CtBP1 R331W missense mutation show severe hypotonia, ataxia, and intellectual disability. However, the cellular and molecular mechanism behind these phenotypes are unknown. In the present study, we addressed possible role of CtBP1 in neurodevelopment by analyzing adult neurogenesis in CtBP1 knock out (KO) mice.

First, we immunohistochemically mapped the expression of CtBP1 in specific cell types of neurogenic lineage in the two major adult neurogenic niches (subgranular zone and subventricular zone) of mice. Next, we generated in-vitro neurosphere cultures of neuronal stem/progenitor cells from the subventricular zone of adult CtBP1 KO and WT mice. We utilized quantitative RT-PCR and immunostainings to characterize neural stem cell proliferation, cell-fate decision and differentiation in these cultured neurospheres. We observed differences in cell proliferation and cell-fate decision in the absence of CtBP1. Collectively, these results will shed light on the role of CtBP1 in the regulation of adult neurogenesis, and provide further insights into transcriptional regulations during this process.

Gene expression analysis of L1CAM deficient human cerebral organoids

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The cell recognition and adhesion molecule L1CAM plays a central role in the development of the nervous system. Mutations of the human L1CAM gene have been shown to cause neurodevelopmental disorders such as X-linked hydrocephalus, spastic paraplegia and mental retardation, collectively known as L1 syndrome (1). Mice lacking L1CAM show various phenotypic features of L1 syndrome. However, research is hampered by the rarity of L1 syndrome and the high prenatal and perinatal mortality in mouse models of L1 syndrome. In this study, we established a novel human cerebral organoid model of L1 syndrome using L1CAM-deficient human embryonic stem cells (H1-hESC) deficient in L1CAM (2). Gene expression analyses of organoids cultured for 60 days in vitro by quantitative real-time PCR or next generation RNA sequencing showed dysregulation in various signaling pathways such as neural precursor proliferation, cell adhesion and neuronal migration. These results suggest, that the manifestation of L1 syndrome occurs already during early neurodevelopment. Future studies will investigate selected neurodevelopmental signaling pathways between L1CAM-deficient human cerebral organoids and L1 syndrome model mice.

Morphological maturation and quantitative changes of microglia cell development in fetal cortex of European wild boar, *Sus scrofa*

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Knowledge on cortical development is largely based on laboratory rodent data. In contrast to these altricial nestlings, ungulates are born with nearly mature sensorimotor system. In particular, the pig is an emerging translational model for human neurodegenerative diseases. We started in recent years to systematically analyse fetal brain development of a non-domesticated ungulate using pre- and postnatal brains of wild boar derived from the forests of the Üfter Mark, Germany, as “breed of choice”. With respect to the maturation of the Neuropeptide Y neuron system in wild boar cortex we reported that the cell types resemble those seen in other mammals, however, the system typical for the adult cortex matures already during prenatal life (Ernst L. et al., Brain Struct Funct 223:3855-3873, 2018). Also recently, we qualitatively described the appearance of Iba-1+ microglia cells during wild boar dorsoparietal cortex development from E45 to P30. Extending this with material from E45 to P90, we now report firstly, that the major switch to a ramified morphology occurs prenatally between E85 and E100, two weeks before birth in pig. Second, prenatal microglial cells are phagocytically highly active as indicated by the presence of stout “polyp”-like (reminiscent of *Hydra spec.*) branches with long thread-like extensions and phagocytic cups. Third, nearest neighbor analyses reveal large average distances between labeled Iba-1+ cells within the laminar compartments at E45 and E60, followed by a substantial shortening of the cell-cell distance between E70 until E85, and a fairly constant average distance at the following ages E110, P5, P30, P90, which by and large is independent from the laminar compartment. Given the massive expansion and gyrification starting from E60, huge numbers of microglial cells presumably become added and integrated into the population to keep the tiling. Fourth, a quantitative assessment of Iba-1+ cells in the laminar compartments MZ, CP, gray matter, SP, IZ/WM and SVZ reveals that the percentage of Iba-1+ cells increases from E60 to P90. For instance, percentage increase in GM from 0.45 % at E60 to ~11 % of all nuclei/ROI at P90. These quantitative results suggest that microglial development proceeds fast during the third fetal month reaching near-adult status already before birth.

Expression of neural stem/progenitor cell-associated glycoepitopes in human cerebral organoids

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In the developing central nervous system, highly specialized cell types are generated from a pool of stem cells. This involves an orchestration of processes such as cell proliferation, differentiation, migration, axon growth and others. In this context, the extracellular matrix (ECM) is of interest, as it contains a huge variety of signaling molecules that are presented to the cells. Glycosylation, the attachment of carbohydrates to a protein, is one of several mechanisms by which the properties of ECM molecules are modified. Distinct motifs, recognized by monoclonal antibodies (mAbs), have been identified in the past that are associated with neural stem/progenitor cells. Among them are LewisX trisaccharide structures and the DSD-1 chondroitin sulfate epitope.

In our study we analyze cerebral organoids derived from human induced pluripotent stem cells (hiPSCs) as a model for very early steps of human central nervous system development (Kandasamy et al. 2017). We performed immunohistochemical stainings with the following mAbs: mAb 487^{LeX} binds terminal LeX motifs, 5750^{LeX} recognizes internal tandem repeats of the motif and mAb 473HD binds the DSD-1 epitope, a sulfation-dependent motif of long chondroitin sulfate glycosaminoglycan chains (Hennen et al. 2011; von Holst et al. 2006). These antibodies were combined with those for cell type-specific markers such as nestin, a neural stem cell marker.

We observed a differential expression pattern of the analyzed glycoepitopes. The 487^{LeX} epitope could be detected on cells in neural tube-like structures, which are formed by nestin-expressing neuroepithelial cells first and by radial glia later. The 5750^{LeX} signal was confined to the lumen of these areas. In contrast, the DSD-1 epitope was prominently expressed at the outer border of the neural tube-resembling structures.

The distinct expression patterns observed in the cerebral organoid model indicate individual functions of the glycostructures in the developing human central nervous system. Differences in the synthesis, transport or also in binding of the epitope-carrying ECM molecules are reflected by the localization of the attached carbohydrates. In the light of functional analyses, blocking experiments with the abovementioned mAbs added to the cell culture might provide more detailed knowledge in the future.

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Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

T2-1 Cell adhesion protein ALCAM interacts with the cytoskeletal protein ERM
Shahzad Munir, Christoph Pille, G. Elisabeth Pollerberg

T2-2 Markerfree axon segmentation with ResNets
Amir Madany Mamlouk, Philipp Grüning, Alex Palumbo, Svenja Kim Landt, Lara Heckmann, Leslie Brackhagen, Marietta Zille

T2-3 Investigating novel functions of brat (brain tumor) in synaptic connectivity
Nicole Kucharowski, Dietmar Schmucker

T2-4 NMDA-receptor subunit GluN2B is required for basal dendritic growth of cortical pyramidal neurons in an early time window
Steffen Gonda, Jan Giesen, Alexander Sieberath, Fabian West, Raoul Buchholz, Oliver Klatt, Tim Ziebarth, Michael Hollmann, Mohammad I. K. Hamad, Andreas Reiner, Petra Wahle

T2-5 Genetically encoded calcium indicators are calcium buffers and can impair dendrite growth of cortical neurons
Ina Gasterstädt, Alexander Jack, Lisa-Marie Rennau, Steffen Gonda, Petra Wahle

T2-6 Molecular mechanisms of structural maintenance and plasticity in neurons
Bahar Aksan, Jing Yan, Javier Sanchez-Romero, Dimitris Missirlis, Daniela Mauceri

Cell adhesion protein ALCAM interacts with the cytoskeletal protein ERM

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Axons of retinal ganglion cells (RGC) grow and navigate depending, among others, on an integral plasma membrane protein, Activated Leucocyte Cell Adhesion Molecule (ALCAM). ALCAM is activated by homophilic trans-interaction triggering intracellular signal pathways and its internalization. ALCAM interacts with intracellular linker proteins of the Ezrin- Radixin-Moesin (ERM) family, anchoring ALCAM to the cortical cytoskeleton. To measure this interaction in RGCs and Neuroblastoma (N2A), we performed double immunostainings which were evaluated by determining the Pearson Correlation Coefficients (PCC). Quantifications revealed that they interact, and that the PCC is higher for the phosphorylated subpopulation of ERMs. Activation of ALCAM by antibody-induced clustering resulted in a persistent increase of the PCC, indicating a boosted binding of activated ALCAM to ERMs. Mutations in the ERM phosphorylation site crucial for ALCAM binding, leading to a permanently phospho-mimicking form or an unphosphorylatable form, showed that the pseudo phosphoryled ERMs increased their interactions with ALCAM and decreased the endocytosis of ALCAM, indicating a protective effect of ERM binding against degradation of ALCAM. This is supported by the finding that the unphosphorylatable form of ERMs did not reduce ALCAM's endocytosis. Inhibition of Ezrin in retinal explants cultures where RGCs extend axons on ALCAM substrate induced massive growth cone collapse and axon retraction, demonstrating the importance of the Ezrin ALCAM interaction for axon elongation and maintenance. Together, these findings for the first time shed light on the crucial role of the ALCAM ERM interaction for axon growth and navigation, and might contribute to improve regeneration paradigms for injured axons.

Markerfree axon segmentation with ResNets

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Automated segmentation of axons in *in vitro* time-lapse recordings remains an important as well as a difficult task. A thorough study of the development and growth of these cells plays a crucial role in a whole range of important applications in medicine and science [1].
Due to their unusual morphology (elongated and highly branched cells), respective data analysis over a long period of time requires dedicated software tools that allow for the precise identification of axonal structures. At the same time, these software tools need to cope with a large amount of data available from imaging where manual inspection is time-consuming, prone to error, and impractical [2].

Most available software packages (such as NeuronMetrics, NeuriteIQ, NeuriteTracer, and NeurphologyJ) trace axonal structures semi-automatically and they require high-contrast images that are only available in fluorescence and not in marker-free phase-contrast microscopy. Unfortunately, fluorescence staining requires either fixation of the cells or genetic modulation, which is less efficient in primary cells and may alter the behavior of the cells as well.

Traditional image processing algorithms (global thresholding, Laplacian or Gaussian filters, and morphological operations) come with several drawbacks: i) They are static and do not react robustly to changes in data collection or the hardware used, ii) most of these procedures are adapted to a particular application scenario and it is unlikely that they generalize well across a wide range of experimental setups and questions, and iii) they are therefore semi-autonomous, i.e. user interaction is required before the data can be collected and automatically evaluated.

Deep Learning now allows searching for fully automatic approaches even in such scenarios. In this work, we used such an approach - based on the well-established ResNet50 [3] - to solve the axon tracing problem. We found that an ensemble of nets has better performance than a single net. Using an oracle approach, we can show that our approach already works close to the optimum. Furthermore, a comparison with segmentation variations across experts' labelings showed that we achieved a level of performance comparable to experts.

The ResNets are pre-trained on the so-called ImageNet dataset and are then trained on the new, labeled axon images through less extensive training. However, the amount of pre-labeled data required sometimes can be further reduced if other image data with similar modalities are also used for training - here, for example, segmented retinal blood vessels.

With these new capabilities, cell behavior can now be analyzed in a completely different way and to a previously unknown extent. This could be particularly crucial for the study of neurodegenerative processes,

for example, to better understand the effect of pharmaceuticals.

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Investigating novel functions of brat (brain tumor) in synaptic connectivity

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We are interested in identifying genes that are involved in specific neuronal wiring mechanisms such as axonal branching, axon maintenance, or synaptogenesis in Drosophila melanogaster. We use a genetic flip-out system that enables to label single cell mechanosensory neurons in the CNS. This system allows visualization of single neurons and its CNS connectivity with an axonal and pre-synaptic marker. It further allows to simultaneously knock-down genes of interest using RNA interference. Using this approach, we identified brat (brain tumor) to be involved in synaptic connectivity of ms neurons.

Brat's function was first described in early embryonic patterning during oogenesis, later studies also showed an important function as tumor suppressor. Brat is categorized as TRIM-NHL protein and its molecular function is dependent on its RNA binding capability which is thought to mediate translational control. Although brat's impact in neuronal precursors is well established, its function in postmitotic neurons is not known yet. We found that knocking down brat in postmitotic mechanosensory neurons alters axon branch patterning and results in aberrant synapse number and distribution. In addition, MARCM clones of different brat loss of function alleles confirmed the RNAi mutant phenotype.

We are now examining whether and how brat-dependent regulation of protein translation is important for axon branching and synaptic specification. We are particularly interested to determine whether local axonal translation might play a role in these brat-dependent processes.

NMDA-receptor subunit GluN2B is required for basal dendritic growth of cortical pyramidal neurons in an early time window

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The importance of calcium signaling and thus of NMDA receptors in the development of cortical neurons has been extensively studied. However, the effects of the individual receptor subunits on the growth of pyramidal cells and interneurons in terms of developmental time were less often examined.

Overexpression of specifically calcium-permeable or flip-spliced AMPA receptor variants or the type-I-TARPS has already shown that depolarization including calcium signaling promotes apical dendritic complexity. NMDA receptors contribute to this effect (Hamad et al., 2011, 2014) because the inhibition of NMDA receptor-mediated currents with APV blocked the growth-promoting effect of AMPA receptor subunits. This shows that NMDA receptors are involved in the growth of cortical neurons.

First, we used the biolistic overexpression strategy successfully employed to test the role of AMPA and kainate receptors (Hamad et al., 2011; Jack et al., 2019). Both constructs delivered currents in HEK-cells showing that both are capable of building functional receptors together with GluN1. Yet, the level of expression was low, and visualization required an immunohistochemical ‘enhancement’. Accordingly, overexpression of GluN2B and GluN2A in organotypic brain slice cultures from rat visual cortex did not increase dendritic complexity of pyramidal neurons.
Unraveling the role of specific subunits becomes possible with antagonists. We therefore inhibited GluN2B-containing receptors with ifenprodil and Ro25-6981, and GluN2A-containing receptors with the novel antagonists TCN201 or NVP-AAM077. In the earlier of the two (DIV 10 and DIV 20) postnatal time windows analyzed, the blockade of GluN2B-containing receptors led to a markedly reduced growth of the basal dendrites of the supragranular and infragranular pyramidal neurons. Apical dendrites were barely affected. This growth delay was recoverable within a few days after wash-out of the antagonists. Blocking the NR2A subunit did not affect the growth of the pyramidal cells. Further, multipolar interneurons did not react to any of the antagonists, which shows that other mechanisms are at work for the dendritic growth of this cell class. Together, GluN2B-containing receptors play a dominant role for basal dendritic growth in pyramidal neurons early after birth.

Genetically encoded calcium indicators are calcium buffers and can impair dendrite growth of cortical neurons

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Genetically encoded calcium indicators (GECIs) with different modifications and therefore different calcium affinities were designed over the recent years to achieve minimal invasive long-term calcium imaging. The calcium affinity of GECIs interfere with the calcium homeostasis and signaling in developing neuronal cells, indicating that GECIs are not only calcium indicators but also calcium buffers. In a pilot experiment, we reported earlier that in particular GCaMP3 expression is detrimental for neurons at 10 postnatal days. We now extended the analysis to five commonly used GECIs: GCaMP3, 5G, 6m, TN-XXL and the recently developed 6m-Xc with a shielded CAM domain. GECIs were biolistically expressed from postnatal day 3 to 22-25 in cortical neurons in organotypic slice cultures of rat visual cortex. First, a high percentage of GCaMP3 and especially TN-XXL transfected neurons show a nuclear accumulation of the indicator protein at day 25 compared to other tested GECIs, and nuclear filling has been discussed as a first step towards cellular degeneration. Second, reconstruction of GCaMP3 and TN-XXL pyramidal neurons, which still display a clear nucleus, reveals an impaired apical dendrite growth. Third, enlarging the sample of GCaMP5G expressing pyramidal cells reveals that infragranular neurons do also suffer from a delay in apical dendrite development, although the rate of nuclear-filled neurons has remained rather low compared to the GCaMP3 and TN-XXL conditions. Fourth, multipolar interneurons were affected by GCaMP3 overexpression resulting in decreased mean dendritic length. In contrast, a prolonged expression of GCaMP3 in already differentiated neurons from DIV 20-30 no longer caused any measurable morphological impairments. Fifth, expressing GCaMP6m and GCaMP6m-Xc has no measurable impact on dendritic arborization in immature and more differentiated neurons. Together, this indicates that GECIs with high calcium-affinity critically disturb sensitive calcium mechanisms linked to structural development in differentiating neurons. Moreover, hypomorphic dendrites can occur in the absence of nuclear filling suggesting that an aberrantly high calcium buffering at the level of the cytosol leads to developmental delays and also neuronal death. GCaMP6m and GCaMP6m-Xc can be declared as neuron-friendly. These results indicate that the use of certain GECIs in undifferentiated neurons, e.g. after transgenic overexpression, has to interpreted with caution.

Molecular mechanisms of structural maintenance and plasticity in neurons

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Pathological changes of the dendrite architecture are hallmarks of many neurological disorders. Therefore, it is crucial to understand the mechanisms of structural aberrations. Despite the fact that dendrites are mostly stable in adult neurons, little is known on the molecular mechanisms of dendrite maintenance and even less on its relation to structural plasticity. Previously, our group identified Vascular Endothelial Growth Factor D (VEGFD) – an angio- and lymphangiogenic factor- as a crucial factor for the maintenance of dendritic morphology and the ability to form long-term memories. Recent unpublished findings show that VEGFD acts like a molecular brake on neuronal morphology: normal expression of VEGFD maintains the dendritic architecture while VEGFD downregulation allows dendritic remodeling. How VEGFD mediates this phenomenon is, however, not known. We characterized the effect of VEGFD on the cytoskeleton by performing atomic force microscopy as well as time-lapse live imaging of actin and microtubules via fluorescent genetically-encoded markers in hippocampal neurons. Furthermore, we monitored the VEGFD-regulated dynamics of dendrite structure. Using a phosphoproteomic screen of cytoskeleton elements and their regulators we generated a list of potential VEGFD-regulated target proteins. We functionally characterized these proteins with gain and loss of function approaches and pharmacological tools and identified an actin-binding protein as a potential mediator of VEGFD signaling.
Our study revealed the mechanisms of VEGFD-mediated dendrite stabilization and thereby contributes to our understanding of pathological dendrite aberrations.

Poster Topic

T3: Developmental Cell Death, Regeneration and Transplantation

Regulation of neural differentiation by artificial polymers and extracellular matrix-derived peptides

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The lack of regenerative capability in the central nervous system is one of the major challenges nowadays and affects patients with traumatic brain injuries as well as patients with neurodegenerative diseases. The replacement of missing cells might be one of the suitable options to counter this problem. Research on hydrogels was performed to create a supportive environment for the injured tissue as well as for new integrated cells. Biocompatible hydrogels mimic the in vivo setting of the central nervous system regarding the stiffness and the 3D encapsulation and thus promote the survival, proliferation und differentiation of neural stem cells (NSCs) (Glotzbach et al., 2020). The modification of the hydrogel with extracellular matrix molecules with their bioactive motives promotes the viability of the stem cells whereby specific peptides and domains support different cellular processes, like proliferation or the differentiation into several neural cell types. The production of a hydrogel with a specific mix of domains and peptides might create a permissive microenvironment for NSCs to proliferate and differentiate into particular cell types and might lead to the replacement of the lost cells after injection into the injured tissue. One promising candidate for hydrogel modification is the glycoprotein tenascin-c (Tnc) which is highly expressed during neurogenesis and in the adult neural stem cell niche (Faissner et al., 2017). Its structure is a hexamer composed of one monomer with several fibronectin III (FNIII) domains, six of which are alternatively spliced in mice. Thereby up to 64 different isoforms of tenascin-c are possible. It is suggested that every isoform or domain combination has its own effect on the proliferation and differentiation behavior of NSCs.

The domain combination FNIII A_1D, CD and $A_{124}BCD$ were found to be expressed in neurospheres, wherefore these domains were analyzed regarding their ability to influence the proliferation, differentiation and migration of neural stem cells. The constantly expressed domain combination FNIII 78 served as a control. The domains were expressed in chinese hamster ovary (CHO) cells and linked to the Fc-fragment of the human IgG due to the special vector composition. The Fc-fragment improved the selective purification and detection of the proteins and ensured a dual appearance of FNIII domain per Fc-fragment. The expression in the eukaryotic CHO cells favored the correct folding and glycosylation of the proteins for an optimal functional morphology. Furthermore, a new hydrogel system with the polymer acryloylmorpholin in combination with stable and degradable crosslinkers was investigated. Therefore, the effect of the stable cationic molecule trimethylaminoethylacrylat (TMAEA) and bioactive motives like the RGD peptide or laminin was tested (Sallouh et al., 2017). In 2D and 3D cultures the adhesion and survival of the glioblastoma cell line U251MG and of NSCs were analyzed. The first experiments revealed that a certain concentration of cations and the addition of bioactive motives were important for the adhesion and the survival of the cells. In future studies, promising FNIII domains will be integrated into a biocompatible hydrogel on acryloylmorpholin-basis and the survival, proliferation, differentiation and migration will be investigated. The combination of a hydrogel with bioactive molecules which trigger particular cellular processes might be a promising tool for regenerative medicine.

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

T4-1 Neural representation of sequence coding in working memory
jacob Khezri, Moein Esghaei

T4-2 Modelling epilepsy seizures in vitro: A kainic acid-induced seizure model for studying the effect of IL-6 on the characteristics and functionality of hPSC-derived cortical neurons
Johanna Lotila, Ropafadzo Mzezewa, Heikki Kiiski, Jukka Peltola, Sanna Hagman, Susanna Narkilahti

T4-3 Simultaneous measurement of neurotransmitters in murine microdialysate by liquid chromatography tandem mass spectrometry
Christin Helmschrodt, Susen Becker, Stefanie Perl, Anja Schulz, Angelika Richter

Neural representation of sequence coding in working memory

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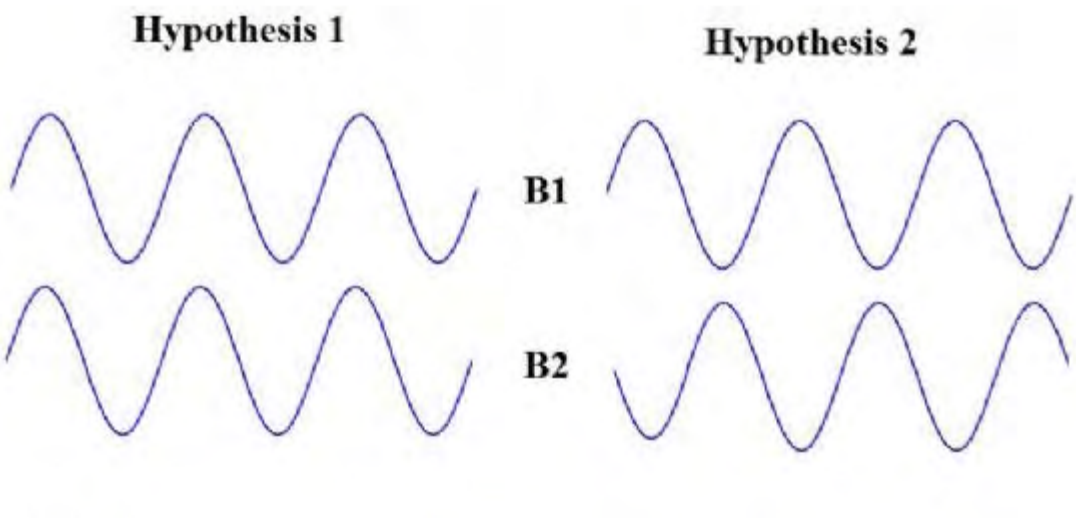
Working memory (WM) enables species to maintain task relevant information in the absence of sensory stimuli. Ongoing neuronal oscillatory activities have been suggested to play a key role in encoding of WM contents, especially in how a specific sequence of entities is internally maintained. Nevertheless, the exact neural mechanism underlying the maintenance of a specific sequence is not yet unveiled.
We analyzed Magnetoencephalography (MEG) data from the human connectome project dataset, recorded from 82 human subjects while performing a 2-back WM task (1). Subjects were presented with consecutive images of tools and faces, where they were asked to report if each image matched the one shown two pictures before. Each picture was shown for an interval of 2 seconds, followed by a 500 ms blank period. The subjects had to report if a given image did or did not match the image preceding the last, by pressing two different buttons.
There are two alternative hypotheses to how the differential sequences in B1 and B2 are encoded; 1) either each memory item is aligned to a constant phase across B1 and B2, or 2) the two items relocate their phases with each other. Here we examine which of the above two hypotheses is the most physiologically plausible one (figure 1).
We focused on pairs of consecutive blank periods (named B1 and B2 here) fulfilling the following criteria: 1. the two pictures maintained in WM were the same across the two blank periods 2. the sequence of the two pictures was the opposite between the two blank periods. To evaluate the hypotheses, we z-scored the LFPs within each pair of B1 and B2 relative to B1 and subtracted them time point by time point for each site. We found the PSD of B2-B1 shows a dominant alpha-beta (6-14) component peaking significantly in patches of parietal areas. Equalization of the power between B1 and B2 led to a similar result, suggesting that the main component of the difference between B1 and B2 is a difference in their phases. This suggests that a specific sequence of items is represented via the configuration of instantaneous phases of the ongoing oscillations.

To calculate the magnitude of the phase shift we aligned B1s to the peak of the alpha-beta in all trials and averaged B1s and B2s for hit and miss trials separately. We next computed the phase of each averaged signal using the Hilbert transform. Hit trials showed a large phase shift between B1 and B2 (about 90 degrees) while this phase shift is about zero in miss trials meaning the sequence was not encoded properly.
In conclusion, our results suggest a mechanistic role of the alpha-beta phase in the temporal representation of working memory items. How exactly a specific item may be bound to a phase remains a question for future studies.

1. Van Essen DC, Smith SM, Barch DM, Behrens TEJ, Yacoub E, Ugurbil K, et al. The WU-Minn Human Connectome Project: an overview. Neuroimage. 2013;80:62-79.


Hypothesis 1
Hypothesis 2
B1
B2

Modelling epilepsy seizures in vitro: A kainic acid-induced seizure model for studying the effect of IL-6 on the characteristics and functionality of hPSC-derived cortical neurons

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Background and aims: Epilepsy is a common neurological disorder characterized by repetitive, unpredictable epileptic seizures. Neuroinflammation and abnormal inflammatory cytokine levels have been associated to epilepsy. Animal and clinical studies have revealed that the levels of interleukin-6 (IL-6) cytokine are elevated after seizures. However, the knowledge of the roles of IL-6 in seizures and its functions on human neurons is minimal. Human pluripotent stem cell (hPSC) -derived neurons are promising tool for in vitro modelling of epilepsy or seizure-like activity. In this study, we developed a kainic acid (KA)-induced seizure model, and studied the effect of IL-6 on the characteristics and functionality of hPSC-derived cortical neurons.

Methods: hPSC-derived cortical neurons were used to model seizure-like activity induced by KA, an analog of the excitatory neurotransmitter glutamate, and the functional activity was investigated with microelectrode arrays (MEAs). The effects of IL-6 and Hyper-IL-6 fusion protein treatment on the viability and seizure-like activity of functionally maturated neuronal networks were studied. Cell viability LIVE/DEAD assay was used to study the effects of KA and cytokine treatments. Moreover, the gene and protein expression levels of IL-6 receptors, IL-6R and glycoprotein 130 (gp130), were studied with quantitative PCR (qPCR) and immunocytochemistry (ICC), respectively.

Results: Functionally mature hPSC-derived cortical neuronal networks responded to KA treatment with an excessive bursting phenotype, but KA treatment was not cytotoxic for the cells. Neuronal cultures expressed both IL-6 receptors, IL-6R and gp130, at the gene and protein level. During maturation of neuronal networks, gene expression level of IL-6R increased. Treatments with IL-6 or Hyper-IL-6 were not cytotoxic for the cells, but increased the gene expression levels of IL-6R. Cytokine treatments did not alter neuronal network activity or KA-induced seizures.

Conclusions: This study showed that hPSC-derived cortical neurons are valuable for modelling in vitro KA-induced seizures in epilepsy. Furthermore, expression levels of IL-6R were also increased after IL-6 and Hyper-IL-6 treatments suggesting that human neurons can respond to IL-6 through classical and trans-signaling pathways. However, we could not show any effect of IL-6 cytokines on the functionality of neuronal networks, suggesting that IL-6 does not affect the induction or modulation of KA-induced seizures. All in all, we propose this model as a valuable tool for research of seizure-like activity in neuronal networks in vitro.

Simultaneous measurement of neurotransmitters in murine microdialysate by liquid chromatography tandem mass spectrometry

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The investigation of neurotransmitter alterations in neurologic disorders requires sensitive multiplex analytical methods. Microdialysis enables to monitor neurotransmission into the extracellular space of freely moving animals. A liquid chromatography-tandem mass spectrometric (LC-MS/MS) method was developed to quantify the following 11 neurotransmitters and metabolites: serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), melatonin (ME), dopamine (DA), levodopa (L-DOPA), 3-methoxytyramine (3-MT), norepinephrine (NE), epinephrine (EP), acetylcholine (ACh), choline (Ch), and y-aminobutyric acid (GABA).
Chromatographic separation of the neurotransmitters was performed using a Kinetex 2.6 µm biphenyl column (150 x 3.0 mm) with gradient elution coupled to an API-QTrap 3200 (SCIEX) tandem mass spectrometer in positive electrospray ionization mode. Microdialysate samples were mixed with 0.5 ng of isotopically labelled standards for analyte quantification.
A rapid LC-MS/MS method was developed and validated for the simultaneous analysis of monoamines, their precursor and metabolites, as well as ACh, Ch and GABA in murine microdialysate within 7.0 min (Helmschrodt et al., 2020, Anal Bioanal Chem.; 412(28): 7777-7787). The limit of detection in artificial CSF ranged from 0.005 ng/ml (ME) to 0.75 ng/ml (NE and GABA). A comprehensive preanalytical protocol was validated. Recovery was between 87 and 117 % for neurotransmitter concentrations from 0.6 to 45 ng/ml with an inter-day accuracy of below 20 %. Basal neurotransmitter values were determined in the striatum of mice over a time period of three hours. First measurements were performed to achieve basal levels in the DYT1 knock-in (KI) mouse, a model of dystonia.
This LC-MS/MS method, including a short and gentle sample preparation, is suitable for simultaneous measurements of neurotransmitters in murine cerebral microdialysate. It enables the determination of basal neurotransmitter levels in specific brain regions to detect disease-related and induced neurochemical changes, as will be done in ongoing examinations in DYT1 mice including effects of deep brain stimulations.

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Poster Topic

T5: G Protein-linked and other Receptors

T5-1 The antidepressant clomipramine induces scratching behavior in mice and itch in individual human subjects
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T5-2 Influence of the alternative trkB receptor activation via 7,8-dihydroxyflavone on trkB overexpressing PC12 cells
Alice Lipinski, Stefan Wiese

T5-3 Neuropathic pain: characterization of adrenoceptor downstream signaling in Spinal Glial cells
Elisa Damo, Manuela Simonetti, Rohini Kuner

T5-4 Molecular and behavioral evaluation of Trojan Exon lines with regard to octopamine function in *Drosophila* larvae
Alexandra Großjohann, Ronja Badelt, Ines Köhler, Dennis Pauls, Andreas S. Thum

The antidepressant clomipramine induces scratching behavior in mice and itch in individual human subjects

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Background&Aims: Pruritus, in simple words itch, is an agonizing sensation and can be one symptom of (pseudo-)allergic drug reactions, which is not restricted to the drug application site but is processed via the spinal cord and the brain. Thus, itch sensation in (pseudo-)allergic drug reactions is modulated by an interplay of mast cells and neuronal cells. MRGPRs, also known as “itch receptors”, are a family of GPCRs expressed on both, mast cells and neuronal cells. Up to now, it remains largely elusive i) if MRGPRs are key players in the interchange of mast cells and neuronal cells and ii) how the organism benefits from expression of MRGPRs on both cell types. Therefore, we aimed to shed light on their role in (pseudo-)allergic drug reactions using the tricyclic antidepressant clomipramine.

Methods: The pruritogenic potential of clomipramine was investigated by assessing scratching behavior in C57BL/6 and MRGPRB2 mutant mice upon intradermal injection of the antidepressant. Activation of MRGPRs in HEK293, mast cells, and dorsal root ganglia neurons (mDRGs) by clomipramine was measured by changes in cytosolic free calcium $(Ca^{2+})_i$ using ratiometric fluorimetry. Mast cell degranulation was determined by means of a β-hexosaminidase assay (ex vivo) and an Evans blue extravasation assay (in vivo) proving local cutaneous anaphylaxis. In healthy volunteers (n = 5), the itch intensity upon intradermal application of clomipramine was quantified on a visual analogue scale. Blood flow was assessed using laser speckle contrast imaging and wheal and flare size were measured using a ruler.

Results: The antidepressant clomipramine effectively and dose-dependently induced scratching behavior in mice. Additionally, it activated murine peritoneal mast cells as well as mDRGs and evoked mast cell degranulation ex and in vivo. In MRGPR-expressing HEK cells, we identified Mrgprb2 and Mrgpra1 as murine target receptors for clomipramine. Scratching behavior was significantly reduced in Mrgprb2 mutants compared to wild-type mice. Moreover, clomipramine activated the human orthologue MRGPRX2 and primary human mast cells in a dose-dependent manner. Intradermal injection of 5 mM clomipramine in five subjects revealed a clear wheal-and-flare reaction and enhanced blood flow in all subjects, while itch sensation varied individually.

Conclusion: Our data strengthen the knowledge about MRGPRs in pseudo-allergic drug reactions. Agonists

of these MRGPRs contribute to IgE-independent mast cell activation and pruritus.

Influence of the alternative trkB receptor activation via 7,8-dihydroxyflavone on trkB overexpressing PC12 cells

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Background: Neurotrophins are essential regulators of cellular processes like proliferation, differentiation or neuronal survival. Thus, the interest in treatment of neurodegenerative diseases by using the activation of neurotrophic receptors is consecutive growing. The neurotrophin BDNF activates the trkB receptor, which is expressed in neuronal cells and exert its effects through tyrosine kinases. It is known that the activation of trkB activates intracellular signaling pathways like Ras/Raf/MAPK and PI3K/Akt, supports the survival of neurons and has an influence on the synaptic transmission. Unfortunately, BDNF is unsuitable as a candidate for disease treatment because of its inability to cross the blood brain barrier as well as its short half-life period. An alternative trkB activation in the absence of BDNF could be the use of the trkB agonist 7,8-dihydroxyflavone (7,8-DHF). We could already show that 7,8-DHF is able to enhance neurite outgrowth as well survival of motoneurons (Tsai et al., 2013). To examine trkB and its possible alternative activation ways in more detail, we generated a PC12 cell line ($PC12^{trkB}$) which is overexpressing human trkB. With different assays we investigated the impact of BDNF independent trkB activation via 7,8-DHF on differentiation, proliferation, apoptosis, viability and signaling activation in our new cell line $PC12^{trkB}$.

Methods: Cells were stimulated with BDNF and different concentrations of 7,8-DHF (0.4 pM - 4 nM) for a different length of time. With DAB staining the average neurite length and the number of neurites were determined after 1 and 5 div, as well the amount of caspase 3 positive cells after 2 div. The proliferation was analyzed by immunological detection of the proliferation markers Ki67, PHH3 and BrdU after 2 div. Furthermore, the viability was investigated after a trypan blue staining whereas the activation of the signaling pathways Ras/Raf/MAPK and PI3K/Akt was tested by western blot analysis. Groups were statistically analyzed via the *students t*-test with Graph Pad Prism.

Results: We could successfully verify the overexpression and the activation of the trkB receptor via 7,8-DHF immunologically and on molecular level with western blot analysis. Assays revealed that 7,8-DHF can significantly lead to an enhanced neurite outgrowth with longer and more neurites especially after 1 div ($p<0.001$). The apoptotic rate was affected concentration-dependent by 7,8-DHF. While 0.4 pM and 4 nM increased the apoptosis ($p<0.001$), all other concentrations reduced it in the same intensity as BDNF ($p<0.001$). Concerning the proliferation rate 7,8-DHF can reduce the amount of Ki67 and BrdU positive cells significantly ($p<0.001$), whereas no significant differences could be observed for PHH3. The viability was mostly significant unaffected by 7,8-DHF. Western blot analysis revealed only a light activation of the Ras/Raf/MAPK and PI3K/Akt pathways after stimulation with 7,8-DHF.

Discussion: In this study, we could demonstrate that the alternative activation of trkB via 7,8-DHF can lead to an enhanced differentiation, but a reduced proliferation of $PC12^{trkB}$ cells. It is also able to support survival, but dependent on concentration. In conclusion, 7,8-DHF can mimic some BDNF traits in our cell line. Furthermore, $PC12^{trkB}$ cells could be used for further studies regarding trkB analysis.

Neuropathic pain: characterization of adrenoceptor downstream signaling in Spinal Glial cells

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Neuro-glial interaction in the spinal cord is important for the development of neuropathic pain after peripheral nerve injury. Injured neurons release many molecules, among those ATP and chemokines, which lead to the activation of microglia. Activated microglia show an increased production of neuroactive and pro-inflammatory factors and the entrance of calcium through the P_2X_4 channels augments the phosphorylation of the mitogen-activated protein kinases (MAPKs), such as p38, ERK and JNK, which are known to participate to central sensitization, astrocytic activation and generation of pain hypersensitivity. It is known that SNRI antidepressants exert analgesic effect in neuropathic condition throughout a not well-defined mechanism. Several preclinical studies using acute, inflammatory and neuropathic pain models, have demonstrated that the descending noradrenergic pathway is crucial to reduce pain at the spinal cord level. Indeed, administration of specific adrenoceptor agonists results in an analgesic effect in mouse models as well as in patients. Multiple mechanisms are implicated in the anti-nociceptive effects of noradrenaline in the spinal dorsal horn, involving both neurons, glial cells and their crosstalk.
However, the precise mechanism of action of glial adrenergic receptors activation in neuropathic pain remains elusive, especially how activation of their downstream signaling in microglial cells influences neuronal activity and synaptic plasticity in the spinal cord.
Here we show that the administration of Clonidine, a specific α_2-adrenoceptor agonist, and Formoterol, a β_2-adrenoceptor agonist, exerts an analgesic effect in neuropathic-model mice. We demonstrate that the treatment of male mice undergone spared nerve injury (SNI) surgery, with intraperitoneal injection of Clonidine administrated three days after SNI operation, was able to significantly revert the increased number of microglia in the ipsilateral spinal dorsal horn, compared to the control saline-injected SNI-operated mice. Furthermore, Clonidine injection is able to block partially the microglial reaction reducing the level of phosphorylation (i.e. activation) of microglial activation markers, namely TAK1, p38 and JNK. Additionally, we show that six days after operation, a single injection of Formoterol reduces the mechanical sensitivity in neuropathic-model mice, effect that is even more pronounced after a second injection given three weeks after operation. Our results demonstrate that α_2-adrenoceptor agonist exerts analgesic effects in a mouse neuropathic pain model, at least partially affecting the activation of microglia in the spinal dorsal horn. Moreover, we show that also the activation of β_2-adrenoceptor using a specific β_2-adrenoceptor agonist partially relieves the mechanical sensitivity developed in SNI-operated mice.
In conclusion, our study supports the analgesic effects of activation of adrenoceptors in neuropathic pain conditions and suggests a mechanism involving the silencing of spinal microglia cells. Our study suggests the activation of microglial cells as a candidate target for an interventional approach using adrenoceptor agonists to reduce neuropathic pain development after peripheral nerve injury.

Molecular and behavioral evaluation of Trojan Exon lines with regard to octopamine function in *Drosophila* larvae

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The invertebrate equivalent of noradrenaline, octopamine, has been reported to play a role in a wide spectrum of physiological and behavioral processes. Accordingly, in adult and larval *Drosophila* octopamine and its related receptors are analyzed to gain further mechanistic insight into the nervous system. The novel genetic tool 'Trojan Exon' by Diao et al. (2015) utilizes a *Minos* mediated integration cassette (MiMIC) and viral T2A factor to promote the translation of a reporter protein product and the related gene of interest from a single transcript. Depending on the insertion site in the gene, a truncated, mutated version of the protein results. However, the expression of the reporter should correspond to the endogenous expression of the related gene. Behavioral analysis of 6 new Trojan octopamine and octopamine receptor lines available at Bloomington stock center shows that only some of the described effects can be reproduced at the developmental, behavioral and anatomical level. Our molecular analysis reveals that in some cases, this was based on the absence of the Trojan exon or additional inserts at the Trojan exon insertion site. Overall, we recommend the Trojan exon method as a possible alternative to perform gene-behavior correlations in *Drosophila* larvae. However, before experiments can be performed, the molecular organization of the respective constructs must be verified, as some lines may contain genetic alterations.

Poster Topic

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

T6-1 Insights into metabolic reprogramming in glioblastoma multiforme: Intratumoral distribution of lactate and the monocarboxylate transporters 1 and 4 and their relationships to other tumor progression-associated processes
Carolin Kubelt, Sönke Peters, Hajrullah Ahmeti, Monika Huhndorf, Lukas Huber, Gesa Cohrs, Jan-Bernd Hövener, Olav Jansen, Michael Synowitz, Janka Held-Feindt

T6-2 Azobenzene photo-switch elicits TRPV4 and TRPA1 calcium signalling in primary astrocytes and heterologus
Diletta Spennato, Massimo Baroncini, Emanuela Saracino, Francesco Formaggio, Roberta Fabbri, Marco Caprini, Roberto Zamboni, Alberto Credi, Valentina Benfenati

T6-3 Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons
Udo Kraushaar, Dominik Loser, Maria G Hinojosa, Jonathan Blum, Jasmin Schaefer, Markus Brüll, Ylva Johansson, Ilinca Suciu, Susanne H Bennekou, Iain Gardner, Timm Danker, Anna Forsby, Marcel Leist

T6-4 The impact of Iba1 silencing on P2x7 functioning – a comparison between intracellular Ca^{2+} transients in BV2 microglia and SNL-activated endogenous macrophages
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T6-7 Novel insights into Kv1 complex assembly in myelinated axons
Nina Kozar, George Kanatouris, Ella Mercer, Dies Meijer

T6-8 Age- and region-dependent changes in intracellular Na^+ and ATP induced by chemical ischemia in mouse forebrain

Insights into metabolic reprogramming in glioblastoma multiforme: Intratumoral distribution of lactate and the monocarboxylate transporters 1 and 4 and their relationships to other tumor progression-associated processes

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Amongst all malignant primary brain tumors, glioblastoma multiforme (GBM) is the most common and most malignant. To develop the aggressive properties responsible for the poor prognosis, tumor cells can undergo metabolic reprogramming (mr), leading to an oxygen-independent glycolysis. This adjustment of metabolism permits a rapid tumor growth, despite a lack of energy substrates. Hence, mr depicts a promising therapeutic target in GBM. In connection with the distribution or membrane passage of lactate produced by oxygen-independent glycolysis, proton-coupled monocarboxylate transporters (MCT) have been observed. The transporters, MCT1 and MCT4, in particular are overexpressed in GBM cells. Only a few studies focus on their role and connection to other tumor progression related processes in GBM till date. In addition, only little is known concerning a possible connection between MCT expression and changes in multi voxel magnetic resonance spectroscopic imaging (MRSI), a noninvasive method for assessing local metabolites. Thus, the aim of this study was to gain more insights into the molecular mechanisms involved in mr. Understanding the underlying mechanism could help contribute to improve diagnosis, therapy and ultimately prognosis of GBM patients in the future.

We examined the distribution of lactate in GBM patients by MRSI and ELISA. Furthermore, we investigated the expression and cellular localization of MCT1, MCT4 and of several markers associated with tumor progression by quantitative PCR and immunofluorescence double-staining in human GBM ex vivo tissues.

The highest lactate concentration was observed at the center of the vital parts of the tumor. MCT1 (p: 0.025) showed a higher gene expression at the center of the tumor compared to the edge. Whereas glial acidic fibrillary protein (p:0.010), the epithelial to mesenchymal transition (EMT) marker ß-catenin (p: 0.039), and the stem-like cell markers, krüppel-like factor4 (p: 0.035) and octamer-binding transcription factor4 (p: 0.026), showed an increased expression at the edge of the tumor. According to the regional gene expression differences of the investigated genes, three main GBM groups could be distinguished.
MCT1 and MCT4 were found on cells undergoing EMT and on tumor stem-like cells. GBM cells which expressed cellular dormancy markers, showed positive staining for MCT4.

Our findings indicate the existence of individual differences in the regional distribution of MCT1 and MCT4 and suggest that both transporters have distinct connections to GBM progression processes, which could contribute to the drug resistance of MCT-inhibitors.

Azobenzene photo-switch elicits TRPV4 and TRPA1 calcium signalling in primary astrocytes and heterologus

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Several studies emphasize the major role of astrocytic intracellular calcium (Ca2+) signaling in brain function such as homeostasis and modulation of synapse as well as in acute or chronic pathologies such as Epilepsy and Alzheimer. Novel tools to modulate of astrocytic calcium (Ca2+) signaling might be relevant to elucidate the mechanisms underpinning astrocytic role in information processing and in pathologies.
Among ion Ca2+ channels underpinning extracellular calcium influx in astrocytes members belonging to the transient receptor potential super family channels (TRP), called Ankirin 1 (TRPA1) and Vanilloid 4 (TRPV4) are of interest of astrocytic ion current and calcium (Ca2+) signaling Azobenzene (Azo) is a molecule that can be switched from its stable trans to metastable cis configuration by light stimuli. Recent evidence indicated that Azo-derived molecular photoswitches can modulate the functionality of neurons. However, it is still unknown whether Azo also specifically affects the astrocytes function. Here, we investigate the effect of Azo in primary cultured astrocytes. By means X-Rhod-1 Ca2+ imaging, we found that photostimulation of Azo elicits Ca2+ signalling in astrocytes. The effect was dependent on the concentration of Azo in the bath. Pharmacological analyses revealed that extracellular Ca2+ influx, intracellular Ca2+ release and the function of the channels TRPV4, TRPA1 are critical for the Azo-induced Ca2+ signal. Experiments in heterologous expression confirmed that TRPA1 and TRPV4 are activated by Azo. Structure-function analyses were performed by using a set of Azo derivatives to describe mechanisms beyond the observed effect. Our results indicate that the photoswitching of Azo could represent a powerful tool to trigger and modulate astroglial Ca2+ signalling and membrane currents, thereby opening a new perspective to study astrocytes role in brain function and dysfunction.
The work was supported by AFOSR grants Astronir (FA9550-17-1-0502) and Astrolight (FA9550-20-1-0386)

Functional alterations by a subgroup of neonicotinoid pesticides in human dopaminergic neurons

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Neonicotinoid pesticides, originally developed to target the insect nervous system, have been reported to interact with human receptors and to trigger signal transduction on rodent neurons. Therefore, we evaluated potential signaling effects on human neurons.

We used here SH-SY5Y neuroblastoma cells as established model of nicotinic acetylcholine receptor (nAChR) signaling. In parallel, we profiled dopaminergic neurons, generated from LUHMES neuronal precursor cells, as novel system to study nAChR activation in human post-mitotic neurons.
Changes of the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) were used as readout, and key findings were confirmed by patch clamp recordings of LUHMES neurons.

Nicotine (1 µM) triggered typical neuronal signaling responses. Antagonists, like tubocurarine and mecamylamine, attenuated the responses. Data obtained with several pharmacological tool compounds suggest a functional expression of α7 and non-α7 nAChRs on LUHMES cells.
In this novel test system, the neonicotinoids acetamiprid, imidacloprid, clothianidin and thiacloprid, but not thiamethoxam and dinotefuran, triggered a signaling response at 10-100 µM.
Strong synergy of the active neonicotinoids with the positive allosteric modulator of α7 nAChRs PNU-120596 suggests the involvement of the nAChR in this effect. Further evidence was provided by complete inhibition of this effect by tubocurarine.
The findings could be confirmed in SH-SY5Y cells, another commonly used model. To provide a third line of evidence for neonicotinoid signaling via nAChR in LUHMES neurons, we studied cross-desensitization: pretreatment with the four active neonicotinoids (at 1-10 µM) blunted the signaling response of nicotine. The pesticides (at 3-30 µM) also blunted the response to the non-α7 agonist ABT 594.

These data show that human neuronal cells are functionally affected by low micromolar concentrations of several neonicotinoids via activation of their α7 and non-α7 nAChRs.

The impact of Iba1 silencing on P2x7 functioning – a comparison between intracellular Ca^{2+} transients in BV2 microglia and SNL-activated endogenous macrophages

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Introduction: Considering the significant gene expression resemblance between microglia and peripheral nerve resident macrophages, we conducted a comparison on the impact a specific silencing of the cytoskeleton Iba1 protein might have on the functioning of P2x7 receptors expressed in BV2 microglial cells (*in vitro* system) versus SNL-activated endogenous macrophages (*in vivo* system). P2x7R are purinergic receptors closely connected to cytoskeleton, which upon ATP-induced activation triggers fast cytoskeleton rearrangements and an intracellular cascade of pro-inflammatory effects. Even though Iba1 protein is not directly coupled with the signalling complex of P2x7 receptor, we hypothesize that its silencing might still modulate the P2x7R kinetics by altering the cytoskeleton architecture.

Materials and methods: BV2 cells were transfected with two-customized siRNA molecules (Iba1 siRNA and scramble siRNA) using Lipofectamine RNAiMAX in Optimem and maintained in culture for 72h until Ca^{2+} imaging recordings. Rat Dorsal Root Ganglia (DRG) were harvested at 5 days after performing Spinal Nerve Ligation (SNL) model of neuropathic pain or after SNL + intra-ganglionic delivery of 4µl Iba1 siRNA. The experiments were carried out in L5 DRG; afterwards, rat primary-isolated macrophages were separated using immunopanning protocol and cultured for 13-15h. All the experiments were approved by the Ethics Committee of the University of Bucharest. In both experimental setups, P2x7 receptors were activated by one brief (20 sec) application of 300 µM native agonist of the P2x7 receptor, Benzoyl ATP (BzATP), and the resulting intracellular changes in $[Ca^{2+}]$ were recorded using ratiometric Ca^{2+} microfluorimetry with Fura-2 (340nm/380nm excitation wavelengths). In order to confirm the P2x7R expression in Iba1-deficient microglia and macrophages, double immunostaining experiments using antibodies against P2x7 were conducted.

Results: The P2x7R response was quantified by analyzing 4 specific parameters: $\Delta F/F_0$, time to 50% decay, decay slopes and area under the curve. In BV2 microglia, the P2x7R response exhibited higher amplitude, longer duration and slower recovery to baseline in Iba1 siRNA condition, corresponding to larger $[Ca^{2+}]_i$ inside the Iba1 siRNA transfected cells (0.8350 ± 0.01967, n=540) as compared to scramble siRNA condition(1.043 ± 0.03596, n=388, $P<0.0001$). In SNL-activated endogenous macrophages, the P2x7R response showed: lower amplitude, shorter duration and faster recovery to baseline, corresponding to a lower $[Ca^{2+}]_i$ after Iba1 siRNA treatment + SNL (4.174 ± 0.3338, n=63) as compared to SNL condition (6.456 ± 0.759, n=27, $P<0.05$).

Conclusion: Iba1 silencing has opposite effects on P2x7 functioning depending on the experimental setup, with significant increase for all the parameters *in vitro*, and significant decrease for most of the parameters *in*

vivo. Our results confirm that *in vitro* data cannot be automatically extrapolated to *in vivo* condition.

I_{Nav} currents during sciatic nerve reconstruction guided by a Nerve Regeneration Assistance System (NerveRAS)

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Introduction: In most cases, the regeneration process after minor peripheral nerve injuries or sectionings is too slow for the axons to correctly rebind with their original pathways, which leaves the affected structure with a reduced sensory or motor function. In this study we investigated if an artificial construct made of biocompatible nanomaterials, can be used instead of the classic nerve grafting techniques for a faster regeneration. Our goal was to find out if the Nerve Regeneration Assistance System (NerveRAS) will aid in a faster recovery of the voltage-gated Na^+ channels activity after a traumatic lesion, and consequently promote a faster recovery of the dorsal root ganglia (DRG) neurons excitability' parameters back to control values.

Materials and methods: Male Wistar rats (200-300g) were assigned to several groups: control, intermediate at 7 days (transected & excision – TE) and end-point at 45 days (sham, TE and TE + NerveRAS). TE consisted in exposing the left sciatic nerve, mid thigh level, transecting and removing a 5 mm nerve fragment. When using NerveRAS the distal and proximal ends were inserted inside an 1 cm tube, providing a minimum of at least 5 mm gap between the two ends inside the tube. All experiments were approved by the Ethics Committee of the University of Bucharest. The action potential (AP) and voltage gated Na^+ currents have been acquired using the patch clamp technique in whole-cell configuration on DRG L4, L5 and L6 neurons cultured for 24 hours. Ion channel activation has been controlled using a voltage protocol with steps ranging from -130 mV to +60 mV (10 mV increment). The conductance-voltage relationship and current density were used to explore I_{Nav} dynamics.

Results: AP amplitude was significantly increased in the end-point TE condition compared to control condition (119.8±3.95 mV, n=12 after TE, vs 107.3±4.31 mV, n=12 in control condition), while in the

presence of the NerveRAS the AP amplitude decreased back towards the control values (105.9±20.1 mV, n=2). The Na^+ current density at the highest activation point did not show any significant differences between the experimental conditions, most probably because of the heterogenous nature of the recorded cells. This decrease in channel activation mirrors the reduced amplitude of the AP when we used the NerveRAS regeneration method.

Conclusion: These results suggest that implanted NerveRAS made of a biocompatible nanomaterial facilitate the recovery of excitability after nerve transection to values comparable to the control condition at only 45 days after the lesion. However, additional experiments are required to confirm if this method is a viable option for long term use in a mammalian organism.

The functioning of voltage gated K^+ channels restores faster after a peripheral nerve lesion in the presence of a biocompatible nanomaterial support system

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Introduction: Although adult mammalian peripheral nerves have the remarkable ability to regenerate after minor injuries or transections, the repair process is slow and often the sensitivity of the injured area does not fully recover. As an alternative to nerve grafts procedures, in this study we investigated the regenerative potential of a Nerve Regeneration Assistance System (NerveRAS) made of biocompatible nanomaterials, implanted at the sciatic nerve level after a transection and excision lesion. The hypothesis was that in the presence of NerveRAS the regeneration will occur faster, which will allow the dorsal root ganglia voltage-gated K^+ channels' kinetics to recover quickly to control values.

Materials and methods: Male Wistar rats were assigned to several experimental conditions: control (without intervention), intermediate at 7 days (transection & excision – TE) and end-point at 45 days (sham, TE and TE + NerveRAS). TE consisted in exposing the left sciatic nerve, mid thigh level, transecting and removing a 5 mm nerve fragment. When using NerveRAS the distal and proximal ends were inserted inside an 1 cm tube, providing a minimum of at least 5 mm gap between the two ends inside the tube. All experiments were approved by the Ethics Committee of the University of Bucharest. Action potentials (AP) and voltage-gated K^+ channels were recorded by whole-cell patch-clamp method, performed on L4, L5 and L6 dorsal root ganglia neurons cultured for 24 hours. A multi-step voltage protocol from -130 mV to +60 mV (+10 mV increment/step) was designed to activate the K^+ channels, and the conductance-voltage relationships and current density analysis were used to evaluate the current kynetics.

Results: At the end-point TE condition the AP amplitude was significantly increased, but it was restored in the presence of NerveRAS (105.9 ± 20.1 mV, n = 2 compared to 119.80 ± 3.957 mV, n = 12 in TE

condition). At the same time point, the current density significantly decreased after TE compared to control condition, but it was restored in the presence of NerveRAS (starting from 122.09 ± 15.18 pA/pF, n = 21 in control, to 67.48 ± 4.42 pA/pF, n = 24 in end-point TE and 93.23 ± 18.19 pA/pF, n = 4 in the NerveRAS). The TE intermedite value was 86.98 ± 6.85 pA/pF, n = 30, suggesting a decrease in current density over time. However, the changes in the activation curve were not as significant as the ones in current density.

Conclusions: These results suggest that implanted NerveRAS made of a biocompatible nanomaterial facilitate the recovery of excitability after nerve transection to values close to control condition at only 45 days after the lesion. However, in order to evaluate the supportive potential for a full recovery, additional experiments at 90 days are required.

Novel insights into Kv1 complex assembly in myelinated axons

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As the interactions between the axon and the neighbouring glial cell lead to ensheathment of the axon with myelin, a large repertoire of voltage-gated ion channels with specific biophysical properties is strategically distributed to the nodes of Ranvier. This allows for salutatory conduction which significantly speeds up the signal transmission and makes the process more energy-efficient.

Although propagation of action potentials is largely reliant on voltage-gated sodium channels, voltage-gated potassium channels (Kv) play an important role in regulation of their shape and rate. Based on sequence homology, Kv channels are divided into 12 subfamilies. First identified in Drosophila, Shaker-type Kv1 channels are sequestered at the juxtaparanode (JXP), a region of myelinated axons flanking the paranodes on each side of the node of Ranvier. Here, they are thought to regulate the membrane potential, protecting the axon from hyper-excitability. Pathology related to Kv1 channels has been reported in epilepsy, neuromyotonia, ataxia and neuropathic pain. Their dissociation from the JXP is also an early sign of axonal demyelination. It is therefore essential that we understand the molecular processes that govern the assembly and maintenance of Kv1 at the JXP.

Previous research has demonstrated that the organisation of Kv1 channels at the JXP is dependent on their association with Caspr2 and Tag-1 molecules, as well as the adaptor protein 4.1B. However, genetic deletion of these proteins did not lead to a complete loss of Kv1 channels. Moreover, the PDZ binding motif of Caspr2, initially proposed to link the channels with the JXP complex, was proven to be dispensable in this process. These findings showed that our understanding of the molecular mechanisms involved in Kv1 localisation at the JXP is still incomplete and suggest that additional proteins are involved in this process.

Recent studies pointed towards the association of Kv1 with the ADAM proteins, in particular ADAM11, ADAM22 and ADAM23. These are known to interact with LGI proteins in a receptor-ligand fashion. The LGI family consists of four secreted proteins, all expressed in the nervous system. Preliminary data from our lab suggest that ADAM23 is present at the JXP where it interacts with LGI2 and LGI3. We employed several genetic mouse models to investigate the involvement of these interactions in clustering and maintenance of Kv1 complexes at the JXP as well as their regeneration after axonal injury. These in-vivo experiments were complemented by in-vitro investigation to further characterise the nature of the molecular interactions that could be taking place within the JXP complex. Our data indicate that the expression of ADAM23 in the axonal membrane within the JXP domain and its interaction with LGI2 and LGI3 are crucial for the organization of Kv1 complexes, not only during development but also in maintenance and axonal regeneration (Fig.1).

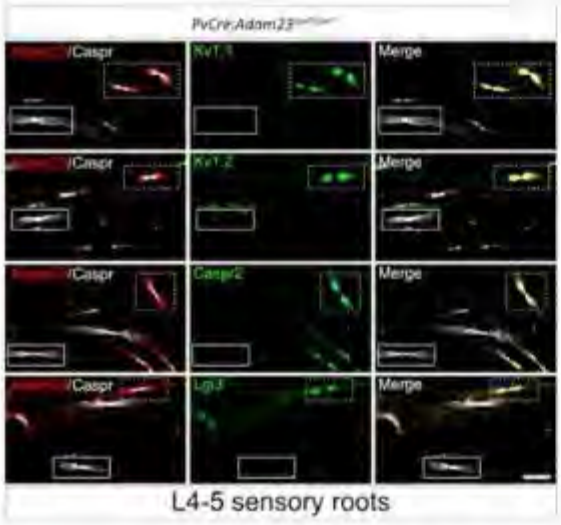

Figure1: Immunohistochemistry on sensory root axons of *PvCre:Adam23*$^{LoxP/LoxP}$ mouse. Removal of Adam23 form the large, parvalbumin-positive fibres results in absence of Kv1 channels, Caspr2 and Lgi3 from the JXP (solid box). Non-recombined axons of the same nerve show normal JXP Kv1 complexes (dashed box).

Scale bar = 25µm.

Age- and region-dependent changes in intracellular Na^+ and ATP induced by chemical ischemia in mouse forebrain

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Ischemic stroke is an often devastating incident which shows an increased prevalence in aged patients. Moreover, periods of ischemia damage different brain areas to a different extent. The reasons for this age- and region-dependent differences are far from understood. Former work has established a direct link between cellular energy metabolism, Na^+/K^+-ATPase activity and intracellular Na^+ regulation. Metabolic failure induces large shifts in Na^+ concentrations of neurons and astrocytes both *in vivo* and *in situ* (e. g. Gerkau et al., Cerebr. Cortex, 2018), indicating that the breakdown of Na^+ homeostasis is among the first consequences of a cellular shortage in ATP.

Here, we have addressed the question if ischemia-induced Na^+ loading differs among different brain regions and with increasing age. To this end, we monitored intracellular [Na^+] in acutely isolated and organotypic brain tissue slices of mouse hippocampus and cortex employing the chemical Na^+ indicator SBFI. Moreover, we analyzed changes in intracellular ATP using the genetically-encoded ATP sensor ATeam1.03^{YEMK}. Transient ischemia was mimicked by perfusion with glucose-free saline, to which sodium azide (NaN_3, 5 mM) and 2-desoxyglucose (2-DG, 2 mM) were added. In organotypic slices expressing ATeam1.03^{YEMK}, chemical ischemia induced a transient decrease in cellular ATP, demonstrating rapid cellular energy failure upon this manipulation. The drop in ATP was significantly stronger in neurons than in astrocytes. ATP depletion increased when increasing the duration of metabolic inhibition from 0.5 to 5 minutes in both cell types. Chemical ischemia was accompanied by a transient increase in $[Na^+]_i$, the amplitude of which mirrored the severity of ATP depletion. Cellular Na^+ loading increased with increasing time in culture (from days *in vitro* 7 to 55). In acute tissue slices derived from hippocampus and cortex, we found that the amplitude and duration of ischemia-induced Na^+ loading increased from postnatal day (P) 2-4 to P14-18 and then remained unchanged until P85-95. Notably, in slices from animals older than P200, peak amplitudes again strongly increased. Hippocampus reacted stronger to chemical ischemia than cortex at all age stages except for animals >P200.

Taken together, our results indicate significant differences in Na^+ increases induced by metabolic failure between hippocampus and neocortex of mice. Moreover, we found that the amplitudes of ischemia-induced Na^+ transients increase significantly during postnatal development. Cellular Na^+ loading therefore seems to mirror the reported age- and region-dependent differences in cellular vulnerability.

Poster Topic

T7: Synaptic Transmission, Pre- and Postsynaptic organization

T7-1 Altered molecular and structural nature of synaptic inputs to the CA2 region in a mouse model of mesial temporal lobe epilepsy
Susanne Tulke, Carola A. Haas, Ute Häussler

T7-2 Synaptic vesicle pools at endbulb of Held active zones upon development and lack of activity
Anika Hintze, Esther Semmelhack, Carolin Wichmann

T7-3 rapidFLIM with ION NaTRIUM Green-2 enables the dynamic quantification of changes in neuronal Na^+

Jan Meyer, Niklas J. Gerkau, Karl W. Kafitz, Matthias Patting, Christine R. Rose

T7-4 Gephyrin isoform knockout mice are generally healthy despite lacking alternatively spliced exons in the central domain
Filip Liebsch, Yvonne Merkler, Günter Schwarz

T7-5 Synapsin Condensates Recruit alpha-Synuclein
Christian Hoffmann, Roberto Sansevrino, Giuseppe Morabito, Chinyere Logan, Marcelo Ganzella, Dragomir Milovanovic

T7-6 Regulation of a subset of release-ready vesicles by the presynaptic protein Mover
Thomas Dresbach, Erwin Neher, Ermis Pofantis

T7-7 Determining the molecular mechanisms that mediate serotonin release from mouse enterochromaffin cells
Cordelia Imig, Ahmed Shaaban, Frederike Maaß, Benjamin H. Cooper

T7-8 Optical control of excitatory transmission in hippocampal slices with photoactive adenosine A1 receptor agonist
Erine Craey, Fabian Hulpia, Serge Van Calenbergh, Inge Van Colen, Jean Delbeke, Kristl Vonck, Paul Boon, Wytse Jan Wadman, Robrecht Raedt

T7-9 Opposing effects of Neuropeptide Y and corticotropin-releasing factor on glutamatergic synaptic transmission in corticotropin-releasing factor-expressing neurons of the bed nucleus of the stria terminalis
Julia Constance Bartsch, Robert Schumacher, Hans-Christian Pape, Kay Jüngling

T7-10 High resolution imaging of evoked dopamine release using a nanosensor paint
James A Daniel, Sofia Elizarova, Abed Chouaib, Ali Shaib, Nils Brose, Sebastian Kruss

T7-11 Effects of short-term pallidal deep brain stimulation on striatal medium spiny neurons in the animal model of the dystonic dt^{sz} mutant hamster
Denise Franz, Marco Heerdegen, Julia Hörnschemeyer, Valentin Neubert, Anika Lüttig, Stefanie Perl, Franz Veit Plocksties, Angelika Richter, Rüdiger Köhling

T7-12 CA3 pyramidal neurons in Fragile X Syndrome
Caroline Zeitouny, Martin Korte, Kristin Michaelsen-Preusse

T7-13 Analysis of the potential TOR interactor Madm, reveals a neuroprotective role of Presynaptic Homeostatic Plasticity in degenerating neurons

Zeeshan Mushtaq, Kumar Aavula, Ingrid Kieweg, Jan Pielage

T7-14 Novel Stim1 splice variants unravel important regulatory functions of Store-Operated Calcium Entry (SOCE) in neuronal and astroglial Ca^{2+} signaling
Lukas Jarzembowski, Girish Ramesh, Mona L. Knapp, Vanessa Poth, Dalia Alansary, Khaled Machaca, Dieter Bruns, Yvonne Schwarz, Barbara A. Niemeyer

T7-15 Analysis of ionotropic glutamate receptor splicing and editing in human RNA-Seq data
Robin Herbrechter, Andreas Reiner

T7-16 Characterization of local and long-range input to S1 cortex layer 1
Julia Ledderose, Timothy A Zolnik, Maria Toumazou, Thorsten Trimbuch , Christian Rosenmund, Britta J Eickholt, Dieter Jaeger, Matthew E Larkum, Robert NS Sachdev

T7-17 Characterization of glutamate dynamics and glutamate receptor activation with optical tools during metabolic stress
Tim Ziebarth, Lisa Kiese, Andreas Reiner

T7-18 Mitochondrial regulation of vesicle recovery after high frequency neurotransmission
Che Ho Yang, Won-Kyoung Ho, Suk-Ho Lee

T7-19 Single vesicle imaging reveals widespread co-release phenomenon in the adult mammalian central nervous system.
Neha Upmanyu, Dr. Sivakumar Sambandan, Prof. Dr. Reinhard Jahn

T7-20 Large, stable spikes exhibit differential broadening in excitatory and inhibitory neocortical boutons
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T7-21 Voltage-gated calcium channels trigger spontaneous glutamate release via nanodomain coupling
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T7-22 Temporal fidelity in principal MNTB neurons of the bat *Phyllostomus discolor*
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T7-23 Examination of perineuronal net formation and synaptic integrity in the visual cortex of quadruple knockout mice
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Altered molecular and structural nature of synaptic inputs to the CA2 region in a mouse model of mesial temporal lobe epilepsy

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Mesial temporal lobe epilepsy (MTLE) is characterized by seizures and extensive cell loss in the hippocampus. CA2 pyramidal cells are, however, preserved while their innervation is pathologically altered. The inhibitory innervation by local interneurons is substantially diminished as interneurons are particularly vulnerable to excitotoxicity. In addition, we have shown aberrant innervation of CA2 pyramidal cell somata by sprouted mossy fibers in MTLE. Here, we set out to characterize the molecular identity of synapses in the CA2 region in the kainate (KA) mouse model of MTLE.

We induced MTLE by unilateral injection of KA into the hippocampus of transgenic mice. For local field potential recording, some mice were implanted with bilateral electrodes into the dentate gyrus and CA2. Thy1-EGFP mice expressing EGFP in a subset of adult granule cells and Rbp4-cre mice expressing cre recombinase in granule cells were used for characterization of synaptic inputs to CA2. Rbp4-cre mice received an adeno-associated virus (phSyn1(S)-FLEX-tdTomato-T2A-SypEGFP-WPRE) injection inducing tdTomato expression in somata and EGFP in mossy fiber synapses. We performed in situ hybridization for glutamic acid decarboxylase 67 (GAD67) mRNA and immunohistochemistry for GAD65, both key enzymes for GABA production, vesicular GABA transporter (vGAT) and potassium-chloride-cotransporter 2 (KCC2) and localized CA2 pyramidal cells with Purkinje cell protein 4 (PCP4) or regulator of G-protein signalling 14 (RGS14), followed by Imaris-based reconstruction of synapses.

Local field potentials recorded in freely behaving mice showed alternating epileptiform activity (EA) and non-EA patterns in the dentate gyrus and CA2 of both hippocampi. Ipsilateral granule cells exhibited an increased somatic Gad67 mRNA expression and strongly upregulated synthesis of GAD65 in mossy fiber terminals innervating CA2 and an increased fraction of EGFP+GAD65-expressing mossy fiber boutons contacting CA2 pyramidal cell somata. Importantly, we did not detect any co-expression of GAD65 with vGAT in mossy fiber terminals, indicating that in case GABA is synthesized by GAD65-mediated decarboxylation of glutamate it is not loaded into synaptic vesicles, rendering classical synaptic GABA release unlikely. Yet, we found a substantial plexus of vGAT-positive fibers in CA2 indicating preservation of inhibitory nerve terminals. KCC2 expression was reduced after KA injection which could result in an altered chloride gradient across the CA2 pyramidal cell membrane and thus a modified GABA reversal potential.

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Synaptic vesicle pools at endbulb of Held active zones upon development and lack of activity

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Endbulbs of Held are the first central synapses of the mammalian auditory pathway. They are formed by the auditory nerve fibers that project onto bushy cells that are located in the anteroventral cochlear nucleus of the lower brainstem (Brawer and Morest, 1975). One endbulb of Held contains hundreds of individual active zones (AZs). During development, endbulbs evolve a fast signal transmission with high temporal fidelity that is essential for their role in auditory processing tasks. The lack of the hair cell specific protein otoferlin ($Otof^{-/-}$) results in an almost abolished exocytosis in the murine cochlea (Roux et al., 2006) and downstream in alterations of endbulb morphology (Wright et al., 2014). In humans, mutations in the OTOF gene result in DFNB9 non-syndromic hearing loss (Varga et al., 2003, Yasunaga et al., 1999).
We hypothesize that the number and distribution of synaptic vesicles (SVs) at endbulb of Held AZs changes upon development or the lack of activity.
In order to analyze morphological SV pools at individual endbulb AZs, we performed high-pressure freezing and freeze-substitution (HPF/FS) followed by electron tomography. HPF/FS leads to a rapid immobilization of the tissue and allows us to determine the number and distances of SVs in a near-to-native state. We compared ultrastructural parameters such as SV numbers of 10-day, 21-day and 6-month-old C57BL/6J wild-type (wt) and $Otof^{-/-}$ mice. We found a comparable vesicle pool size between wt and $Otof^{-/-}$ of 10-day and 21-day old mice. However, the SV number increased upon maturation towards adulthood at wt AZs, but decreased at $Otof^{-/-}$ AZs. The average number of docked SVs remained unaltered in all groups. Our results indicate a correlation between synapse activity and the number of SVs at individual AZs.

rapidFLIM with ION NaTRIUM Green-2 enables the dynamic quantification of changes in neuronal Na^+

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Fluorescence lifetime imaging microscopy (FLIM) is based on the principle of *Time Correlated Single Photon Counting* (TCSPC). In combination with chemical ion indicators, FLIM enables the quantitative measurement of ion concentrations based on the lifetime of the fluorophore. In contrast to intensity-based imaging, the latter is not affected by changes in the dye concentration, photo-bleaching or a drift in focus. This represents a fundamental advantage over intensity-based techniques as it enables quantitative imaging under challenging conditions as e.g. induced by ischemic cell swelling. With classic TCSPC, however, the time required for photon acquisition to allow a stable fitting of the fluorescence lifetime is usually rather high (many sec to >1 min). This is partly due to the so-called *dead-time artefacts*, which strongly reduce the amount of usable photons. *Dead-time artefacts* describe the shift towards shorter lifetimes when the photon count rate surpasses a certain threshold (usually 1-5% of the laser repetition rate). To overcome this limitation, rapidFLIM was recently introduced, reducing the dead-time from ~100 ns to ~ 0.7 ns. *Dead-time artefacts* are therefore virtually non-existent, resulting in much higher possible photon count rates and a drastically improved temporal resolution.

Here, we employed this approach for dynamic, intensity-independent quantification of changes in Na^+ in CA1 pyramidal neurons in acute mouse brain tissue slices. We first tested the suitability of the chemical Na^+ indicator ION NaTRIUM Green-2 (ING2) for rapidFLIM-based determination of $[Na^+]$ in vitro. These measurements demonstrate that fluorescence lifetimes of ING2 (τ_{AVG}) increase with increasing $[Na^+]$ and show that they are largely unaffected by other monovalent cations, changes in viscosity or different ionophores. Full in situ calibrations of ING2, performed simultaneously for intensity and lifetime in CA1 neurons in organotypic hippocampal tissue slices, confirmed the suitability of ING2 for rapidFLIM of Na^+ inside cells. Using this technique enabled dynamic detection of glutamate-induced Na^+ signals of CA1 neurons at a full-frame temporal resolution of 0.5-1 Hz. Moreover, it revealed that even brief metabolic inhibition (2-5 minutes) results in an unexpectedly large increase in neuronal $[Na^+]$, accompanied by substantial cellular swelling. Taken together, we conclude that dynamic rapidFLIM will pave the way to a better understanding of the mechanisms of cellular Na^+ signaling and Na^+ dys-regulation in the brain.

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Gephyrin isoform knockout mice are generally healthy despite lacking alternatively spliced exons in the central domain

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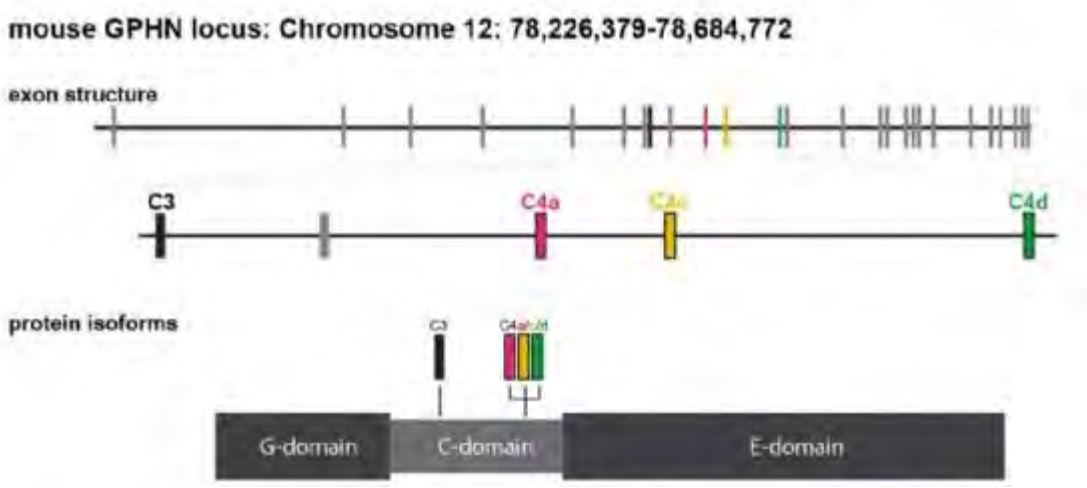
In neurons, gephyrin (Gphn) acts as a scaffold at GABAergic and glycinergic inhibitory synapses. Gphn is ubiquitously expressed and alternatively spliced, a process through which multiple protein isoforms can be produced from a single gene in a tissue-specific manner. Neuronal alternative splicing generates Gphn isoforms without (i.e. P1) or with the C4a, C4b (only in humans), C4c, and/or C4d cassettes. In contrast, Gphn in non-neuronal cells is required for the synthesis of the molybdenum cofactor and contains the C3 splice cassette. These cassettes are located in the central domain (C-domain) of Gphn. Knockout experiments demonstrated that both functions of Gphn, namely clustering of inhibitory neurotransmitter receptors and molybdenum cofactor biosynthesis, are necessary for survival in mice.

To study neuronal Gphn isoforms, low levels of Gphn P1, C4a, C4c, and C4d were expressed in dissociated murine hippocampal and cortical neurons using adeno-associated viruses. Analysis of Gphn in neuronal and non-neuronal tissues in vivo was achieved by generating knockout mice lacking either of the alternatively spliced exons (i.e. C3, C4a, C4c, or C4d). In agreement with RNA sequencing data retrieved from the splicecode database, we found that P1 is the predominant splice variant expressed in the adult mouse brain, followed by C4a; minor amounts of the other variants were detected. Our analysis demonstrated that all isoforms have the ability to localize to inhibitory synapses in vitro. Surprisingly, mice lacking the alternatively spliced exons are overall healthy and develop normally to adulthood. Gphn was detected with a lower apparent molecular weight in the liver of C3 knockout mice, indicating that they express the P1 isoform, which is normally only present in neurons. However, molybdenum cofactor synthesis was not affected, as normal sulfite oxidase (a molybdenum cofactor-dependent enzyme) activity was detected. Overall, our data suggest that the alternatively spliced cassettes of Gphn are not essential for survival and development to adulthood in mice. Mice lacking alternatively spliced exons in the C-domain of Gphn provide a tool to study their physiology on molecular, cellular, and behavioral levels.



Synapsin Condensates Recruit alpha-Synuclein

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Neurotransmission relies on the tight spatial and temporal regulation of the synaptic vesicle (SV) cycle. Nerve terminals contain hundreds of SVs that form tight clusters. These clusters represent a distinct liquid phase in which one component of the phase are SVs and the other synapsin 1, a highly abundant synaptic protein. Another major family of disordered proteins at the presynapse includes synucleins, most notably α-synuclein. The precise physiological role of α-synuclein in synaptic physiology remains elusive, albeit its role has been implicated in nearly all steps of the SV cycle. To determine the effect of α-synuclein on synapsin phase, we employ the reconstitution approach using natively purified SVs from rat brains and the heterologous cell system to generate synapsin condensates. We demonstrate that synapsin condensates recruit α-synuclein, and while enriched into these synapsin condensates, α-synuclein still maintains its high mobility. The presence of SVs enhances the rate of synapsin/α-synuclein condensation, suggesting that SVs act as catalyzers for the formation of synapsin condensates. Notably, at physiological salt and protein concentrations, α-synuclein alone is not able to cluster isolated SVs. Excess of α-synuclein disrupts the kinetics of synapsin/SV condensate formation, indicating that the molar ratio between synapsin and α-synuclein is important in assembling the functional condensates of SVs. Understanding the molecular mechanism of α-synuclein interactions at the nerve terminals is crucial for tackling synucleinopathies, where α-synuclein, synaptic proteins and lipid organelles all accumulate as insoluble intracellular inclusions.

Regulation of a subset of release-ready vesicles by the presynaptic protein Mover

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Neurotransmitter release occurs by regulated exocytosis from synaptic vesicles. Evolutionarily conserved proteins mediate the essential aspects of this process, including the membrane fusion step and priming steps that make synaptic vesicles release competent. Unlike the proteins constituting the core fusion machinery, the synaptic vesicle protein Mover does not occur in all species and all synapses. Its restricted expression suggests that Mover may modulate basic aspects of transmitter release and short-term plasticity.

To test this hypothesis, we analyzed synaptic transmission electrophysiologically at the mouse Calyx of Held synapse in slices obtained from wildtype mice and mice lacking Mover. Spontaneous transmission was unaffected, indicating that the basic release machinery works in the absence of Mover. Evoked release and vesicular release probability were slightly reduced, and the paired pulse ratio was increased in Mover knockout mice.
To explore, whether Mover's role is restricted to certain subpools of SVs, we analyzed our data in terms of two models of priming. A model assuming two SV-pools in parallel showed that knocking out Mover decreased the release probability of so-called 'superprimed vesicles', while 'normally-primed' vesicles were unaffected. The second model, which holds that vesicles transit sequentially from a loosely docked state to a tightly docked state before exocytosis, showed that knocking out Mover selectively decreased the release probability of tight state vesicles.
These results indicate that Mover regulates a subclass of primed synaptic vesicles in the mouse Calyx of Held.

Determining the molecular mechanisms that mediate serotonin release from mouse enterochromaffin cells

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Enterochromaffin cells are secretory cells in the gut epithelium that store and release the vast majority of the body's serotonin and form an important relay station for sensory information transmitted along the microbiome-gut-brain-axis. Impairments in enterochromaffin cell signaling have been directly linked to many diseases including obesity, inflammatory bowel diseases, and visceral pain. To treat these diseases, a better understanding of fundamental molecular and cell biological mechanisms regulating serotonin secretion is required. Enterochromaffin cells function as chemo- and mechanoreceptors, are electrically excitable and release serotonin in response to a variety of stimuli including nutrients, metabolites, and physical forces. Importantly, these cells express components of the neuronal molecular neurotransmitter release machinery and are positioned close to neuronal processes, indicating fast and directed cell-to-cell communication reminiscent of that at synaptic junctions between neurons in the brain. To study enterochromaffin cell function and serotonin release from individual secretory vesicles, we have established an experimental workflow combining mouse genetics, intestinal 3D organoid and 2D monolayer cultures, light- and electron microscopy, and electrochemistry, that allow us to monitor the serotonin release process with extremely high spatiotemporal resolution and on the single-cell level. We provide functional and ultrastructural evidence that enterochromaffin cells release serotonin from large-dense core vesicles in vitro and show that the vesicle fusion reaction is mediated by key molecular components of the presynaptic neurotransmitter release machinery in the brain. Our data indicate that targeting components of vesicle fusion machinery in enterochromaffin cells is a promising avenue to modulate serotonin secretion, which will become important for the treatment of diseases associated to altered serotonin release in the gut.

Optical control of excitatory transmission in hippocampal slices with photoactive adenosine A1 receptor agonist

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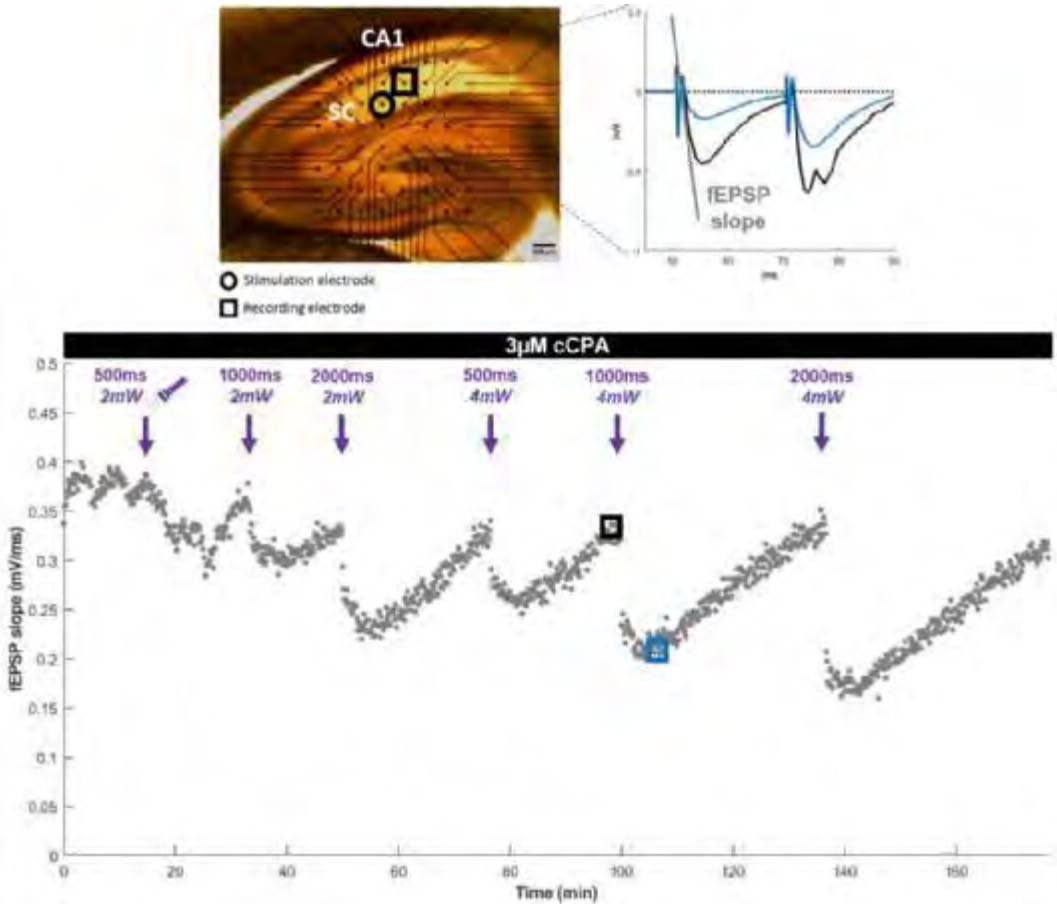
Adenosine A1 receptors are capable of modulating neuronal activity by presynaptic as well as postsynaptic routes, offering promising possibilities for therapeutic intervention. Unfortunately, their use is limited by the ubiquity of the A1 receptors, highlighting the need for focal activation. Optopharmacology allows site-specific agonist release and receptor activation using light-sensitive caged compounds. Here we investigated the UV-triggered activation of the coumarin-caged A1 agonist N6-cyclopentyladenosine (cCPA) in the CA1 region of acute rat hippocampal slices.

Rat hippocampal CA1 field potentials (fEPSPs and population spikes(PS)) were evoked by electrical stimulation of the Schaffer collaterals with variable intensity as single or double pulses. They were recorded using 60-channel multielectrode arrays (MEAs). Superfusion with 3µM cCPA and UV light-pulses (LED: 405nm) at two power intensities (2 and 4 mW) for 500 (n=3), 1000 (n=5) and 2000 ms (n=5) duration induced transient releases of CPA that modulated synaptic transmission and neuronal excitability as quantified in the local field potentials. They reduced fEPSP slopes for both power intensities and the three durations to respectively 81 ± 2%, 55 ± 7% and 40 ± 5% of baseline value. Within this working range the physiological effects and probably the CPA release are proportional to the time integrated stimulation. The effects are reversible as demonstrated by the stable and repeated modulation of A1 signalling within the same slice. A similar effect but of larger magnitude was observed for the PS and reflected a substantial change in neuronal excitability. A computational model that takes into account slice wash-in/out dynamics, A1 receptor occupation and G-protein activation, accumulation and deactivation, all as a function of time, allowed us to interpret the observed temporal transients in neuronal excitability. It also enabled the generation of illumination strategies that can fine tune excitability modulation over longer periods of time.

These data provide first proof that UV-triggered uncaging of cCPA can be used for controlled transient inhibition of excitatory transmission in slices, making optopharmacology a promising tool for focal modulation of neuronal activity in disease models like epilepsy.


CA1
SC
fEPSP
slope
Stimulation electrode
Recording electrode
3μM cCPA
500ms
2mW
1000ms
2mW
2000ms
2mW
500ms
4mW
1000ms
4mW
2000ms
4mW
fEPSP slope (mV/ms)
Time (min)

Opposing effects of Neuropeptide Y and corticotropin-releasing factor on glutamatergic synaptic transmission in corticotropin-releasing factor-expressing neurons of the bed nucleus of the stria terminalis

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The anterior bed nucleus of the stria terminalis (BNST) and the endogenous neuropeptide systems of corticotropin-releasing factor (CRF) and neuropeptide Y (NPY) are involved in shaping stress and anxiety responses. Existing data support largely opposing behavioral effects of NPY and CRF on stress and anxiety. NPY, CRF and their specific G-protein-coupled receptors are also expressed in the BNST. It has already been shown that NPY and CRF bi-directionally modulate inhibitory synaptic transmission in BNST at the Y2 receptor (Y2R) and the CRF receptor 1 (CRFR1). Here, we used whole-cell patch-clamp recordings in acute slices of anterior dorsal BNST from transgenic CRF reporter mice to characterize the impact of both neuropeptides on excitatory synaptic activity. While selective pharmacological activation of Y2R inhibited glutamatergic synaptic transmission in both CRF-positive and CRF-negative neurons via a presynaptic mechanism, selective activation of CRFR1 preferentially enhanced glutamatergic synaptic transmission in CRF-positive neurons, likely through a postsynaptic mechanism. Taken together, our results suggest potential anatomical and cellular substrates for a functional antagonism of NPY and CRF in the anterior dorsal BNST. Thus, BNST outputs from CRF-expressing neurons in this critical hub could form a distinct part of an anxiety- and stress-modulating circuitry governed by NPY- and CRF-driven neuromodulation.

High resolution imaging of evoked dopamine release using a nanosensor paint

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Dopamine (DA) is critical in everyday human behaviours and prevalent neurological diseases. The axonal structures that release DA, called varicosities, are the fundamental units of dopaminergic transmission. Dopamine-secreting varicosities are highly functionally diverse. However, the molecular basis of this diversity and the molecules that control DA release are mostly unknown. The precise study of DA release requires DA detection with high spatial and temporal resolution, which traditional methods lack. To address this limitation, we developed ‘Adsorbed Nanosensors Detecting Release of Dopamine’ - AndromeDA. This method uses cultured dopaminergic neurons, which are surrounded by millions of individual carbon-based fluorescent nanosensors ‘painted’ onto the glass coverslip. The nanosensors are DA-selective and respond to nanomolar quantities of DA. These sensors register DA in their local environment, providing a readout of dopamine release and diffusion. AndromeDA shows highly localised, transient fluorescence ‘hotspots’ upon neuronal stimulation that correlate with the position of single varicosities. We also find that fluorescence hotspots are not observed at all varicosities, an observation consistent with the presence of 'silent' varicosities. Overall, AndromeDA provides an imaging-based approach for analyzing how DA release is regulated by cellular and molecular processes with unprecedented resolution.

Effects of short-term pallidal deep brain stimulation on striatal medium spiny neurons in the animal model of the dystonic dt^{sz} mutant hamster

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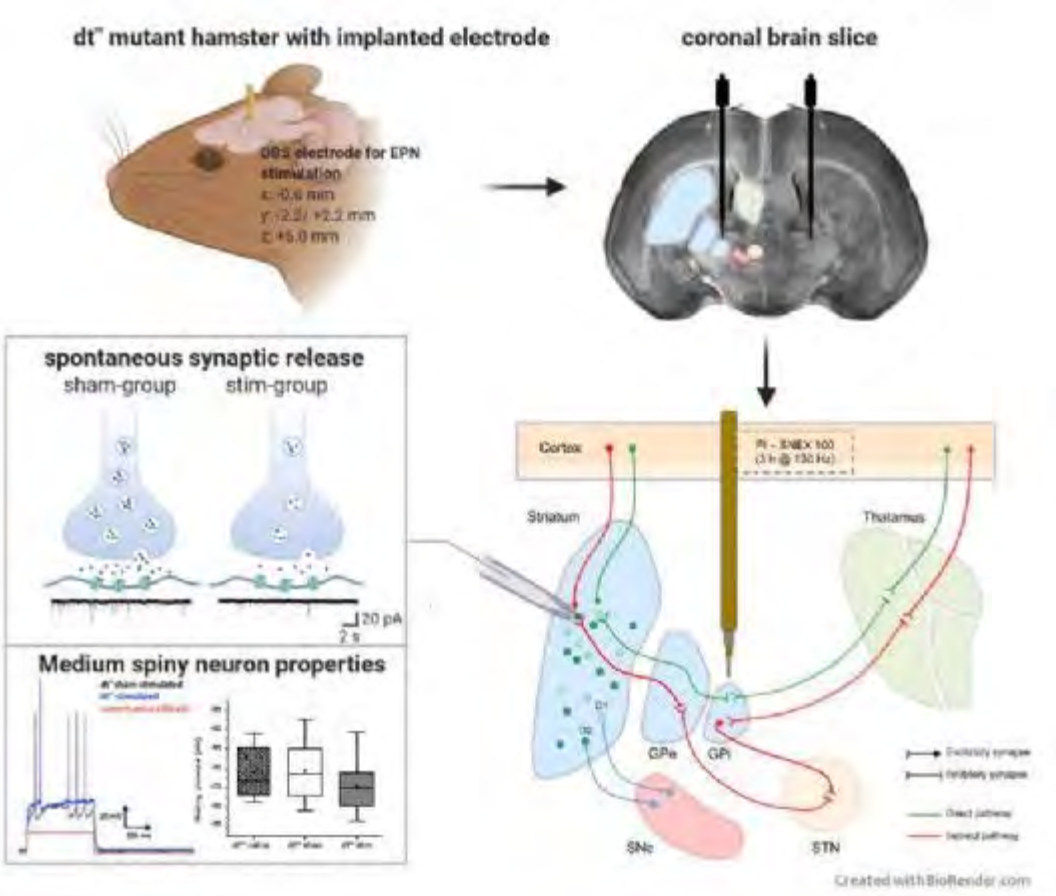
Electric stimulation of the deep brain nuclei, such as globus pallidus internus (GPi), indicated the most relevant therapeutic option for patients with severe dystonias. However, the mechanisms of the deep brain stimulation (DBS) of the GPi are far from understood. Dystonia, a hyperkinetic movement disorder, is thought to result from an imbalance in the direct and indirect pathway arises from disturbance in the striatum. In the dt^{sz} mutant hamster, a model of inherited generalized, paroxysmal dystonia, it has been already shown that the number of parvalbumin-positive gabaergic interneurons declined, which leads to uncontrolled projections via the medium spiny neurons (MSN). We hypothesized that DBS via backfiring, or indirectly via thalamic and cortical coupling, modifies striatal network function.

The dt^{sz} mutant hamsters were bilaterally implanted with stimulation electrodes targeting the entopeduncular nucleus (EPN; equivalent of the human GPi). DBS (130 Hz, rectangular pulse of 50 µA and 60 µs) and sham-DBS were performed in vivo in unanaesthetized animals for three hours. Acute brain slices of stim- and sham-groups were immediately prepared after the 3 h DBS. Additionally, acute brain slices were obtained from untreated dt^{sz} mutant hamsters (native group) to assess the effects of electrode implantation. With whole-cell patch clamp recordings, we investigated the spontaneous cortico-striatal synaptic activity and the characteristics of the D1- (direct pathway) and D2- (indirect pathway) MSN, which are capable of being differentiated by adding the D2-agonist sumanirole.

In view of spontaneous release activity from cortical projections, our study indicated a strong dampening effect on the frequency of spontaneous miniature excitatory postsynaptic currents (mEPSC) of the stim-group in contrast to sham-group. The specific cellular parameters of D1- and D2-MSN, which include resting membrane potential, input resistance, membrane capacity, rheobase, and firing properties of action potentials, did not differ between native-, sham-, and stim- group. In summary, while EPN-DBS obviously dampens spontaneous presynaptic glutamate release at cortico-striatal synapses, there is no alteration on MSN properties.

Future studies will consider the effects of long-term stimulation on synaptic plasticity by a novel implantable stimulation system which enables DBS over weeks.

This study is supported by the German Research Foundation (DFG) within the Collaborative Research Centre (SFB 1270/1 ELAINE 299150580).


dt" mutant hamster with implanted electrode
coronal brain slice
spontaneous synaptic release
sham-group
stim-group
20 pA
2 s
Medium spiny neuron properties
Cortex
Striatum
Thalamus
GPe
GPi
SNc
STN
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CA3 pyramidal neurons in Fragile X Syndrome

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Abstract
The Fragile X Syndrome (FXS) is the leading monogenetic cause of cognitive impairment and autism. Patients with FXS can show attention deficits and autism spectrum disorder (ASD) symptoms like stereotypic behavior and excessive adherence to patterns thereby indicating perturbed hippocampal networks. The hippocampus is indeed one of the most important brain regions involved in pattern separation/ completion during memory formation. In this respect the CA3 region is of special interest because of the auto-associative nature of the recurrent inputs. Unlike most other cells in the cortex, these cells actually are connected to a high degree to themselves. In contrast to this, the exceptionally strong and sparse mossy fiber input onto CA3 neurons is described as a ‘detonator’ synapse, directing plasticity and information processing in the recurrent network.
In the FXS the transcriptional silencing of the fragile X mental retardation protein (FMRP) leads to an abnormal development of dendritic spines which form the postsynaptic compartment. While spines have mostly been described as immature in FXS patients and the FXS mouse model (fmr1 KO), we could recently uncover a novel role of FMRP in restricting synapse development of mossy fiber inputs as the postsynaptic thorny excrescences (TEs) on CA3 neurons are premature during development in fmr1 KO mice. A detailed analysis of regular spines formed by the collateral input in different CA3 subfields showed that spine number was increased only in the Ca3b and Ca3c subregions in adult (6-7 months old) Fmr1 KO mice compared to Wt controls. In juvenile animals (p21), however, no alterations were observed in any of the subregions. Our data provide therefore evidence for a strong age-dependent dysregulation of synapse maturation in a synapse-type specific manner in the CA3 subregion, a fact that will most likely result in detrimental outcome for information processing in CA3 neurons and for hippocampal function as a whole.

Analysis of the potential TOR interactor Madm, reveals a neuroprotective role of Presynaptic Homeostatic Plasticity in degenerating neurons

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The precise coordination of synaptic connectivity and release properties is essential to maintain functional neuronal circuits. In a genetic screen of the Drosophila kinome, we identified the pseudo-kinase Madm (Mlf-1-adapter-molecule) as a novel regulator of structural and functional synaptic plasticity at the neuromuscular junction (NMJ). We demonstrate that presynaptic Madm is required for NMJ extension, maintenance and function. In addition, Madm controls NMJ growth in conjunction with the TOR pathway. In contrast, postsynaptic Madm contributes to synaptic organization and stability by enhancing presynaptic neurotransmitter release independently of TOR. This postsynaptic function of Madm is not essential for physiological presynaptic homeostatic plasticity (PHP) expression, but relies on the PHP presynaptic machinery. Strikingly, amelioration of presynaptic release alone via induction of PHP is sufficient to partially restore NMJ organization and maintenance in Madm mutants. Together, our study identifies Madm as a novel co-factor of synaptic TOR signalling and defines a physiological and neurotherapeutic role for PHP in the trans-synaptic control of structural plasticity.

Novel Stim1 splice variants unravel important regulatory functions of Store-Operated Calcium Entry (SOCE) in neuronal and astroglial Ca^{2+} signaling

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Store-operated $Ca2^{+}$-entry (SOCE) mediated by endoplasmatic reticulum (ER) localized STIM Ca^{2+} sensor proteins and highly Ca^{2}+ selective Orai channels is a major Ca^{2+} influx pathway in many cell types triggered by depletion of ER Ca^{2+} stores following receptor-stimulation. While its importance in immune function is well established, the molecular mechanisms of SOCE's and ER Ca^{2+} stores' contribution to neural transmission and astroglial Ca^{2+} signaling have been largely unknown, despite prominent expression in the central nervous system. We recently identified the uncharacterized Stim1 splice variants Stim1A and Stim1B[1] with high and mutual exclusive expression in astrocytes and neurons, respectively. In both cases modification of C-terminal residues by insertion of additional aminoacids and/or truncation confers distinct SOCE phenotypes that include a reduction of I_{CRAC} and subsequent cytosolic Ca^{2+} levels. Similarly, Stim1A overexpression in murine primary astrocytes reduced SOCE. Strikingly, overexpression of the neuronal variant Stim1B in hippocampal neurons revealed targeting to presynaptic boutons where Stim1B specifically converts short-term depression (STD) into short-term enhancement (STE) of synaptic transmission at high-frequency stimulation (>20 Hz). These effects were dependent on Ca^{2}+ and Orai channels and not present in Stim1 overexpressing neurons, which contrary to previous reports showed primarily a somatic localisation. These results highlight a hitherto unknown regulatory role of alternatively spliced SOCE components in synaptic plasticity. In addition, we created a splice-deficient mouse model lacking expression of Stim1A and Stim1B but not Stim1, that will be further characterized on effects in synaptic physiology, gliotransmission and behaviour.

1. Knapp et al. Alternative splicing switches STIM1 targeting to specialized membrane contact sites and modifies SOCE. (2020) bioRxiv, https://doi.org/10.1101/2020.03.25.005199

Analysis of ionotropic glutamate receptor splicing and editing in human RNA-Seq data

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Alternative splicing and RNA editing are posttranscriptional modifications which increase protein diversity and drive evolution, especially in higher eukaryotes. The regulation of alternative splicing and editing is tissue dependent and tightly regulated during development, which allows contextual gene expression adjustments. RNA Seq allows quantification of isoforms generated by splicing and editing and gives the power to identify new isoforms without previous sequence knowledge.
Ionotropic glutamate receptors (iGluRs) are ligand gated ion channels, which mediate glutamatergic neurotransmission within the central nervous system. The 18 iGluR members are divided into AMPA, NMDA, KA and Delta receptors and have immense neurophysiological importance. Former studies cloned and characterized iGluRs transcripts mainly from rodents and revealed the massive impact of the mentioned posttranscriptional modifications on receptor function, e.g. affecting receptor trafficking, kinetics, and pharmacology. However, alternative splicing and editing of human iGluRs is less well studied and specie-specific splicing shows the urgency of an investigation in humans.
We analyzed alternative splicing and RNA editing of iGluRs in the human brain using existing RNA Seq data (SRA, NIH). Next to analyzing previously annotated splicing and editing events, we identified new potential iGluR isoforms using custom written de novo identification tools. We detected 772 potential new iGluR splice junctions, most of them with low coverage (90% ≤ 8804 reads). However, some junctions occur with relative abundances of more than 20%, i.e. with similar frequency as known functional isoforms. Further validation of these events focuses on exon coverages, species comparisons and RT-PCRs on independent RNA samples. Moreover, we identified 10 edited sites within the open reading frame of iGluRs, which correspond to previously described sites. Our data give the first comprehensive overview of alternative splicing and RNA editing of iGluRs in the human brain.

Characterization of local and long-range input to S1 cortex layer 1

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Neocortical layer 1 is thought to be a locus of dynamic interactions between feedback and feedforward inputs. While we know that this layer contains interneurons and apical dendrites of L2/3 and L5 pyramidal neurons that are the targets of a variety of axons, our current understanding of how long-range and local inputs interact in L1 is incomplete. Here, we quantitatively assessed the local excitatory and inhibitory inputs to primary somatosensory cortex, using FB retrograde tracing, with or without Cre-dependent rabies virus for L5 intratelencephalic and pyramidal tract neurons. Our work shows first that a large portion of the input to layer 1 is local and that the bulk of the cortical local and long-range inputs arise from L2/3 and L5; second, that local intra-telencephalic neurons provide functionally stronger and numerically larger input to L1 than pyramidal tract neurons do, and that local L6b input to L1 is cell-type specific; third, that local inhibitory neurons from all layers provide input to L1. Using a synaptophysin version of the rabies virus, we also show that the input to the intra-telencephalic and pyramidal tract neurons most likely spans the entire column, the entire extent of a pyramidal neuron. Combined retrograde tracing with standard rabies shows that some local L1 projecting neurons also target intra-telencephalic and pyramidal tract neurons. The long-range input that targets L1 primarily connects to pyramidal-tract, and not the intra-telencephalic neurons. Taken together this work highlights the potential for complex interactions between the local input to and long-range feedback to L1 that can modulate the output of cortex.

Characterization of glutamate dynamics and glutamate receptor activation with optical tools during metabolic stress

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Disruption of energy supply to the central nervous system, e.g., ischemia, causes depolarization of neurons and astrocytes. This in turn can trigger an increased release of neurotransmitters, while at the same time glial neurotransmitter uptake becomes impaired. It is hypothesized that under these conditions ionotropic glutamate receptors (iGluRs) play a key role in perpetuating metabolic stress: High glutamate concentrations in the extracellular space may cause iGluR over-activation and subsequent cell death, commonly referred to as 'excitotoxicity'. However, little is known about how individual iGluR subtypes are affected during metabolic stress. Several mechanisms, such as AMPA and KA receptor desensitization or NMDA receptor inhibition by zinc and protons might safeguard against over-activation.

Our aim is to probe these processes by measuring the glutamate dynamics and the response of different iGluR subtypes during metabolic stress in real time, using an organotypic slice culture model. Brief periods (2-4 min) of chemical ischemia are evoked by withdrawing glucose and adding azide and 2-deoxyglucose to inhibit ATP synthesis and glycolysis, respectively.

To monitor changes in the extracellular glutamate concentration, we expressed variants of iGluSnFR, a genetically encoded glutamate sensor. Fluorescence imaging showed spontaneous glutamate release events, which during chemical ischemia increased in frequency and caused accumulation of extracellular glutamate. Then release stopped abruptly. After several minutes, normal activity recovered in many cases. Our experiments now focus on the role of individual iGluRs during the ischemic period. For this we rely on an optogenetic approach using modified sensor-iGluRs controlled by photoswitchable ligands (L-MAGs). Light-stimulation during whole-cell recordings of cortical layer II/III neurons should provide real-time information on whether particular iGluRs remain in their resting state, get activated or become desensitized/inhibited. In parallel, we use heterologous expression in HEK cells to investigate the behavior of selected sensor-iGluR homo- and heteromers using defined glutamate applications. Overall, these results will help to better understand glutamate dynamics and iGluR signaling during ischemic conditions.

Mitochondrial regulation of vesicle recovery after high frequency neurotransmission

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Previously we demonstrated that presynaptic mitochondria not only absorb but also release Ca^{2+} during high frequency stimulation (HFS) when presynaptic [Ca^{2+}] is kept low by high cytosolic Ca^{2+} buffer or strong plasma membrane calcium clearance mechanisms under physiological $[Ca^{2+}]_o$ (1.2 mM). Mitochondrial calcium release (MCR) during HFS elevated local [Ca^{2+}] near synaptic sites, and thus vesicular release probability, at interspike intervals to enhance short-term facilitation (STF) and to support stable synaptic transmission under physiological $[Ca^{2+}]_o$ at mature calyx synapses, but not at immature calyx or at 2 mm $[Ca^{2+}]_o$. Under the latter high cytosolic calcium conditions, MCR slowly occurred not during, but after HFS ceased. Here we show that this slow MCR after strong stimulation is responsible for the acceleration of slow endocytosis by using capacitance measurements from presynaptic terminals before and after the application of the confirmed mitochondrial Na+ /Ca^{2+} exchanger (mNCX) specific inhibitor, 2 μM tetraphenylphosphonium (TPP^+). Whereas TPP^+ reduced STF under low cytosolic calcium conditions, it strongly slowed down the decay rate of slow endocytosis after stimulations of high cytosolic calcium conditions. The post-train MCR may be physiologically responsible for the residual calcium after cytosolic [Ca^{2+}] has returned close to basal levels, and accelerate slow endocytosis required for recovery of synaptic vesicles.

Single vesicle imaging reveals widespread co-release phenomenon in the adult mammalian central nervous system.

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Quantitative synaptic biology provides the necessary framework to model and understand synaptic communication. In contrast to conventional understanding of synaptic function where one type of neurotransmitter is released at a time (Dale's original proposition), recent evidences clearly establish that in the mammalian central nervous system, neurons in specific brain regions release more than one type of transmitter at the same synapse (co-release). Although co-release of transmitters represents novel logic operations at both local and circuit level, there is no brain wide understanding of such a phenomenon and if they are released from the same vesicle pool. Here by using DyMIN STED super resolution microscopy we provide quantitative information on the capacity of the whole brain for multi-transmitter release in adult rats. Using single vesicle imaging, we mapped colocalization of different transporters of classical neurotransmitters and classified synaptic vesicles into 28 types based on the phenotype of transporters expressed. Our data show that 30% of the total synaptic vesicles express more than one classical neurotransmitter transporter, out of which 45% of glutamatergic and 40% of GABAergic vesicles display corelease competence. Furthermore, using biochemical and imaging assays, we show that the presence of one transporter on a vesicle influence the transmitter uptake activity of the other, indicating that co-localization of multiple transporters has direct implications for presynaptic function. Our data suggests that localization of multiple transporters on synaptic vesicles as a prevalent phenomenon in the brain and lays foundation for a rigorous probe into its functional aspects on presynaptic regulation.

Large, stable spikes exhibit differential broadening in excitatory and inhibitory neocortical boutons

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Presynaptic action potential spikes control neurotransmitter release and thus interneuronal communication. However, the properties and the dynamics of presynaptic spikes in the neocortex remain enigmatic because boutons in the neocortex are small and direct patch-clamp recordings have not been performed. Here, we report direct recordings from boutons of neocortical pyramidal neurons and interneurons. Our data reveal rapid and large presynaptic action potentials in layer 5 neurons and fast-spiking interneurons reliably propagating into axon collaterals. For in-depth analyses we establish boutons of mature cultured neurons as models for excitatory neocortical boutons, demonstrating that the presynaptic spike amplitude is unaffected by potassium channels, homeostatic long-term plasticity, and high-frequency firing. In contrast to the stable amplitude, presynaptic spikes profoundly broaden during high-frequency firing in layer 5 pyramidal neurons but not in fast-spiking interneurons. Thus, our data demonstrate large presynaptic spikes and fundamental differences between excitatory and inhibitory boutons in the neocortex.

Voltage-gated calcium channels trigger spontaneous glutamate release via nanodomain coupling

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Neurotransmitter release occurs either synchronously to action potentials or spontaneously, yet whether molecular machineries underlying evoked and spontaneous release are identical, especially whether voltage-gated Ca^{2+} channels (VGCCs) can trigger spontaneous events has been in debate. To elucidate this issue, we characterized Ca^{2+} dependency of miniature excitatory postsynaptic currents (mEPSCs), in autaptic cultured hippocampal neurons. We found that spontaneous release shows Ca^{2+} cooperativity comparable to evoked release, and most of $[Ca^{2+}]_o$-dependent mEPSCs was attributable to VGCCs. Coupling distance between VGCCs and Ca^{2+} sensors was estimated as tight for both types of release. In hippocampal slices, VGCCs trigger mEPSCs to a different extent depending on areas and ages. At the calyx of Held synapses, mEPSCs showed VGCC-dependence in type 1 mature synapses with nanodomain coupling, but not in immature synapses. These data suggest that the distance between VGCCs and Ca^{2+} sensors is the key factor to determine VGCC dependence of spontaneous release.

Temporal fidelity in principal MNTB neurons of the bat *Phyllostomus discolor*

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The medial nucleus of the trapezoid body (MNTB) is a mammalian brainstem nucleus involved in the processing of sound localization. Each principal MNTB neuron receives a single axosomatic calyceal synapse from the contralateral anteroventral cochlear nucleus. The strong excitatory input is rapidly converted in glycinergic inhibition, mainly targeting the ipsilateral medial and lateral superior olive and nuclei of the lateral lemniscus. MNTB neurons have been extensively investigated in various mammals with high and low frequency hearing ability, but biophysical data for a small-headed, high frequency hearing specialist, like the bat, is missing.

Here, we examined the membrane and synaptic properties of principal MNTB neurons of the bat Phyllostomus discolor. We detected similarities with MNTB neurons of non-echolocating mammals in their resting potential, membrane time constants and input resistance. We noticed that near current threshold, the success rate of action potential generation in response to hair comb stimulations drops in a frequency dependent manner that may be caused by prolonged afterhyperpolarization. We described the typical DTX-sensitive potassium currents and confirmed the somatic expression of the underlying Kv1.1 channels. Synaptic EPSCs mediated through the calyx of Held are large, fast and show frequency dependent facilitation followed by depression. In response to stimulation of 300 Hz and above, onset facilitation occurs. Synaptic depression appears smaller and less stimulus frequency dependent compared to rodents. We note that in a fraction of neurons, a small portion of slowly replenishable, superprimed synaptic vesicles might play a role in the short-term plasticity.

In order to probe the effect of STP and input size on the input-output fidelity of principal MNTB neurons, we created conductance templates that mimic trains of synaptic inputs. For one set of templates, the first pulse matched the amplitude of a single input, and for the other, it was set at 10% above conductance threshold. Both sets were paired with or without synaptic plasticity. MNTB neurons were able to fire with high success rate and temporal fidelity during stimulation trains with single input size. Failures were observed only at high frequencies (e.g. 800 Hz) and increased when short-term plasticity was added. MNTB neurons failed to follow through high frequency stimuli near their conductance threshold. Driven by STP, however, and independent of initial conductance amplitude, the latency of evoked action potentials increased during the stimulus duration, indicating poor temporal fidelity of the spike generator.

We thus propose that the large excitatory inputs promote information transfer with high fidelity, but with low temporal constancy. Therefore, bat MNTB neurons are exquisite at relaying sound onset with precision.

Examination of perineuronal net formation and synaptic integrity in the visual cortex of quadruple knockout mice

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Aim: The role of the extracellular matrix (ECM) in the formation of perineuronal nets (PNNs) in the central nervous system is well documented. In this study, we examined the structure of PNNs and synaptic integrity in the visual cortex of quadruple knockout (KO) mice, that lack the four ECM molecules *brevican*, *neurocan*, *tenascin-C* and *tenascin-R* (Geissler et al. 2013; Gottschling et al. 2019). Due to the accessibility of the visual cortex and the absence of four crucial ECM molecules, this model could offer an opportunity for future studies regarding the influence of these ECM components on visual processing, PNN structure and synaptic integrity.

Methods: PNNs were stained immunohistochemically in visual cortices of wildtype (WT) and quadruple KO mice using anti-aggrecan and *Wisteria floribunda lectin* (*WFA*) (N=7). Anti-synaptophysin, anti-vesicular GABA transporter (VGAT) and anti-gephyrin were used as pre- and postsynaptic markers (N=7). Inhibitory fast-spiking neurons were specifically stained with anti-parvalbumin and anti-calretinin (N=7). Stainings were visualized by confocal laser-scanning microscopy and statistically evaluated by Student *t*-test (Statistica). Additionally, PNN structure and VGAT distribution on PNN-enveloped cells was analyzed by super-resolution microscopy (N=7). Furthermore, visual cortex tissue was used for mRNA analyses (N=5) of *Acan* (*aggrecan*), *Vcan* (*versican*), *Slc32a1* (*VGAT*) and *Sema3a* (*semaphorin3a*) by quantitative real-time PCR (RTq-PCR). For RTq-PCR analyses, groups were compared using a pairwise fixed reallocation and randomization test (REST© software).

Results: Immunohistochemical analyses showed a significantly reduced number of WFA-positive cells (WT: 99.2±5.7 vs. KO: 64.64±7.4; $p<0.001$) and aggrecan-positive cells (WT: 95.44±8.9 vs. KO: 63.5±15.9; $p<0.001$) in the visual cortex of KO compared to WT mice. The staining of synaptophysin and gephyrin was comparable in both groups ($p>0.05$). However, the VGAT staining was significantly reduced in the KO group (WT: 29.1±3.2 VGAT+ area [%] vs. KO: 13.94±6.9 VGAT+ area [%]; $p<0.01$). Interestingly, super-resolution microscopy revealed a significantly impaired PNN density in the KO (WT: 3.4±1.7 WFA/PNN [%] vs. KO: 1.4±0.9 WFA/PNN [%]; $p=0.008$). No alterations in the PNN volume were observed ($p>0.05$). Remarkably, the number of VGAT positive puncta that perforated the PNNs were significantly reduced in the KO (WT: 560.3±283.2 VGAT+ puncta vs. KO: 154.4±197.1 VGAT+ puncta; $p=0.009$). The number of parvalbumin-positive interneurons was significantly decreased in the KO (WT: 75.9±5.7 vs. KO: 57.36±18.1; $p=0.024$), while the number of calretinin-positive interneurons was comparable in both groups ($p>0.05$). Finally, RT-qPCR analyses showed comparable *Acan* and *Vcan* expression levels ($p>0.05$), but significantly reduced *Slc32a1* ($p=0.03$) and *Sema3a* levels ($p=0.04$) in KO mice.

Conclusion: Our study showed that the absence of four ECM molecules causes impaired PNN and inhibitory GABAergic synapse formation in the visual cortex. Also, the number of parvalbumin positive interneurons was reduced. Collectively, our findings indicate an important role of the four ECM constituents in PNN formation and synaptic integrity. Therefore, quadruple KO mice could represent a suitable model for investigating PNN formation as well as synaptic integrity.

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Plasmalogens and their role in synaptogenesis and synaptic transmission

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Plasmalogens are a special class of phospholipids characterized by a vinyl-ether bond at the sn-1 position of the glycerol backbone and highly enriched in nervous tissue. The relevance of plasmalogens in human health has been highlighted by the severe clinical presentation of Rhizomelic Chondrodysplasia Punctata (RCDP), a lethal autosomal recessive peroxisomal disorder caused by defects in the biosynthesis of plasmalogens. RCDP patients display neurologic alterations, with severe psychomotor impairment, epilepsy, and progressive impairments of visual-evoked and brain-evoked potentials, highlighting the relevance of plasmalogens in the nervous tissue. To investigate plasmalogens' function and the consequences of their deficiency, we are using the Gnpat knockout (KO) mouse, which has a generalized plasmalogen defect. Neurons are specialized cells capable of communicating with other neurons, and with other cells through synapses. Synaptic dysfunction has been shown to underlie many brain disorders. Therefore, understanding the neuropathophysiological mechanisms involved in RCDP will provide a better understanding of this disorder. For this work, we have performed a proteomic analysis comparing proteins from WT and Gnpat KO mice and observed many proteins that are either up- or downregulated in our KO mice. A subset of these proteins (e.g. GAD1, Complexin 1/2) was already validated using western blot, demonstrating a dysregulation in the synapses. Combined with cortical neuron cultures, which show fewer synaptic sites in Gnpat KO neurons, our results support an important role of plasmalogens in the regulation of synaptogenesis and synaptic transmission.

Poster Topic

T8: Synaptic Plasticity, LTP, LTD

T8-1 GLIOGLY - THE INVESTIGATION OF GLYCOBIOLOGY OF GLIOBLASTOMA
Ugne Kuliesiute, Vidhya Madapusi Ravi, Jasmin von Ehr, Urte Neniskyte, Dieter Henrik Heiland, Kevin Joseph

T8-2 Entorhinal cortex lesion induces homeostatic synaptic plasticity of CA3 pyramidal neurons in vitro.
Pia Kruse, Andreas Vlachos, Maximilian Lenz

T8-3 Expansion microscopy in honeybee brains: a new attempt to push the limits of neuroanatomical analyses in social insects
Nadine Kraft, Thomas S. Muenz, Christian Werner, Markus Sauer, Claudia Groh, Wolfgang Rössler

T8-4 TNFα modulates synaptic plasticity in a concentration-dependent manner through synaptopodin-associated intracellular calcium stores
Dimitrios Kleidonas, Matthias Kirsch, Andreas Vlachos

T8-5 Proton magnetic resonance spectroscopy study of GABA neurotransmitter in motor cortex and basal ganglia of healthy subjects following anodal transcranial direct current stimulation
Harshal Jayeshkumar Patel, Chang-Hoon Choi, Lea-Sophie Stollberg, Elene Iordanishvili, Ezequiel Farrher, N. Jon Shah, Ferdinand Binkofski

T8-6 Depletion of neurocan in the prefrontal cortex impairs temporal order recognition, cognitive flexibility, and perisomatic GABAergic innervation
David Baidoe-Ansah, Hector Carceller, Hadi Mirzapourdelavar, Marta Pérez-Rando, Constanze Seidenbecher, Rahul Kaushik, Juan Nacher, Alexander Dityatev

T8-7 Extracellular matrix balances principal cell excitability and synaptic plasticity
Hadi Mirzapourdelavar, Inseon Song, Jeet Sing, Alexander Wirth, Daniel Minge, Rahul Kaushik, Maura Ferrer-Ferrer, Jeissica Mitlöhner, Andre Zeug, Christian Henneberger, Evgeni Ponimaskin, Renato Frischknecht, Constanze Seidenbecher, Alexander Dityatev

T8-8 CtBP1 attenuates the suppression of synaptic transmission under metabolic stress
Enes Yagiz Akdas, Debarpan Guhathakurta, Hui-Fen Chen, Anna Fejtova , Seda Salar

T8-9 The functional effects of the Rasopathy-related KRASV14I mutation in the brain
Seda Salar, Debarpan Guhathakurta, Eva-Maria Weiss, Deniz Madencioglu, Saeideh Nakhaeirad, Lena Marx, Gürsel Caliskan, Martin Zenker, Oliver Stork, Muhammed Reza Ahmadian, Anna Fejtova

T8-10 All-trans retinoic acid induces synaptic plasticity in the human neocortex.
Maximilian Lenz, Pia Kruse, Amelie Eichler, Julia Muellerleile, Jakob Straehle, Peter Jedlicka, Jürgen Beck, Thomas Deller, Andreas Vlachos

T8-11 Delineation of pathways behind impaired neuroplasticity in a mouse model of Noonan Syndrome
Cathleen Höke, Anna Fejtova, Seda Salar

T8-12 Vesicle recruitment is not accelerated during LTP at neocortical layer 5 pyramidal neurons
Iron Weichard, Stefan Hallermann, Jana Nerlich

T8-13 Modulation of synaptic plasticity by ketamine
Stefan Vestring, Claus Normann

GLIOGLY - THE INVESTIGATION OF GLYCOBIOLOGY OF GLIOBLASTOMA

Ugne Kuliesiute[1,2], Vidhya Madapusi Ravi[1], Jasmin von Ehr[1], Urte Neniskyte[2], Dieter Henrik Heiland[1], Kevin Joseph[1]

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Objective:
Glioblastoma (GBM) is the most malignant brain tumour hallmarked by the aggressive infiltration of tumour cells into neighbouring brain regions. Alterations in the glycobiology of cancer cells is closely associated with malignant properties, including invasiveness and metastatic potential. In particular, the composition of glycocalyx, a layer of multifunctional glycans that covers the surfaces of cells and the expression of sialic acids (the terminal moieties of glycoconjugates, which compose the glycocalyx) were shown to be altered in GBM. However, the glycobiology of glioblastoma remains poorly investigated. Here, we aim to explore the role of glycobiology in glioma and map sialylation levels using an organotypic neocortical slice model.

Methods:
Bioorthogonal CLICK chemistry was adapted to assess *de novo* synthesis of sialic acid in human organotypic brain slice culture and patient-derived glioblastoma cells. Longitudinal imaging was used to assess migratory capacity of GBM cells. Calcium imaging was used to investigate glioblastoma cells network activity. Multi-electrode array technique was used to obtain detailed reconstructions of the calcium singling waveforms allowing to perform detailed analysis and clustering. Expression level of genes involved in sialylation was evaluated by spatial transcriptomics (Visium 10X) and RNA-sequencing of tumour-infiltrated patient samples and patient-derived GBM cell lines.

Results:
Visualization and quantitative analysis of newly synthesised sialic acid confirmed that glioblastoma cells demonstrate high rate of *de novo* sialic acid synthesis. We found a significant loss of migratory capacity after inhibition of sialic acid. Feature extraction of longitudinal imaging data indicated a significant reduction of cellular velocity in the group of inhibited synthesis of sialic acid. Further we quantified inter- and trans-cellular Ca^{2+} signalling which was found to be strongly reduced upon inhibition of sialic acid biosynthesis. At a global scale, we found an abolished scale-free topology of the functional network after inhibition of sialic acid synthesis suggesting that glycobiology is critical to establish cellular connectivity. Spatial transcriptomics and RNA-sequencing results indicate high expression of sialidases, enzymes that cleave sialic acid from glycoconjugates, in both tumour-infiltrated patient samples and patient-derived GBM cell lines, suggesting the metabolism of sialylation being potentially involved in tumour growth. Additionally, we showed that human organotypic brain slice culture technique can be used as a robust framework for glycobiology research allowing metabolic labelling of sialic acid moieties and quantifying changes in glycocalyx.

Conclusion:

In summary, our results provide new insights into the role of sialic acid in the functional activity of GBM and highlight the importance of functional relationships and cellular migration. In the future, these interactions have the potential to be targeted therapeutically.

Entorhinal cortex lesion induces homeostatic synaptic plasticity of CA3 pyramidal neurons in vitro.

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A common feature of neurological diseases is the loss of central neurons, which leads to deafferentation of connected brain regions. In turn, the remodeling of denervated networks plays an important role for the postlesional recovery. In previous work, we demonstrated that *in vitro* entorhinal cortex lesion causes homeostatic synaptic adaptions of excitatory neurotransmission onto dentate granule cells, which aim at compensating for perturbations in network activity. In this study, we aimed at extending these findings to CA3 pyramidal neurons, which receive input from the entorhinal cortex and from (partially denervated) dentate granule cells. We employed whole-cell patch-clamp recordings in organotypic entorhino-hippocampal tissue cultures and investigated excitatory neurotransmission onto dentate granule cells and CA3 pyramidal neurons following entorhinal cortex lesion (ECL). Partial denervation following ECL leads to a strengthening of excitatory synapses of dentate granule cells and CA3 pyramidal neurons. Notably, this homeostatic adjustment occurs predominantly in the fraction of the strongest excitatory synapses as shown by a hierarchical analysis of spontaneous excitatory postsynaptic currents. In this context, we are currently testing for changes in synaptic transmission at the mossy fiber/CA3 synapse after a partial, i.e., distal denervation following ECL. We conclude that ECL *in vitro* induces homeostatic synaptic plasticity of dentate granule cells and CA3 pyramidal neurons that may lead to complex heterodendritic synaptic adaptations of hippocampal networks. Supported by Else-Kröner Fresenius Stiftung (EKFS)

Expansion microscopy in honeybee brains: a new attempt to push the limits of neuroanatomical analyses in social insects

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Immunolabeling using fluorophore-conjugated antibodies combined with confocal laser scanning microscopy have proven to be invaluable tools to investigate structural neuronal plasticity in social insects for many years. Particularly in our field of interest, the mushroom body (MB) calyces, these methods have paved the way to obtain a comprehensive knowledge on age-, experience- and learning-related structural plasticity in MB microcircuits, thus bringing us another step closer to identifying the neuronal basis of behavioral plasticity. On the path to deciphering ultrastructural details of synaptic fundamentals, however, we are confronted with constraints posed by the diffraction limit of conventional microscopy systems. Modern super-resolution microscopy techniques such as STED, SIM or STORM are able to overcome this limitation, but they come at the cost of expensive additional equipment and, often, increased complexity. However, with the introduction of protein-retention expansion microscopy (proExM; Tillberg et al., *Nat Biotechnol*, 2016), which allows for an isotropic physical expansion of conventionally immunostained tissue, an easy, affordable tool for increasing the resolution of light microscopic systems has become available. Using swellable polymers, immunolabels locked to the gel can be linearly expanded by a factor of 4.5 (using the most basic protocol), thus achieving an enhanced resolution of ~60-70 nm. For the first time, we have now established the expansion microscopy (ExM) protocol in a social insect species, the honeybee *Apis mellifera*. In this study, our focus lies on the structure and plasticity of synaptic microcircuits (microglomeruli) in the MB calyces, high-order sensory integration and memory centers in the insect brain. Using ExM, we analyzed the colocalization of two synaptic proteins - the synaptic vesicle-associated protein synapsin and the active zone-associated protein Bruchpilot - and performed first quantitative analyses applying this new super-resolution technique now available for honeybee brains. We additionally applied this technique to further specify the γ-aminobutyric acid (GABA) feedback system within the MBs and its interference with the cholinergic system. Furthermore, we used the enhanced resolution of ExM to shed new light on a novel nuclear structure within postsynaptic MB Kenyon cells, detected by a Kenyon-cell specific antibody. Currently, we are also working on expanding selective neuronal tracings from fluorescent dye injections to gain more detailed insight into the connectivity between projection neurons and MB Kenyon cells. Altogether, ExM constitutes a promising tool to revolutionize the basic concept of structural neuronal analyses. We demonstrate the potential of this method for different neuroanatomical techniques and neuronal structures in the honeybee brain, which establishes a basis for future analyses of plasticity in synaptic microcircuits in social insects. Supported by a DFG grant to Claudia Groh (GR3305/2-1) and the University of Würzburg.

TNFα modulates synaptic plasticity in a concentration-dependent manner through synaptopodin-associated intracellular calcium stores

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The pro-inflammatory cytokine tumor necrosis factor-α (TNFα) has been firmly linked to the ability of neurons to express synaptic plasticity. However, the precise molecular mechanisms and neuronal targets through which TNFα modulates synaptic plasticity remain not well-understood. Here, we hypothesized that the actin-binding protein synaptopodin, which is a marker and essential component of the spine apparatus organelle, is required for TNFα-mediated synaptic changes. In order to address this question we employed organotypic entorhinal-hippocampal tissue cultures prepared from synaptopodin-deficient mice and their wild type littermates, and we studied structural and functional properties of CA1 pyramidal neurons in control and TNFα-treated tissue cultures. Our results reveal that a “high” concentration of TNFα (6nM, 24h) promotes synaptic plasticity by increasing the content of calcium-permeable, GluA2-lacking AMPA receptors at excitatory postsynapses, without affecting baseline synaptic transmission. Experiments performed in synaptopodin-deficient preparations reveal that synaptopodin mediates these effects of TNFα on excitatory neurotransmission. Strikingly, a lower concentration of TNFα (60pM, 24h) enhanced excitatory neurotransmission by increasing GluA2-lacking AMPA receptors at excitatory postsynapses. Microglia seem to play an important role in orchestrating the plasticity promoting effects of TNFα. These results extend previous work on the effects of TNFα on excitatory synaptic plasticity by demonstrating a concentration-dependent and synaptopodin-mediated accumulation of calcium-permeable, i.e., GluA2-lacking, AMPA receptors at excitatory postsynapses. (Supported by DFG).

Proton magnetic resonance spectroscopy study of GABA neurotransmitter in motor cortex and basal ganglia of healthy subjects following anodal transcranial direct current stimulation

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Introduction

Previous work on functional connectivity has shown the influence of anodal transcranial direct current stimulation (tDCS) over the primary motor cortex and the interconnected basal ganglia region [Naegel et al. 2018]. It escalates a possibility of neurochemical link between primary motor cortex and basal ganglia. The neurochemical mechanism of cortical excitability after anodal tDCS has been investigated by measuring the GABA neurotransmitter in the motor cortex [Patel et al. 2019]. However, the neurochemical mechanism between the motor cortex and the basal ganglia, under the influence of anodal tDCS, is not known. We aim to explore GABA modulation between the motor cortex and the basal ganglia following anodal tDCS by consecutive and repeated (1H) proton magnetic resonance spectroscopy (MRS) from left M1 and basal ganglia up to 30 minutes.

Materials and Methods

Two righthanded healthy volunteers were recruited at the RWTH Aachen University Hospital, Germany. All experiments were performed on a 3T Siemens PRISMA wholebody scanner using a 20 channel 1H head coil for signal reception. As shown in Figure 1a, GABA spectroscopy measurements from left M1 and basal ganglia were carried out consecutively three times before and after the stimulation, respectively. Two mA anodal tDCS were applied for 10 minutes with the anodal electrode placed over the left M1 and cathodal electrode placed on the right supraorbital area. GABA edited 1H spectra were independently acquired from a volume of interest of 3×3×3cm (27mL) and 2.5×4×2cm (20mL) on the left M1 and the basal ganglia, respectively as shown in Figure 1b & 1c. MEGAPRESS Jediting was used for GABA detection in M1 (TR/TE = 2000/68 ms and 42 averages) and in BG (TR/TE = 2000/68 ms and 56 averages). Data preprocessing and quantification of GABA spectra were performed with FID-A toolkit [Simpson et al. (2017)] and LCModel, respectively.

Results

Normalized GABA signal from the left motor cortex and basal ganglia following anodal tDCS are shown in Figure 2. The GABA signal decreases immediately after anodal stimulation and recovers by the end of the measurement in the motor cortex and in basal ganglia.

Discussion and Conclusion

Our study showed a similar trend of GABA modulation between the motor cortex and basal ganglia. The result showed immediate decrease of GABA concentration and a sign of recovery in the motor cortex and basal ganglia following anodal tDCS as measured with proton MRS. Our preliminary results are relevant to understand the neurochemical mechanism underlying neuronal plasticity in the basal ganglia motor cortical circuit. We intend to employ a large number of subjects in future for further analysis.

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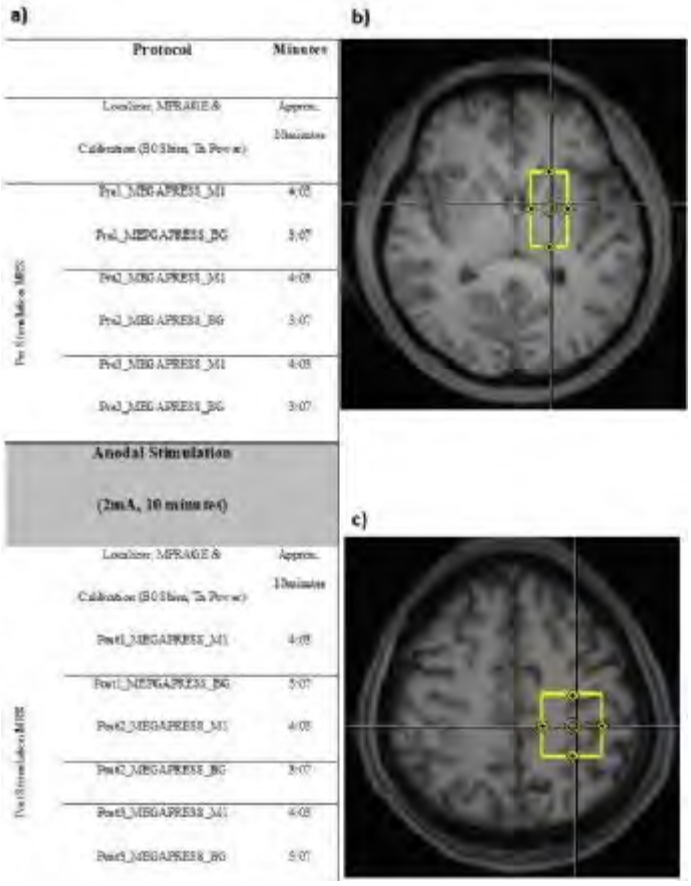


% Change in GABA/Baseline
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
1.00
1.18
1.04
1.17
0.90
1.06
0.74
1.18
0.85
1.11
0.82
BG
M1
Pre1
Pre2
Pre3
Post1
Post2
Post3
Measurements

Depletion of neurocan in the prefrontal cortex impairs temporal order recognition, cognitive flexibility, and perisomatic GABAergic innervation

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The condensed form of neural extracellular matrix (ECM), perineuronal nets (PNNs), are predominantly associated with parvalbumin-expressing (PV+) interneurons in the cortex and hippocampus. PNNs are enriched in several lecticans, including neurocan (Ncan). The *Ncan* gene polymorphism rs1064395 has been associated with changes in the hippocampus-dependent memory function, variation of prefrontal cortex structure, schizophrenia and mania in humans. Ncan knockout (KO) mice show related behavioral abnormalities (hyperactivity) and there is a decrease of Ncan expression in the medial prefrontal cortex (mPFC) in the pentylene tetrazole model of epilepsy. Here we focused on studying how dysregulation of Ncan specifically in the mPFC of mice may affect cognitive and synaptic functions using an adeno-associated virus (AAV) delivery to express shRNA against Ncan under a universal promoter. Analysis of PNNs in Ncan shRNA AAV-injected mice revealed a reduction in PNN labeling by *Wisteria floribunda* agglutinin (WFA) around PV+ interneurons. Reduced Ncan expression resulted in a loss of the mPFC-dependent temporal order recognition and impaired reversal spatial learning in the labyrinth (dry maze) task. As potential synaptic substrate of these cognitive abnormalities, we report a reduction in the perisomatic GABAergic innervation of PV+ cells in Ncan constitutive knockout and Ncan shRNA AAV-injected mice. GABAergic innervation of principal neurons was also reduced in Ncan shRNA AAV-injected mice, but compensated in Ncan-knockout mice. Thus, our findings highlight a functional role of Ncan in supporting perisomatic GABAergic inhibition, temporal order recognition and cognitive flexibility, as one of the important cognitive resources depleted in neuropsychiatric disorders.

Extracellular matrix balances principal cell excitability and synaptic plasticity

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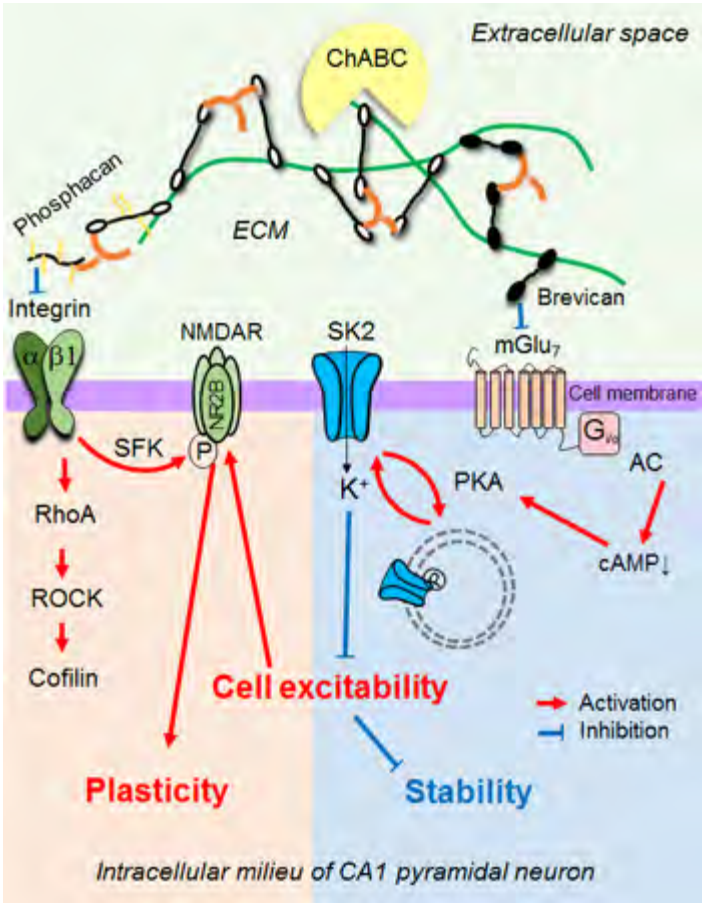
The extracellular matrix (ECM) regulates both physiological and pathophysiological processes in the brain. Paradoxically, ECM attenuation is reported either to enhance or to impair synaptic plasticity. Here, we uncover the molecular mechanisms behind this dualism. We demonstrate that enzymatic attenuation of ECM by chondroitinase ABC (ChABC) decreased CA1 pyramidal cell excitability and thus restrained long-term potentiation (LTP) in CA3-CA1 synapses. This effect was mediated by a loss of ECM proteoglycan brevican, triggering increased cell surface expression of small conductance (SK) Ca^{2+}-activated K^+ channels through a mechanism involving metabotropic glutamate receptor mGlu7 and protein kinase A. Blocking SK channels restored principal cell excitability and LTP in brevican knockout mice. It also led to a beta1 integrin- and NR2B-dependent enhancement of LTP after enzymatic attenuation of ECM, which was mimicked by the knockdown of phosphacan. Thus, ECM attenuation counterbalances the augmented predisposition of synapses to undergo modifications by reduced cell excitability. This homeostatic regulation may protect neurons from unspecific excessive potentiation/saturation of synaptic inputs.


Extracellular space
ChABC
Phosphacan
ECM
Integrin
Brevican
NMDAR
SK2
mGlu7
Cell membrane
α β1
NR2B
SFK
P
RhoA
ROCK
Cofilin
K+
PKA
AC
cAMP↓
Cell excitability
Activation
Inhibition
Plasticity
Stability
Intracellular milieu of CA1 pyramidal neuron

CtBP1 attenuates the suppression of synaptic transmission under metabolic stress

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C-terminal binding protein 1 (CtBP1) is a transcriptional co-repressor protein that shuttles between nucleus and presynapse in an activity- and metabolic state-dependent manner. It regulates the expression of neuroplasticity-related genes in the nucleus and the synaptic vesicle recycling in the presynapse. A de novo CtBP1 mutation in humans was linked to hypotonia, ataxia and mental retardation. The sensitivity of CtBP1 to NADH and its involvement in expressional regulation of mitochondrial pore-forming proteins such as Bax and Bim implicate its putative role(s) in the metabolic regulation of neuroplasticity.

In this project, we investigated the role of CtBP1 as a link between metabolic regulation and neuroplasticity. First, we recorded extracellular field excitatory postsynaptic potentials (fEPSPs) in the hippocampal Schaffer collaterals (SC) to CA1 pathway in acute slices from adult CtBP1 KO and wild type (WT) mice. We observed impaired in vitro LTP, but intact basal transmission in CtBP1 knock out mice (KO). It was shown that an application of the glycolytic inhibitor 2-deoxyglucose (2-DG) acutely suppresses neurotransmission and subsequent withdrawal of it induces potentiation in the SC to CA1 pathway. Since 2DG regulates activity of CtBP1, we compared the effect of 2-DG on fEPSPs suppression and subsequent recovery in WT and CtBP1 KOs. In addition, we measured the mitochondrial oxidative capacity in the same slices using mitochondrial succinate dehydrogenase activity-dependent MTT assay.
We observed accelerated suppression and delayed recovery of fEPSPs in response to a transient application of 2-DG in slices from CtBP1 KO mice. Moreover, neurotransmission more frequently failed to recover fully upon 2-DG withdrawal. The MTT assay indicated a decreased mitochondrial respiratory activity in the CtBP1 KO hippocampal slices even under metabolic stress-free conditions.

Our results indicate that CtBP1 has a modulatory role in hippocampal energy metabolism and promotes resilience against suppression of synaptic transmission upon glycolytic stress. This will not only shed light on the regulatory mechanism(s) linking neuroplasticity and metabolism but also provide possible treatment alternatives against the pathogenesis in patients carrying CtBP1 mutation.

The functional effects of the Rasopathy-related KRASV14I mutation in the brain

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Noonan Syndrome (NS) is a RASopathy, resulting from a germline KRASV14I mutation that leads to overactive KRAS and subsequently the dysregulation of the downstream RAS/MAPK pathway. Patients with NS have mild to moderate cognitive impairments along with facial dysmorphisms and cardiac deficits. In order to investigate the effect of the mutation specifically in the brain and its role in the excitation/inhibition balance, we generated a mouse strain expressing KRASV14I mutant from its natural promoter but restricted to the excitatory and glial cells of the forebrain (KI).

The biochemical analyses confirmed increased KRAS activity in brain lysates of KRASV14I KI animals. Analysis of the signaling downstream of KRAS revealed the dysregulation in ERK, AKT and JNK pathways. In addition, immunostainings in the brain slices confirmed increase RAS/MAPK activation and uncovered alterations in the excitation/inhibition balance in KI mice. Electrophysiological analysis showed normal spontaneous and carbachol-induced hippocampal oscillations but aberrant LTP in the Schaffer collaterals to CA1 hippocampal circuit in KI mice. Behavioral analysis confirmed impaired remote memory in this mouse model of NS.

Our results shed light on the functional effect and pathogenesis of the KRASV14I mutation on neuroplasticity in the hippocampus as well as create a basis for reversal studies in an attempt to develop medical interventions against mental impairments in NS patients.

All-trans retinoic acid induces synaptic plasticity in the human neocortex.

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A prominent feature of the central nervous system is its ability to adapt structural and functional properties of synaptic contacts. However, direct experimental evidence for coordinated structural and functional synaptic plasticity in the adult human cortex is currently missing. Here, we studied the structural and functional properties of superficial (layer 2/3) pyramidal neurons in acute slices prepared from mouse cortex or human cortical access material. Using whole-cell patch-clamp recordings and immunohistochemistry, we tested for the plasticity-promoting effects of all-trans retinoic acid (atRA), which has recently been suggested as medication for the treatment of neuropsychiatric disorders. Our experiments reveal increased excitatory neurotransmission in mouse and human cortical neurons upon atRA application. atRA-mediated synaptic adaptation requires mRNA-translation and is accompanied by increased spine volumes and ultrastructural remodeling of the calcium-storing spine apparatus organelle. We conclude that atRA is a potent mediator of synaptic plasticity in the mouse cortex and the adult human cortex. Supported by DFG and the EQUIP Medical Scientist Program, Faculty of Medicine, University of Freiburg.

Delineation of pathways behind impaired neuroplasticity in a mouse model of Noonan Syndrome

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RASopathies are a disease family linked to mutations in genes encoding components of the RAS/MAPK-pathway. Noonan Syndrome (NS), a neurodevelopmental syndrome affecting the heart, craniofacial features and cognition, belongs to the RASopathies and was linked to mutations in PTPN11, SOS1, RAF1, NRAS, SHOC2, CBL or KRAS. In patients, KRASV14I mutation resulting in an increase of active KRAS presents with mild to moderate cognitive defects.

The previous experiments indicated *in vivo* memory alterations and *in vitro* baseline synaptic transmission and LTP impairments in mice expressing KRASV14I mutation restricted to excitatory and glial cells of the forebrain (KI) compared to wild-type mice (WT) (see poster of S. Salar).

Our current aim is to characterize the mechanisms leading to these neuroplasticity impairments in order to develop treatment alternatives against cognitive changes seen in NS patients. To this end, we carried out extracellular field potential recordings from the CA1 stratum radiatum region evoked by the stimulation of Schaffer Collaterals in acute cortico-hippocampal brain slices from 7-8 week-old WT and KI mice. We evaluated the effects of pharmacological treatments, which 1) suppress the RAS/MAPK pathway directly or indirectly and 2) interfere with the excitatory/inhibitory balance, on baseline synaptic transmission, short-term plasticity and LTP induction.

The delineation of functional effect of the KRASV14I mutation may bring new insights to the role of RAS/MAPK pathway in neuroplasticity as well as help us to develop better treatment strategies against the cognitive deficits seen in NS patients.

Vesicle recruitment is not accelerated during LTP at neocortical layer 5 pyramidal neurons

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Long-term potentiation (LTP) is a potential mechanism for experience-dependent learning and memory. LTP can be expressed both pre- and postsynaptically. The mechanisms of presynaptic transmitter release and its regulation during plasticity remain poorly understood. Particularly, the speed of vesicle recruitment is an important parameter determining synaptic efficacy but difficult to quantify. It has not been studied carefully whether vesicle recruitment can be accelerated during LTP. To address this question we focused on LTP at synapses of layer 5 pyramidal neurons of the somatosensory cortex, which have been shown to exhibit presynaptic LTP referred to as redistribution of the synaptic efficacy. We performed whole cell recordings from layer 5 pyramidal cells in acute cortical slices with extracellular stimulation of local excitatory inputs and measured paired-pulse ratio, short-term plasticity during high-frequency transmission and recovery from depression before and after the induction of LTP. After LTP the EPSC amplitude and the depression during high-frequency transmission was increased, concomitant with a decrease in the paired pulse ratio. Interestingly, the steady state level was potentiated in addition, which would be consistent with an increase in vesicle recruitment kinetics. However, further mechanistic analyses including the investigation of the recovery from synaptic depression revealed that LTP increased the vesicular release probability and the number of readily releasable vesicle but not the rate of vesicle recruitment. In contrast, a second slow component occurred in the recovery from depression after LTP. Our data therefore indicate that the kinetic of vesicle recruitment is not accelerated during LTP at neocortical layer 5 neurons suggesting a biophysically maximized recruitment rate per release site which cannot be further enhanced during plasticity.

Modulation of synaptic plasticity by ketamine

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Introduction

Since clinical trials have shown that a single intravenous infusion of a subanaesthetic dose of ketamine is able to induce rapid antidepressant effects in patient suffering from major depressive disorder, this NMDA-receptor (NMDAR) antagonist came into focus in the development of new antidepressant drugs.

Recent findings suggest a decisive role of neuroplasticity in the pathophysiology of depression and the mechanism of action of antidepressants, particularly long-term synaptic plasticity (long-term synaptic potentiation, LTP; long-term synaptic depression, LTD). In previous work, we have shown that LTD is facilitated in animal models of depression and that antidepressants directly inhibit LTD. Moreover, correlates of synaptic plasticity in depressed patients are altered.

Given the decisive role of glutamatergic targets in synaptic plasticity, theoretical models predict a modulation of synaptic plasticity by ketamine; however, comprehensive experimental evidence is missing. Therefore, a systematic study to evaluate modulation of synaptic plasticity by ketamine was executed.

Methods

Basal transmission, associative and homosynaptic forms of LTP and LTD were elicited in CA3-CA1 synapses of hippocampal brain slices from wildtype mice (C57BL/6). Ketamine (K), the enantiomer S-ketamine (SK) and the metabolite (2R,6R)-hydroxynorketamine (HNK) were applied in the bath solution or by intraperitoneal (i.p.) injection. Experiments were performed in the presence or absence of the GABAA receptor antagonist picrotoxin (PIC) which blocks inhibitory interneurons. Ca2+ transients were measured by fluorescence imaging. Chronic behavioural despair (CBD) was used as an animal model of depression. The Mann-Whitney test was used to compare group differences (56 experimental series, $n>10$ each, significance level $p<0.05$).

Results

Basal synaptic transmission remained unchanged by K, SK and HNK. In the presence of PIC, NMDA-dependent forms of synaptic plasticity (LTP, homosynaptic LTD) were blocked by K, SK and HNK. Associative LTD (NMDAR-independent) was not altered by K and SK but inhibited by HNK. In the absence of PIC, LTD induction was blocked, and LTP remained unchanged by K, SK and HNK. In the CBD animal model, LTP was impaired, which was reversed by i.p. K. Postsynaptic AMPA currents and intracellular Ca2+ transients were augmented by HNK, but not by K.

Conclusion and Discussion

This study is the first to show that an “antidepressant pattern” (i.e. LTP maintenance and LTD inhibition) by K and SK is only observed if PIC is absent, pointing out the major role of interneurons in the MOA of ketamine. The blockade of NMDA-receptors on inhibiting interneurons leads to an enhanced glutamate release which might translate into an upregulation of BDNF signalling further downstream. These results favour the earlier described “disinhibition hypothesis”.

Moreover, this study provides novel insights in the unique characteristics of HNK. The blockade of LTD by HNK independent of PIC can be explained by a positive AMPA modulation and enhanced intracellular Ca2+

transients. Ongoing experiments examine the modulation of plasticity by a newly described additional target of ketamine on the BDNF-TrkB receptor.
We conclude that a complex modulation of synaptic plasticity, presumably involving several distinct targets, might underlie the rapid antidepressant action of ketamine.

Poster Topic

T9: Glia, Glia-Neuron Interactions

T9-1 Analysis of perineuronal net properties in primary hippocampal neurons after an acute treatment with microglia secreted factors
David Wegrzyn, Nadja Freund, Georg Juckel, Andreas Faissner

T9-2 Lactate originates from brain glycogen stores
Katja Fink, Nina Vardjan, Robert Zorec, Marko Kreft

T9-3 Noradrenergic cAMP-signaling in astrocytes of the murine olfactory bulb
Jessica Sauer, Antonia Beiersdorfer, Timo Fischer, Christine Gee, Christian Lohr

T9-4 Role of the electrogenic sodium bicarbonate cotransporter 1 (NBCe1) during energy failure in mouse cortical astrocytes
Katharina Everaerts, Eleni Roussa, Christine R. Rose

T9-5 Reactive astrocytes are necessary for spontaneous improvement of functional connectivity and behavior after stroke
Markus Aswendt, Ulrika Wilhelmsson, Frederique Wieters, Anna Stokowska, Felix Schmitt, Niklas Pallast, Yolande de Pablo, Lava Mohammed, Mathias Hoehn, Marcela Pekna, Milos Pekny

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T9-23 Selective triggering of Astrocytes Calcium signaling by Graphene-Based Devices
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Analysis of perineuronal net properties in primary hippocampal neurons after an acute treatment with microglia secreted factors

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Perineuronal nets (PNNs) are dense reticular structures consisting of extracellular matrix (ECM) molecules that cover the soma and proximal dendrites of a certain neuronal subpopulation. These highly specialized structures are frequently associated with fast-spiking parvalbumin positive interneurons and contribute to the regulation of synaptic plasticity (Gottschling et al. 2019). There is a growing evidence that PNNs are disrupted in neuropsychiatric disorders like schizophrenia. However, the exact cause for these disturbances is unknown. Interestingly, evidence is mounting that inflammatory processes appear in the central nervous system (CNS) of schizophrenia patients (Wegrzyn et al. 2020). Here, especially microglia, the immune cells of the CNS, might play an important role for disease progression. Microglia possess a repertoire of cytokines and matrix metallo proteinases (MMPs) that might influence PNN integrity and alter electrophysiological network properties. However, less is known about the impact of microglia secreted factors on PNN stability.
To shed light on this question, we performed an acute treatment study of cultured hippocampal neurons with conditioned medium of activated microglia. First, primary cortical microglia were cultured and treated for 24 h with polyinosinic-polycytidylic acid (Poly I:C; 50 µg/ml). Poly I:C acts through toll-like receptor 3 (TLR-3) signaling and induced a morphological rounding of microglia after 3 h of treatment. Via cytokine array analysis various members of the CCL- and CXCL-motif chemokine family could be identified in the supernatant of activated microglia. In addition, interleukin-6 (IL-6) and tumor necrosis factor-α; (TNF-α) were also observed in the supernatant. Besides, MMP-2 and MMP-9 could be verified on mRNA level. The treatment of hippocampal neurons with microglia conditioned medium was performed after a cultivation time of 12 days in vitro (DIV), when dense PNNs were visible. Then a 25 % (v/v) medium exchange was performed and after a subsequent 24 h incubation further analysis was done. Interestingly, immunocytochemical visualization of the PNN main component Aggrecan could prove a significant disruption in PNN intensity and area size. This disturbance went along with a decrease of structural γ-aminobutyric acid-(GABA)ergic and an increase of glutamatergic synapse numbers. On PNN negative neurons, a reduction of both, excitatory and inhibitory synapse numbers was observed. To investigate, whether the disruption of PNNs and the alteration of synapse numbers affect neuronal network properties, electrophysiological recordings were performed using multielectrode array (MEA) technology. Interestingly, a strong and significant increase of single occurring action potentials could be observed 24 h and 48 h after the addition of microglia conditioned medium. In summary, we demonstrate that activated microglia can alter PNN integrity as well as synaptic plasticity in an indirect manner.

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Lactate originates from brain glycogen stores

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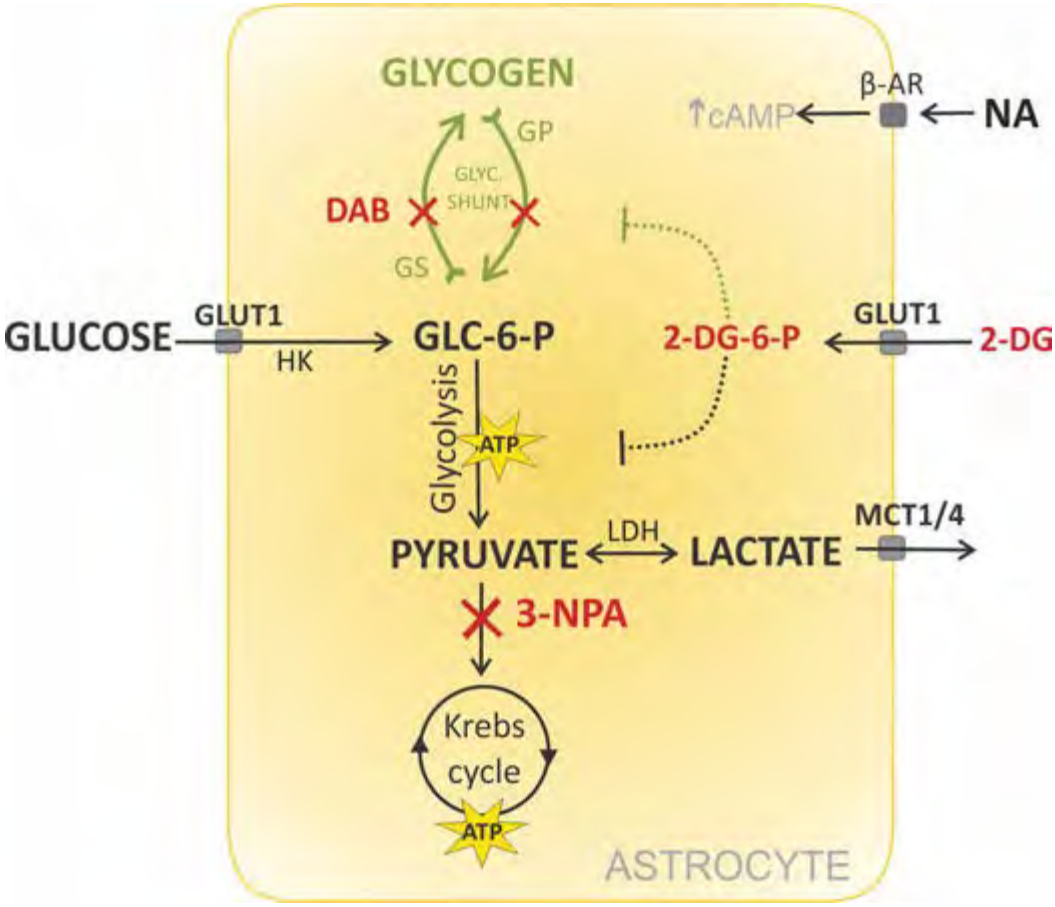
Approximately 20% of additional energy is required during cognitive efforts mediated by local neuronal networks. This is mediated by chemical messengers such as noradrenaline (NA), which targets astroglial aerobic glycolysis, the hallmark of which is the end-product L-lactate, a fuel for neurons. Biochemical studies have revealed that astrocytes exhibit a prominent glycogen shunt, in which a portion of D-glucose molecules entering the cytoplasm is transiently incorporated into glycogen, a buffer and source of D-glucose during increased energy demand. Here, we studied single astrocytes by measuring cytosolic L-lactate ($[lac]_i$) with the FRET nanosensor Laconic. We performed a calibration of the Laconic sensor in astrocytes with β-escin perforated plasma membranes. To block intracellular lactate production, we added oxamate (1 mM), which blocks L-lactate dehydrogenase and inhibits the conversion of pyruvate to L-lactate. We generated a calibration curve which was used to estimate $[lac]_i$. Next, we examined whether NA-induced increase in [lac]i is influenced by: i) 2-deoxy-D-glucose (2-DG, 3 mM), a molecule that enters the cytosol and inhibits the glycolytic pathway; ii) 1,4-dideoxy-1,4-imino-d-arabinitol (DAB, 300 µM), a potent inhibitor of glycogen phosphorylase and glycogen degradation; and iii) 3-nitropropionic acid (3-NPA, 1 mM), an inhibitor of the Krebs cycle. The results of these pharmacological experiments revealed that D-glucose uptake is essential for the NA-induced increase in $[lac]_i$, and that this exclusively arises from glycogen degradation, indicating that most, if not all, D-glucose molecules in NA-stimulated cells transit the glycogen shunt during glycolysis. Moreover, under the defined transmembrane D-glucose gradient, the glycolytic intermediates were not only used to produce L-lactate, but also to significantly support oxidative phosphorylation, as demonstrated by an elevation in [lac]i when Krebs cycle was inhibited. We found that a large proportion of D-glucose at rest is metabolized in the Krebs cycle, since the resting $[lac]_i$ is only 0.003 mM. This is increased to 0.620 mM after 3-NPA treatment, which blocks the Krebs cycle. This is in line with the view that the oxidative metabolism in mitochondria may metabolize 200x more D-glucose in comparison to D-glucose being metabolized to L-lactate in the glycolytic pathway at rest. However, NA stimulation further increases $[lac]_i$ by a factor of 3 (to 1.870 mM). This indicates that substantial aerobic glycolysis occurs only upon adrenergic stimulation of astrocytes. We conclude that L-lactate production via aerobic glycolysis is an essential energy pathway in NA-stimulated astrocytes; however, oxidative metabolism is important at rest.


GLYCOGEN
GP
GLYC.
SHUNT
DAB
GS
β-AR
↑cAMP
NA
GLUCOSE
GLUT1
HK
GLC-6-P
2-DG-6-P
GLUT1
2-DG
Glycolysis
ATP
PYRUVATE
LDH
LACTATE
MCT1/4
3-NPA
Krebs
cycle
ATP
ASTROCYTE

Noradrenergic cAMP-signaling in astrocytes of the murine olfactory bulb

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Astrocytes respond to a variety of neurotransmitters and modulators by activation of receptors and transporters. Most of those signaling cascades have been extensively studied in means of calcium signaling. However, many astrocytic receptors are coupled to G_i or G_s and initiate an intracellular rise or fall of cyclic adenosine 3´,5´-monophosphate (cAMP) concentration. cAMP is a ubiquitous second messenger and plays a central role in gene expression and synaptic transmission. While calcium imaging is a well-established method with a variety of different chemical and genetically encoded sensors, cAMP sensors have been lacking until recently and hence cAMP signaling is not well studied in astrocytes yet. Flamindo2 from Odaka et al. (PLOS One 9:6, 2014) is a genetically encoded cAMP sensor, which fluorescence intensity decreases in response to increased cAMP levels. In this study we investigated cAMP signaling in astrocytes of the main olfactory bulb by confocal cAMP imaging using Flamindo2 in acute brain slices. Therefore, we performed a retrooribital injection of the AAV-packed Flamindo2 construct controlled by the astrocyte-specific promotor GFAP. The olfactory bulb is innervated by noradrenergic projections from the locus coeruleus as main source of norepinephrine (NE). Those noradrenergic terminals are found in the glomerular layer and modulate neuronal plasticity and thus olfactory processing. Astrocytes express different NE-receptors; while α1-receptor-mediated calcium transients in astrocytes have been shown, we asked whether NE also evokes cAMP signaling. Our results show that the application of NE results in changes in cAMP levels in astrocytes via different G-protein-coupled receptors. Bath application of 10 µM NE (30 s) induced transient increases in cAMP concentration. Suppression of neuronal activity by use of a mix of glutamatergic and GABAergic antagonists as well as tetrodotoxin did not affect NE-induced cAMP signals. The application of the β receptor agonist isoprenaline evoked large cAMP signals, while α1 and α2 agonists phenylephrine and xylazine evoked small but measurable cAMP-signals. NE-induced cAMP signals could be significantly reduced with a combination of antagonists prazosin (α1), rauswolscine (α2) and ICI 118,551 (β). Our results show that NE evokes cAMP signaling in olfactory bulb astrocytes mainly by activation of β receptors and to a lesser extent by α1 and α2 receptors.

Role of the electrogenic sodium bicarbonate cotransporter 1 (NBCe1) during energy failure in mouse cortical astrocytes

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The electrogenic sodium bicarbonate cotransporter 1 (NBCe1; NBC) is a major regulator of extra- and intracellular pH in the brain. It is highly expressed by astrocytes and, owing to its stoichiometry of $1Na^+:2HCO_3^-$, can operate in inward or outward direction depending on the respective ion concentrations and membrane potential. Under conditions of energy failure, as e.g. observed upon brain ischemia and stroke, astrocytes experience a significant Na^+-loading, which may promote outward operation of NBC, thereby aggravating ischemia-induced intracellular acidosis.

To address this question, we performed wide-field imaging of intracellular H^+ and Na^+ employing the H^+-sensitive fluorescent indicator BCECF-AM and the Na^+-sensitive indicator SBFI-AM, respectively, in organotypic tissue slice cultures and primary cultures of astrocytes of the mouse cortex. In CO_2/HCO_3^--buffered saline, increasing extracellular $[K^+]$ to 7 mM resulted in an intracellular alkalinization as well as an increase in astrocytic $[Na^+]_i$. Both were significantly reduced by 30 µM S0859, suggesting that the $[K^+]$-induced depolarization resulted in inward operation of NBC. To model ischemia-like conditions, cells were then perfused with glucose-free saline, to which the metabolic inhibitors sodium azide (NaN_3, 5 mM) and 2-desoxyglucose (2-DG, 2 mM) were added. Induction of chemical ischemia for 2 minutes was accompanied by an acidification and an increase in astrocytic $[Na^+]_i$. Addition of S0859 dampened both the ischemia-induced acidosis as well as the Na^+ load, suggesting inward operation of astrocytic NBC. Finally, we analyzed astrocytes in organotypic tissue slices derived from NBCe1-deficient mice. These not only showed significantly reduced $[K^+]$-induced alkalinization and $[Na^+]_i$ increase, but also stronger acidosis and lower Na^+ load in response to chemical ischemia, confirming the results obtained with pharmacological inhibition of NBC.

Taken together, our results strongly suggest that short periods of energy failure result in activation of inwardly-directed NBCe1 in astrocytes. While NBC-mediated inward transport of HCO_3^- protects the cells from intracellular acidification, it augments the accompanying Na^+ load. Because astrocytic Na+ elevations reduce the driving force for Na^+-dependent glutamate uptake, this may result in exacerbation of extracellular glutamate accumulation and promote excitotoxicity.

Reactive astrocytes are necessary for spontaneous improvement of functional connectivity and behavior after stroke

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Objective

The intermediate filaments glial fibrillary acidic protein (GFAP) and vimentin are upregulated in reactive astrocytes surrounding a focal brain lesion, such as in ischemic stroke. The glial boundary zone serves disease progressing and protective functions (Pekny and Pekna, Physiol Rev 2014). Previously, we have shown that *GFAP-/-Vimentin-/-* mice develop smaller, cortical infarcts, induced by photothrombosis, as well as reduced functional recovery and CST axonal remodeling (Liu et al., Glia 2014). The aim of the current study was to monitor longitudinally the sensorimotor behavior as well as the functional neuronal network changes related to GFAP/Vimentin-deficiency in mice with cortical stroke using repetitive behavioral testing and in vivo Magnetic Resonance Imaging (MRI). We wanted to identify if there is a different baseline (pre-stroke) connectivity in knockout animals and if there is characteristic connectivity change between sensorimotor regions.

Methods

Two-months-old male *GFAP-/-Vim-/-* mice carrying a null mutation in the GFAP and vimentin genes (Pekny et al. Neurochem Res 1999), and male wild-type (WT) mice entered the study (n=14 WT and n=15 *GFAP-/-Vim-/-*). Mice underwent photothrombotic stroke and a longitudinal experimental protocol composed of repetitive MRI (GE-EPI rs-fMRI and T2-weighted MRI, 9.4T Bruker) and three different sensorimotor behavior tests (rotating beam, cylinder and grid wlk test) until 4 weeks post stroke as described previously (Aswendt et al., Transl Stroke Res 2020). The MRI data was registered with the Allen Mouse Brain atlas and processed using our software tools AIDAmri and AIDAconnect (https://github.com/maswendt/, (Pallast et al. 2020 Neuroimage, Front Neuroinform. 2019)). Photothrombosis was performed by injecting 1.5 mg Rose Bengal i.p. followed by 50 mW laser radiation at 561 nm for 15 min targeting the primary somatosensory forelimb area. Immunohistochemistry of Iba1, Sox2, S100beta and GAP43 was quantified in the ipsilesional and contralesional cortex 4 weeks after stroke.

Results and Conclusion

In a detailed atlas-based lesion mapping approach for the in vivo T2-weighted MRI and histology, we verified that the attenuation of reactive gliosis in *GFAP-/-Vimentin-/-* mice did not significantly change the lesion size and location compared to WT mice. In the acute phase, at 1 day post stroke, functional deficit determined by behavioral testing was increased and functional connectivity measured by rs-fMRI in the sensorimotor network decreased with no differences between the groups. However, in the following three

weeks, *GFAP-/-Vimentin-/-* recovered significantly slower and remained with a higher functional deficit. In line with the behavioral data, functional connectivity in terms of number of connections and connectivity strength remained lower and did not reverse back to baseline levels in *GFAP-/-Vimentin-/-* compared to WT mice. In contrast, there was an increased gain and loss of different connections compared to the WT mice. These findings, together with the increased expression of the axonal plasticity marker Gap43 in the *GFAP-/-Vim-/-* peri-infarct cortex, suggest that attenuation of reactive gliosis leads to suboptimal re-organization of functional neuronal networks and maladaptive plasticity responses after stroke. We conclude that reactive gliosis is required for normalization of functional connectivity and recovery after stroke.

Microglial cells sense neuronal activity indirectly via astrocyte GABA release in the postnatal hippocampus

Francesca Logiacco[1,2], Pengfei Xia[1], Svilen Georgiev[1], Celeste Franconi[1], Yi-Jen Chang[1], Bilge Ugursu[1,3], Susanne Wolf[1,3], Ralf Kühn[4], Helmut Kettenmann*[1,5], Marcus Semtner*[1]

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Microglia are the resident macrophages in the central nervous system. Besides the abundant expression of various immune receptors, microglia also express receptors for classical neurotransmitters, such as GABA and glutamate, suggesting their potential for sensing synaptic activity. To detect microglial Ca^{2+} in response to neuronal activity, we generated novel transgenic mouse lines which express the fluorescent Ca^{2+} indicator GCaMP6m specifically in microglia. We demonstrate that electrical stimulation of the Schaffer collateral pathway results in transient microglial Ca^{2+} responses at early postnatal, but not adult hippocampus. Microglial responses propagated in a wave-like fashion from the stimulus towards CA1. Preceding the microglial responses, we observed a similar wave-propagation of calcium responses also in astrocytes. Calcium wave propagation in both cell types was sensitive to TTX but not to ionotropic GABA and glutamate receptor blockers. Blocking astrocytic glutamate uptake or GABA transport abolished microglial responses due to Schaffer collateral stimulation. GABAB receptors, functionally expressed in microglia, are activated by Schaffer collateral stimulation, but their activation depends on functional glutamate and GABA transport in the astrocytes. Our data therefore suggest that the neuronal activity induced glutamate uptake and release of GABA by astrocytes which triggers the activation of GABAB receptors in microglia. This novel neuron, astrocyte and microglia communication pathway might modulate microglia activity, having in turn critical impact on developing neuronal networks

A mathematical model for ion homoestasis at the tripartite synapse during metabolic stress

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[2]University of Twente, Applied Mathematics, Drienerlolaan 5, 7522NB Enschede, Netherlands
[3]University of Twente, Applied Mathematics, Drienerlolaan 5, 7522NB Enschede, Netherlands
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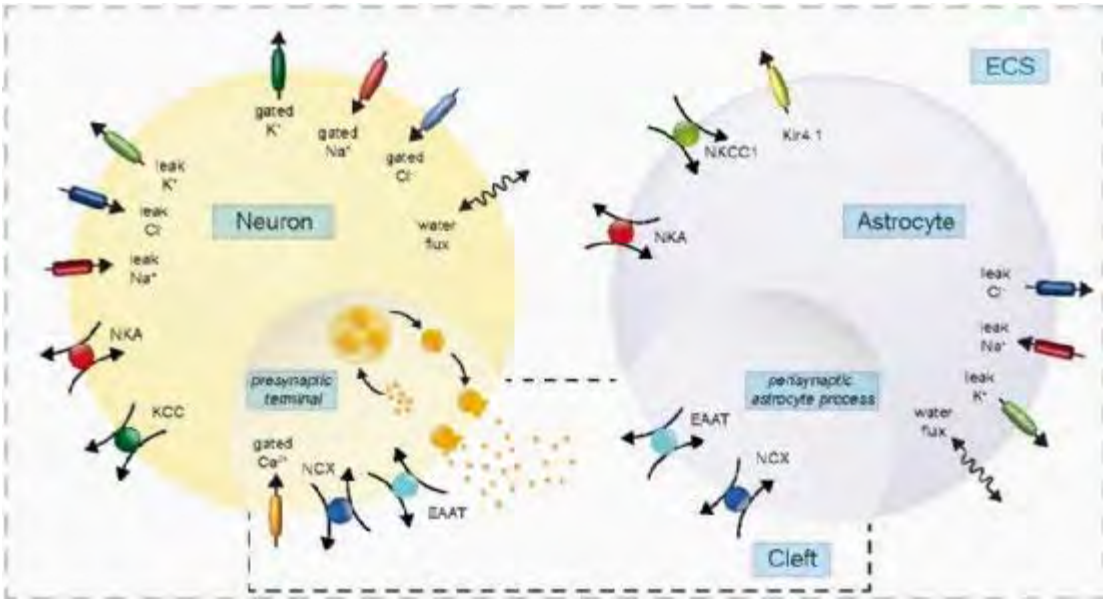
The anatomical and functional organization of neurons and astrocytes at `tripartite synapses' is essential for reliable neurotransmission, which critically depends on ATP. In low energy conditions, synaptic transmission fails, accompanied by a breakdown of ion gradients, changes in membrane potentials and cell swelling. The resulting cellular damage and cell death are causal to the often devastating consequences of an ischemic stroke. The severity of ischemic damage depends on the age and the brain region in which a stroke occurs, but the reasons for this differential vulnerability are far from understood. In the present study, we addressed this question by developing a comprehensive biophysical model of a glutamatergic synapse to identify key determinants of synaptic failure during energy deprivation. Our model is based on fundamental biophysical principles, includes dynamics of the most relevant ions, i.e., Na^+,K^+, Ca^{2+},Cl^- and glutamate, and is calibrated with experimental data. It confirms the critical role of the Na^+/K^+-ATPase in the maintenance of ion gradients, membrane potentials and cell volumes. Our simulations demonstrate that the system exhibits two stable states, one physiological and one pathological. During energy deprivation, the physiological state may disappear, forcing a transit to the pathological state, which can be reverted when blocking voltage-gated Na^+ channels.Our model predicts that the transition to the pathological state is favoured if the extracellular space fraction is small. A reduction in the extracellular space volume fraction, as e.g. observed with ageing, will thus promote the brain's susceptibility to ischemic damage. Our work provides clinically relevant insights into the neuropil's ability to recover from energy deprivation, with translational relevance for diagnosis and treatment of ischemic stroke. We also present an easy-to-use graphical user interface (GUI) for simulating our model, which can be used to compare with relevant experimental data.


ECS
gated K+
gated Na+
gated Cl-
leak K+
leak Cl-
leak Na+
Neuron
water flux
NKA
KCC
presynaptic terminal
gated Ca2+
NCX
EAAT
NKCC1
Kir4.1
NKA
Astrocyte
leak Cl-
leak Na+
leak K+
water flux
perisynaptic astrocyte process
EAAT
NCX
Cleft

The Role of Serotonergic Signaling in Astrocytes

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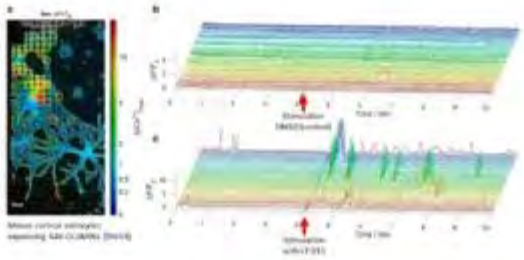
Astrocytes are an important component of the cellular network and crucial for proper brain function. They express receptors for serotonin (5-HT), an important neurotransmitter regulating various cellular processes and physiological functions. We have recently demonstrated that the serotonin receptor 4 (5-HT_4R) is expressed in hippocampal astrocytes, both *in vitro* and *in vivo*. Using fluorescence microscopy, we established that 5-HT_4R activation leads to morphological changes of astrocytes by triggering RhoA activity via Gα13-mediated signaling, which then boosts filamentous actin assembly. Further, 5-HT_4R-RhoA signaling changes glutamatergic synaptic transmission: It increases the frequency of miniature excitatory postsynaptic currents (mEPSCs) in mixed cultures and reduces the paired-pulse-ratio (PPR) of field excitatory postsynaptic potentials (fEPSPs) in acute slices. These findings render astrocytic 5-HT_4R signaling as a previously unrecognized molecular pathway involved in the functional regulation of excitatory synaptic circuits[1]. Moreover, we developed a novel multi-threshold event detection (MTED) approach to analyze astrocyte Ca^{2+} dynamics shaped by serotonergic signaling and morphological changes. By stimulating primary astrocytes with 5-HT receptor subtype specific agonists, we receive distinct responses of astrocytic Ca^{2+} event patterns, which indicates a direct impact of the 5-HT signaling pathway (Fig. 1).

1. Müller, F. E. et al. Serotonin receptor 4 regulates hippocampal astrocyte morphology and function. Glia n/a, (2020).

Figure 1: a, Color-coded Ca^{2+} activity of primary cortical astrocytes presented as Max $\Delta F/F_0$ of GCaMP6s. **b,** Time traces of 10 min recordings show no Ca^{2+} activity response to stimulation with DMSO. **c,** Stimulation of the same region with LP-211, a selective 5-HT_7R agonist, results in increased Ca^{2+} activity.

This study is supported by the DFG and Rebirth - Cluster of Excellence.



Divergent microglia morphological alterations in brain regions associated with hyper- and hipofunctional neuronal pathways in a rodent model of schizophrenia

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Schizophrenia (SCZ) is a mental disorder characterized by a plethora of symptoms, from hallucinations to cognitive deficits and disruption of social interactions, associated with the hyper- and hypofunctional state of specific dopaminergic pathways. Microglia cells screen the activity of newborn synaptic contacts and subsequently reinforce or eliminate them according to their level of activity. This fact led us to hypothesize that alterations in microglia morphology (which conditions function) during critical periods of neurodevelopment may underlie the hyper- and hypofunction of the aforementioned pathways.

In the present study we used the methylazoxymethanol acetate (MAM) rodent model of SCZ (based on the injection of the toxin, MAM, to pregnant Wistar rats at gestational day 17). Male and female descendants were subjected to neurodevelopmental tests and, at postnatal day 30 (adolescence), to cognitive and social behavioral tests. Brains were then collected to characterize microglia morphology in specific regions.

MAM males and females presented a delay in neurodevelopmental milestones associated with maternal odor discrimination/emotional state and involving the sensorial system. At adolescence, both sexes presented social deficits paralleled by an atrophy of microglia in hyperfunctional pathways (namely in the hippocampus and nucleus accumbens) and without morphologic alterations in the prefrontal cortex (hipofunctional pathway).

The fact that microglia from hyper- and hipofunctional pathways in the MAM model undergo differential morphologic changes may suggest a dual involvement of these cells in the genesis of SCZ during brain development, a working hypothesis deserving further investigation.

The contribution of microglial dysfunction to the pathogenesis of Prader-Willi syndrome

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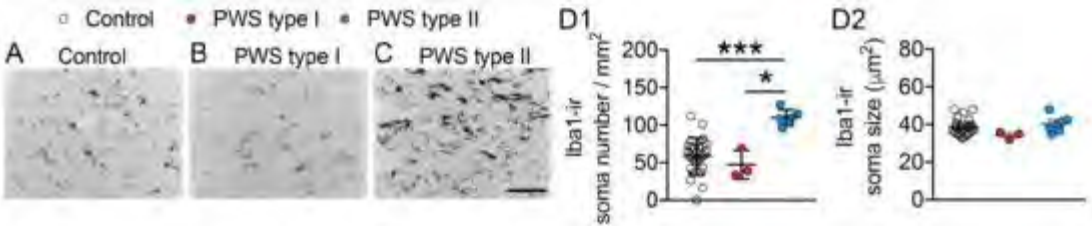
Background: Prader Willi Syndrome (PWS) is a rare neurodevelopmental disease characterized by hyperphagia, morbid obesity and mental retardation. PWS patients have a paternal deletion at chromosome 15q11-q13, which can be further divided into two subtypes. Patients with a type I (T1) deletion miss an extra chromosomal segment compared to type II (T2) and present a more severe phenotype. Hypothalamic dysfunction in controlling body weight and food intake is deemed the basis of hyperphagia and obesity in PWS. Microglia are the brain innate immune cells that coordinate brain immunity and are essential for keeping local brain microenvironment optimal for neuronal functioning and survival. Notably, microglial dysfunction is involved in hypothalamic neural dysregulation of food intake and energy homeostasis, both in mice and humans. Particularly disrupted microglia phagocytic function can lead to an unhealthy microenvironment for neurons due to the accumulation of synaptic and cellular debris. Here, we explored the association between the hypothalamic neurons and microglia and PWS subtypes.
Methods: To delineate PWS T1 from PWS T2 deletion, a Multiplex Ligation-dependent Probe Amplification (MLPA) method was used to perform genotyping of the formalin-fixed paraffin-embedded (FFPE) postmortem hypothalamic tissue donated by PWS patients to the Netherlands Brain Bank. We then profiled the hypothalamic microglia and neuronal populations involved in energy homeostasis. Immunoreactivity of neuropeptide Y (NPY) / pro-opiomelanocortin (POMC) / oxytocin (OXT) and arginine-vasopressin (AVP) were used to evaluate the cell number and soma size of these neurons. Immunoreactivity of ionized calcium-binding adapter molecule 1 (IBA1) was used to characterize microglia. Finally, CD68 immunoreactivity within IBA1 positive cells was analyzed among age-matched lean controls.
Results: We identified three subjects with T1 deletion (ages range 6 months – 49 years old) and seven subjects with T2 deletion (age range 3 -67 years old) and compared with the age-matched controls (age range 1 month – 77 years old). We observed an increased number of microglia in the hypothalami of the PWS T2 subjects compared to the controls. However, in PWS T1 microglia number is comparable to matched controls. Surprisingly, the microglia in T1 deletion are highly dysmorphic, with severe fragmentation of their cell bodies and processes. Microglia morphology is untimely connected with its immunosurveillance function. A constant retraction and expansion of their processes is an essential step of their phagocytic roles. Disruption in cell morphology eventually leads to defective immunosurveillance by microglia. Thus, we investigated CD68-immunoreactivity as a functional redoubt of its function. The microglia in T1 deletion has increased phagosome volume, whereas microglia in T2 deletion is comparable

to the controls. Taken together, the morphological disruption and abnormal accumulation of phagolysosome indicate a severe defect in microglial immunity in PWS T1. Regarding the profile of neurons, we observed an overall dampening in neuronal signature in PWS hypothalami, regardless of the genotype. Strickling, one PWS T1 subject has increased number of anorexigenic neurons. This is an infant, and thus before the onset of hyperphagia and obesity characteristic of PWS patients.

In summary, hypothalamic neuronal and microglial changes in PWS T2 mirror the pattern observed in non-PWS subjects. Whereas in PWS T1, the severe dysfunction of hypothalamic microglia, even before the disruption of the anorexigenic neuronal populations, suggests an association between the defective microglial immunity and the more severe phenotype found in PWS T1 deletion.



Investigation of astroglial heterogeneity in the human cortex and caudate nucleus

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Astroglia and neurons populate the human cerebral gray matter in a 1:1 ratio. While there is much information available on the diversity of neuronal populations, relatively little is known about that of the astroglia. We aimed to quantitatively investigate different astroglial populations present in the human cortex and caudate nucleus with morphometric and topographic analyses. We focused on the dorsolateral prefrontal cortex whose involvement in neuropsychiatric disorders is already demonstrated. Human brain tissue was provided by the Netherlands Brain Bank and Oxford Brain Bank.

Our results showed that GFAP+ and ALDH1L1+ astroglial populations were distributed in a partially overlapping pattern in the dorsolateral prefrontal cortex. The GFAP+ population was preferentially located in L1 and L6, whereas the ALDH1L1+ population was predominantly found in L2-L5. Furthermore, two times more ALDH1L1+ than GFAP+ astroglia was found in both the cerebral cortex and caudate nucleus.

Our study indicates diverse astroglial populations distributed in the human cerebral cortex and caudate nucleus in a complementary fashion. Furthermore, our results suggest that the use of GFAP in routine pathological investigations only informs about approximately one-third of the cortical astroglia. Regional distribution of diverse astroglial populations was mapped quantitatively in the human grey matter which will allow future investigations of potential astroglial alterations in conditions such as autism spectrum disorder and schizophrenia.

P2Y1 receptor-dependent neuron-glia communication in the mouse olfactory bulb

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Purine nucleotides such as ATP modulate the communication between cells throughout the nervous system and elicit a wide range of physiological effects. In the mouse olfactory bulb (OB), the first olfactory relay station of the olfactory sensory system in the CNS, ATP is released from nerve terminals of the sensory axons together with glutamate as a neurotransmitter and stimulates calcium signaling in glial cells as well as neuronal network activity in the OB.

To further dissect the contribution of purinergic signaling to the processing of olfactory signals we used spatiotemporal defined photolysis of caged ATP in acute mouse OB slices. By releasing ATP locally restricted to glomeruli, specific processing units of the OB, we mimicked the release of ATP in the course of incoming odor signals and recorded the response in output neurons (mitral cells) conveying to this glomerulus by whole-cell patch clamp.

Photoapplication of ATP led to a P2Y1 receptor-dependent increase in synaptic activity resulting in a prominent depolarization of MC. This effect is indirect and not dependent on common neurotransmitters such as GABA or glutamate. TTX almost completely abolished the ATP-induced mitral cell response, indicating a neuronal component in the signaling pathway. By means of confocal calcium imaging we identified a population of juxtaglomerular neurons selectively activated by ATP via P2Y1 receptors. In addition, the ATP-induced depolarization of mitral cells is almost completely inhibited in astrocyte-specific P2Y1 receptor knock out mice. This suggests that neurons contribute to P2Y1 receptor-dependent signaling pathways upon ATP release, but astrocytes play a significant role in modulating the P2Y1 receptor-dependent neuronal activity.

ATP not only is an important signaling molecule in normal brain physiology but also plays a crucial role in immune responses and is involved in the pathology of neurodegenerative diseases. Unpublished data shows, that the olfactory bulb is amongst the brain regions strongly affected by neurodegeneration and immune cell infiltration during experimental autoimmune encephalomyelitis (EAE), the primary animal model for multiple sclerosis research. To assess the influence of inflammatory conditions on P2Y1 receptor-dependent neuromodulation, we compared ATP-induced responses in mitral cells in acute EAE and healthy control animals.

Precise cortical myelin replacement after demyelination at a single cell level

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Myelin, rather than being a static insulator of axons, is emerging as an active participant in circuit plasticity. This requires precise regulation of oligodendrocyte numbers and myelination patterns. Here, by devising a laser ablation approach of single oligodendrocytes, followed by in vivo imaging and correlated ultrastructural reconstructions, we report that in mouse cortex demyelination as subtle as the loss of a single oligodendrocyte can trigger robust cell replacement and remyelination timed by myelin breakdown. This results in reliable reestablishment of the original myelin pattern along continuously myelinated axons, while in parallel, patchy isolated internodes emerge on previously unmyelinated axons. Therefore, in mammalian cortex, internodes along partially myelinated cortical axons are typically not reestablished, suggesting that the cues that guide patchy myelination are not preserved through cycles of de- and remyelination. In contrast, myelin sheaths forming continuous patterns show remarkable homeostatic resilience and remyelinate with single axon precision.

Role of astrocytic $GABA_B$ receptors on γ-hydroxybutyric acid induced absence seizures

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Absence seizures are non-convulsive epileptic events characterized by brief losses of consciousness, unresponsiveness to stimuli and are commonly observed in pediatric or juvenile epilepsies. The weak $GABA_B$ receptor agonist γ-hydroxybutyric acid (GHB) mimics generalized spike and slow wave discharges (SWDs) characterizing absence epilepsy and is therefore used as a pharmacological model of absence seizures. Given the role of astroglia in modulating and sustaining neuronal synaptic activity and their involvement in many different pathological scenarios, we investigated the role of astrocytic $GABA_B$ receptor in the genesis and progression of GHB-induced absence seizures. To this aim, we took advantage of the CreERT2-LoxP system to induce time-controlled astrocyte-specific gene deletion of the GABAB1 subunit resulting in lack of functional $GABA_B$ receptors in astrocytes. *Ex vivo* GHB administration in presence of the voltage-gated Na^+ channel blocker tetrodotoxin induced longer intracellular Ca^{2+} signals with higher amplitudes in astrocytes expressing the genetically encoded Ca^{2+} indicator GCaMP3 and imaged by two-photon laser-scanning microscopy (2P-LSM). Mice lacking functional astrocytic $GABA_B$ receptors showed unaltered Ca^{2+} signals compared to baseline. *In vivo* GHB induced highly synchronous Ca^{2+} waves in cortical astrocytes immediately after intravenous injection in control animals. On the contrary, in conditional knock-out animals Ca^{2+} signals after GHB injection were lower in amplitude compared to sham-treated animals. Moreover, loss of astrocytic $GABA_B$ receptors resulted in the alteration of GHB-induced dose-response assessed through *in vivo* telemetric electroencephalographical recording of brain activity and behavioural video monitoring. GHB-induced alterations lasted shorter independently of the administered dose. Taken together, these results suggest a role of astrocytic $GABA_B$ receptors in the mechanisms underlying GHB-induced absence seizures. This makes this receptor a promising target for the treatment of absence epilepsy, which is still effective in only half of the patients.

Photonic Gold-Nanoclusters (AuNCs) to probe the Morphological and Bioelectrical Properties of Primary Astrocytes

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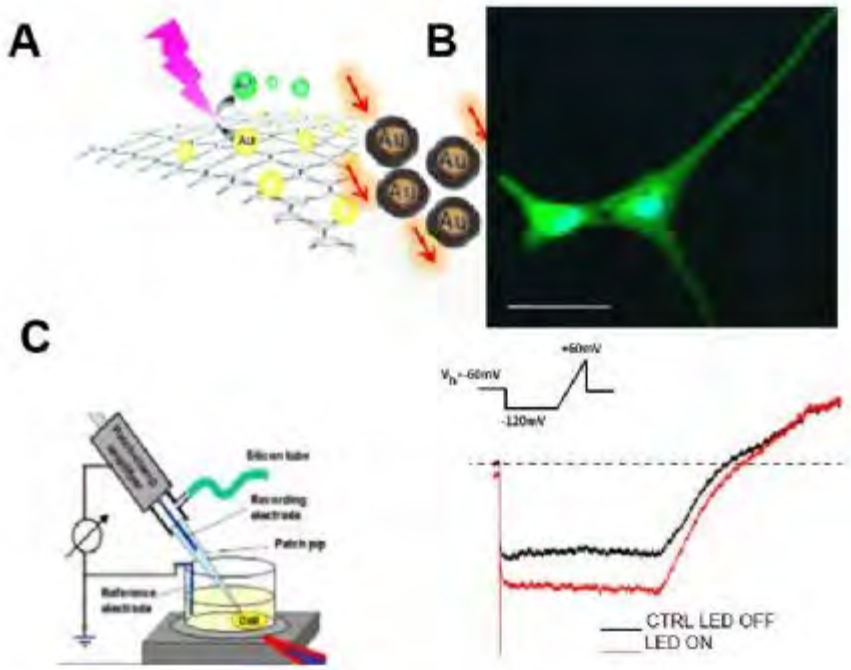
Over 40 year evidences indicated that astrocytes have a critical role in sensing chemo-physical changes occurring in the brain extracellular environment. Crucial in maintaining the homeostatic balance of the central nervous system (CNS) in vivo are the expression of ion channels and aquaporins at nano-microdomains of astrocyte endfeet. However, how astrocytes modulate their response to different chemo-physical stimuli is still unknown. Herein we propose, ultrasmall size (1-4 nm) metal nanoclusters (NCs) as a new class of nanoprobes useful in understanding the complex chemistry and physics of biological systems. We investigate the effect of GoldNCs (AuNCs) on primary rat cortical astrocytes structure and function in vitro.

By means of viability assay, time lapse imaging and confocal immunofluorescence, we found that treatment with AuNCs are not toxic for astrocytes and induce a formidable morphological differentiation in astrocytes. The tremendous cytoskeleton rearrangement demonstrates a huge cortical expression of actin in treated cells; data were followed by stimulated emission depletion microscopy (STED) imaging to qualitatively reveal the expression of inward rectifier potassium channels (KIR 4.1) and aquaporin-4 (AQP4), also revealed by western blot experiments. Moreover, perform scanning electric microscopy (SEM) was performed to reveal the possible localization of the cluster inside the astrocytes. Patch-clamp experiments indicated that differentiation was accompanied by the expression of potassium (K+) and chloride (Cl-) current increase in the whole-cell membrane currents upon photo-stimulation. Calcium Imaging was measured in order to collect Ca2+ fluxes across the cell membrane in response to trigger selective activation of transmembrane receptors and channels in treated cells.

These findings suggest that AuNCs offer a 1) a biocompatible, nanoscale powerful method to probe and sense astroglial bioelectrical properties acutely and chronically 2) a transformative approach to study astrocytic homeostatic regulation 3) a model to study the glial/neural network involvement in diseased brain physiology.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Uncovering neural populations and transcriptional networks underlying stress resilience in hippocampus

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Chronic stress can have major impacts on human health. After stress, though, the majority of individuals are resistant to mental dysfunction, they are called as resilient. However, a small portion of individuals, called susceptible, develops stress disorders such as anxiety and depression. Upon chronic stress, distinct brain circuits, involving several brain regions and cell types, are recruited. Even though the impact of chronic stress is rather well described at the behavioral levels, the molecular mechanism underlying stress resilience has remained largely unclear. In this project, by using the cutting-edge technique of single-cell RNA-seq the neural cell populations, and their individual transcriptomes were investigated in stress resilient and vulnerable mice. To this end, hippocampal cells from the left and right hemisphere, but also the dorsal and ventral part, respectively, were isolated from resilient and susceptible mice. Transcriptomes were analyzed from several thousands of single cells. After detailed computational analyses of these transcriptomes, we observed that the main cell populations, i.e., neurons, astrocytes or oligodendrocytes are conserved between the groups. We observed however that some specific signaling pathways are different between resilient and susceptible mice. For example, we observed that myelination and axonal ensheathment are upregulated in resilient mice and downregulated in susceptible mice as compared to controls. These different pathways are closely associated with specific genes dysregulated in distinct cell populations. Moreover, in resilient mice, we highlighted an interesting cross talk between the different cell populations, suggesting the stress resilient phenotype to be a complex process involving different cell types. In summary, identified cell populations and molecular mechanisms related to stress resilience were uncovered, opening new avenues for understanding the mechanisms underlying stress resilience.

Phagocytosis efficiency differs between M1 and M2 microglia

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Abstract:
Background: Microglia represent a resident macrophage population of the central nervous system (CNS). They play a critical role in various processes of the immune response after brain injury or infection, including phagocytosis and the release of inflammatory mediators. Accumulating evidence shows that microglial activation in the CNS is heterogeneous and two major polarization states have been defined as M1 and M2 microglia. In general, the M1 microglia exacerbate neuroinflammation, while the M2 microglia promote tissue repair and mediate anti-inflammatory effects. The reparative and anti-inflammatory actions of M2 microglia are closely related to their phagocytic activity. In the present study, we established in vitro assays to investigate the phagocytosis of M1/M2 microglia in more detail.
Methods: Cerebral cortices were dissociated from C57Bl/6 mice aged for 3-5 days and the cells were cultured for 7 days to obtain mixed primary glia cultures. The proliferation, activation, and polarization of microglia were induced for further 7 days by macrophage colony-stimulating factor (M-CSF) plus interleukin 4 (IL4) or granulocyte-macrophage colony-stimulating factor (GM-CSF) plus Interferon-gamma (INFγ). Morphometric analyses were carried out following immunocytochemistry using ImageJ. Gene expression analyses were performed using quantitative real-time PCR (qPCR). Phagocytosis was examined using fluorescently labeled rabbit IgG latex beads or E.coli particles in combination with immunocytochemistry. Data were analysed using GraphPad Prism software (Shapiro-Wilk test, QQ plots, Student's t-test., Mann-Whitney-U-test, ANOVA, Kruskal-Wallis-Test followed by post-hoc tests, Values are presented as mean ± standard error of the mean (SEM); $p^{*}<0.05$, $p^{**}<0.01$, $p^{***}<0.001$.
Results: We found various morphological changes between M1 and M2 microglia following induction such as size and circularity. M-CSF + IL4 treated microglia showed significantly increased mRNA expression of the M2 phenotype markers ARG1, and MRC1. GM-CSF + INFy treated microglia showed significantly increased mRNA expression of the M1 markers iNOS and MHC2. No differences between M1 and M2 microglia were found in the mRNA expression of IBA1, a common marker of microglia. M2 microglia showed a higher efficacy for the phagocytosis of IgG beads than E.coli particles. Conversely, M1 microglia showed a higher efficacy for the phagocytosis of E.coli particles than IgG beads.
Conclusion: We found that phagocytosis efficacies for IgG and E.coli particles differs between M1 and M2 microglia. The results further suggest that the phagocytic functions of M1/M2 polarized microglia differ in pathological conditions such as CNS injuries and infections.
Keywords: M-CSF; IL-4;GM-CSF; INFy microglia; M2; M1; phagocytosis; E. coli; IgG-latex beads.

Microglia regulate synaptic plasticity induced by repetitive magnetic stimulation.

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Microglia are the resident immune cells of the brain. Beyond their role in neuroinflammatory processes, recent studies revealed that microglia play an important role in physiological brain function.
To learn more about the role of microglia in neural homeostasis and plasticity, we studied CA1 pyramidal neurons in organotypic entorhino-hippocampal tissue cultures, which were treated with the CSF1R inhibitor PLX3397. Plasticity of excitatory neurotransmission was probed by 10 Hz repetitive magnetic stimulation (rMS). PLX3397 treatment resulted in a reliable microglia depletion from organotypic tissue cultures. No major alterations in basic structural and functional properties of CA1 pyramidal neurons were observed in the absence of microglia. In contrast, CA1 pyramidal neurons of PLX3397-treated tissue cultures did not express rMS-induced synaptic plasticity.
Transcriptome analyses are currently employed to learn more about the role of microglia in rMS-induced synaptic plasticity. Our results demonstrate that basic structural and functional properties of neurons are maintained in the absence of microglia, while the presence of microglia is required for the induction of synaptic plasticity.
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Molecular-physiological modeling of calcium homeostasis in cultured astrocytes

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Cultured astrocytes have long been used to investigate fundamental properties of cellular calcium regulation. Nevertheless, our understanding of homeodynamic calcium fluxes in cellular models such as astrocytes is rather limited. One reason is that the endoplasmic reticulum (ER) is the main intracellular calcium store, but the spatiotemporal profile of ER calcium dynamics is not well investigated. Here, we compared two methods of direct ER calcium imaging: targeted-esterase-induced dye loading (TED) with the low-affinity calcium indicator Fluo5N-AM and ER calcium imaging with the genetic indicator ER-GCaMP6-150. For an unbiased comparison of both imaging approaches, we developed objective ER signal analysis criteria using Python. In comparison, the TED method enabled faster detection of changes in ER calcium concentration. ER-GCaMP6-150 had the advantage to show less bleaching.
Due to its higher bleach-resistance, ER-GCaMP6-150 was better suited for a dual color calcium imaging approach in which ER calcium was imaged in green fluorescence, while cytosolic calcium was imaged with a red fluorescent calcium indicator. Moreover, to better understand the toolkit of calcium signaling mediators, we determined the mRNA transcriptome. Our data show that ER calcium responses in astrocytes are not stereotypic, but rather flexible towards extracellular calcium removal, albeit calcium signals are likely to be mediated by a rather small set of proteins of the calcium toolkit. For instance, handling of resting and induced store-operated calcium entry seems to depend on one highly expressed SERCA (Atp2a2) and proteins including ORAI1/2/3, STIM1/2. Trp ion channel proteins were barely expressed, only TrpC1 appeared at relevant levels in the transcriptome. Surprisingly, we found expression of the voltage-gated calcium channel Cacna1a (P/Q-type VGCC). To verify the physiological relevance of this finding, we showed that potassium-induced calcium influx in astrocytes could be blocked with agatoxin, an antagonist of P/Q-type calcium channels. Also, the metabotropic adenosine receptor Adora1a showed extraordinarily high expression. Accordingly, a rather low concentration of adenosine was sufficient to induce calcium oscillations in astrocytes. The preferential mediators of ER calcium release during these astrocytic calcium oscillations are IP_3 receptors (Itpr1/2), as ryanodine receptors (Ryr1-3) are almost absent in this cell model.
Based on these results, we offer an updated signaling model for homeodynamic calcium fluxes in cultured astrocytes. These data can also be used for a retrospective look at previous calcium data that were monitored in this prototypical cell model.

Activity staining and morphological analysis of olfactory bulb astrocytes in context with olfactory enrichment

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Astrocytes are involved in brain homeostasis and fulfill multiple functions associated with neuronal integration. As part of the tripartite synapse, they modulate synaptic processing on several levels. It is known that exposure to an enriched environment is generally beneficial for cognitive performance in human and mice. The influence of enriched environment on neuronal processing and morphology has intensively been studied within the last decade. In contrast, little is known about the influence of enriched environment on astrocyte morphology and the consequences on that. In the present pilot study, we evaluate the impact of olfactory enrichment on the morphology of astrocytes in the mouse olfactory bulb.

To first assess whether a complex odorant mixture leads to significant neuronal activation in the olfactory bulb, we analyzed c-Fos expression after 2 hours of olfactory stimulation and observed neuronal activation in stimulated mice as compared to un-stimulated controls. To analyze the impact of olfactory enrichment on astrocyte morphology, mice were exposed to the stimulating odorant mixture for 3 weeks. Following tissue clearing and immunhistological staining, 3D reconstruction and filament tracing of GFAP-positive structures were performed using the image analysis software Imaris. In total, 660 astrocytes from un-stimulated control mice and 935 astrocytes of mice housed in olfactory enriched environment were evaluated regarding several morphological characteristics. So far, analysis of the astrocytic skeleton, represented by GFAP-positive processes with a diameter larger than 1.5 µm, indicate a reduction of the complexity of these large-scale astrocytic branches in odorant-stimulated mice compared to un-stimulated mice.

Cisplatin-induced activation and functional modulation of satellite glial cells lead to cytokine-mediated modulation of sensory neuron excitability contributing to chemotherapy-induced painful neuropathy

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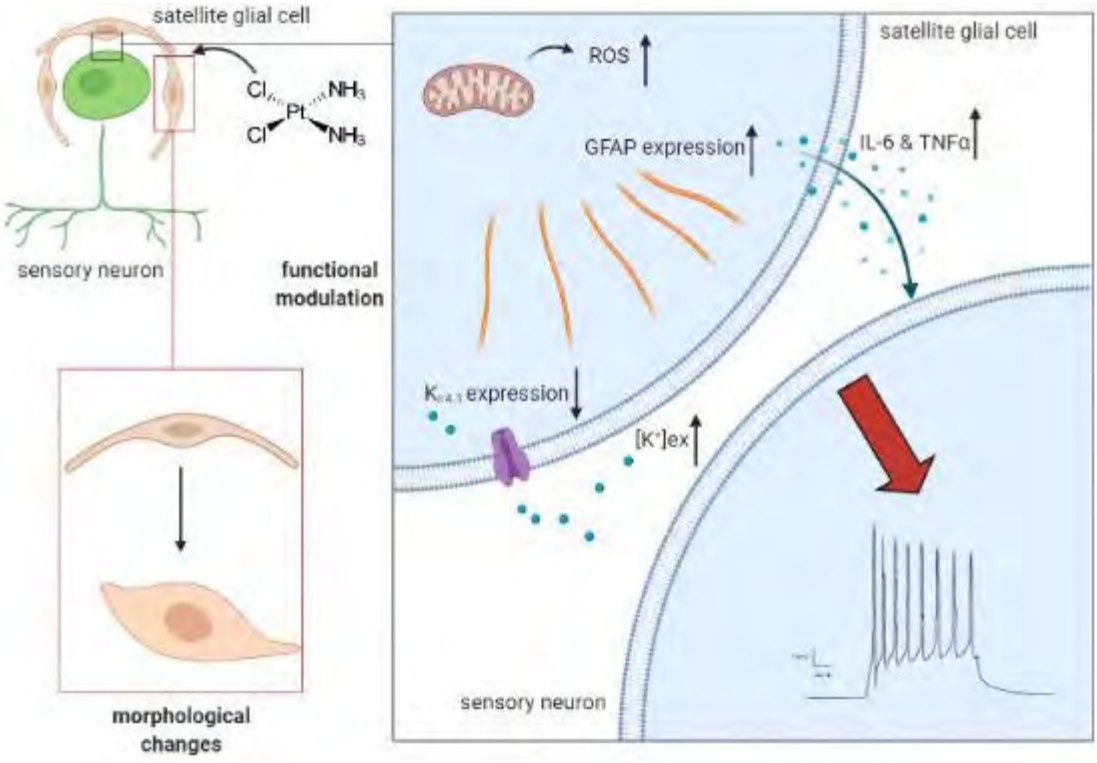
Cisplatin plays an essential role in the treatment of various cancers. Cisplatin exhibits high efficacy, but it often leads to severe neurotoxic side effects, such as chemotherapy-induced polyneuropathy (CIPN). The mechanisms leading to the genesis of CIPN are not fully understood with several potential pathomechanisms suggested, including the disturbance of calcium homeostasis in the dorsal root ganglia (DRG), mitochondrial dysfunction of DRG neurons, axonal loss, and apoptosis of neurons induced by platinum-deoxyribonucleic acid (DNA)-adducts. Interestingly, most described mechanisms leading to CIPN focus on the effects of chemotherapeutics on the function of DRG neurons, while surrounding satellite glial cells (SGCs) are more or less unnoticed.

Satellite glial cells play an essential role in regulating and maintaining extracellular matrix proteins and the release of various cytokines, adenosine phosphate (ATP), and other chemical messengers. Due to the encapsulation of neurons, SGCs precisely regulate the diffusion of molecules through the neuronal cell membrane. Satellite glial cells act as regulators of the neural environment and are characterized by their electrical properties, similar to astrocytes.

We investigated the influence of cisplatin on the function of SGCs and the direct influence on DRGs. Satellite glial cells were isolated from dorsal root ganglia of Wistar rats (4 weeks) and exposed to 0.1, 1, 10 or 100 µM cisplatin for 2 h, 4 h, and 24 h. Using immunocytochemical staining (ICC) and Western blot analysis, the expression of the glial fibrillary acid protein (GFAP), reactive oxygen species (ROS), and inward rectifier potassium channel 4.1 (Kir4.1) was determined. An increase in the immune reactivity (IR) and protein levels of GFAP and ROS was measured, and a reduction of IR and protein level of Kir4.1 was detected. A decrease in the current density of these channels was observed using the whole-cell patch-clamp recording. Furthermore, we observed morphological changes in SGCs after exposure to cisplatin. The release of the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor α (TNFα) from SGCs increased after cisplatin exposure, while the release of interleukin-1β (IL-1β) was decreased. The SGC-secreted factors in the supernatant after cisplatin treatment led to a modulation of excitability of cultured DRG neurons (Fig. 1).

Taken together, the modulation and function of different SGC proteins could be linked to a direct impact of cisplatin. Further, SGC-secreted factors influenced the excitability of sensory neurons. Overall, SGCs could be a potential target in the prevention and treatment of chemotherapy-induced neuropathic pain.


satellite glial cell
Cl
Pt
NH3
NH3
sensory neuron
functional modulation
morphological changes
ROS
satellite glial cell
GFAP expression
IL-6 & TNFα
K+4.1 expression
[K+]ex
sensory neuron

Altered Gap Junction Network Topography in Mouse Models for Human Hereditary Deafness

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One hallmark of the auditory system is the tonotopic organization of neuronal circuitry that is preserved from the cochlea, throughout auditory brainstem nuclei up to the auditory cortex. The formation of proper circuitry requires spontaneous neuronal activity in the pre-hearing animal. However, in CaV1.3 knock-out (KO) and otoferlin KO mice, where auditory brainstem nuclei are deprived of cochlea-driven neuronal activity, this precise organization is impaired in the lateral superior olive (LSO). Up to now, it remained unknown whether an impaired neuronal circuitry is followed by an altered astrocyte network topography. To answer this question, we analyzed the expression of astrocytic connexin (Cx) 43 and Cx30 in auditory brainstem nuclei of CaV1.3 KO and otoferlin KO mice by immunohistochemistry. Furthermore, we used acute brainstem slices prepared from animals at postnatal days 10-12 and whole-cell patch-clamp to load single sulforhodamine 101 labeled LSO astrocytes with the gap junction permeable tracer neurobiotin. After visualization of the tracer with avidin-conjugated Alexa Fluor 488, tracer coupled networks were documented at a confocal microscope and the shape and orientation of tracer-coupled networks analyzed using a vector based approach in combination with an intensity-based cell detection method. We found a strong elevation of Cx30 immunoreactivity in the LSO of CaV1.3 KO mice, while Cx43 levels were only slightly increased. In otoferlin KO mice, LSO showed a slight increase in Cx43 as well, whereas Cx30 levels were unchanged. The total number of tracer-coupled cells was unaltered and most networks were anisotropic in both KO strains. In contrast to wild type animals, however, LSO networks were predominantly oriented parallel to the tonotopic axis and not orthogonal to it. Taken together, our results show that spontaneous cochlea-driven neuronal activity is not required per se for the formation of anisotropic LSO astrocyte networks. Furthermore, they suggest that proper formation of LSO astrocyte networks necessitates neuronal input from the periphery, indicating a critical role of neuron-glia interaction during early postnatal development in the auditory brainstem.

Selective triggering of Astrocytes Calcium signaling by Graphene-Based Devices

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Within the last four decades the traditional understanding of brain functionality has dramatically changed, since the crucial role of astrocytes in the physiology and pathophysiology of the central nervous system has been widely recognized. Despite astrocytes have long been considered as passive glue supporting neurons, emerging knowledge indicates that they are actively involved in the control of brain homeostasis and modulation of synaptic transmission. The exploration of biomolecular mechanisms underlying the astrocytes functionality such as variation in intracellular calcium concentrations ([Ca2+]i) represents a major challenge for the development of innovative neuroscience tools and neuroengineering applications.
Graphene based nanomaterials have been successfully used as bio-interfaces in recent years, due to their unique combination of biocompatibility, mechanical and electrical properties. In vitro studies have shown the positive impact of graphene nanoflakes and functionalized graphene-oxide (GO) membranes on viability and physiological properties of astrocytes.
The present work aims to study the effect of extracellular electrical stimulation by Graphene-based devices on astrocytes [Ca2+]i signaling. We performed Fluo-4 calcium imaging experiments on primary rat cortical astrocytes plated on indium tin oxide coated with GO (ITO-GO) devices. Astrocytes were also grown on ITO and on ITO-reduced graphene oxide (ITO-rGO) devices. Unprecedentedly, we demonstrate the possibility to trigger different [Ca2+]i dynamics in astrocytes by exploiting different properties of GO-devices. Upon electrical stimulus, astrocytes on conductive substrates of ITO and rGO show rapid oscillatory dynamics mediated by the Ca2+ release from the cytoplasmic stores. Conversely, astrocytes on insulating GO devices display slower transients typical of extracellular Ca2+ influx, whose dynamics appear to correlate with the GO layer thickness. Our findings suggest the great potentialities of graphene substrates to generate advanced interfaces and devices devoted to the selective investigation and control of astrocytes processes in the study and therapy of brain functions and dysfunctions.
Supported by AFOSR-3D Neuroglia, ASTROMAT; EU-GRAPHENE FLAGHIP

Poster Topic

T10: Aging and Developmental Disorders

T10-1 Reversal of Altered Auditory Reactivity and Filtering in the *Cntnap2* Knock-Out Rat Model for Autism by Selective Activation of $GABA_B$ Receptors with R-Baclofen
Dorit Moehrle, Wenxuan Wang, Shawn Whitehead, Susanne Schmid

T10-2 Auditory processing disruptions in Cntnap2 knock-out rats
Susanne Schmid, Kaela Scott, Rajkamalpreet Mann, Dorit Moehrle, Brian Allman

T10-3 Effects of late-in-life spermidine on mTOR and Ca2+ signalling cascades
Alexander Wirth, Andre Zeug, Franziska Müller, Evgeni Ponimaskin

T10-4 Loss of CtBP1 in mice leads to decline in motor function and skeletal muscle atrophy
Debarpan Guhathakurta, Juliana Monti, Cosima Rhein, Liubov Kalinichenko, Christian P. Müller, Said Hashemolhosseini, Anna Fejtová

T10-5 Inhibition of microglia over-activation restores neuronal survival and maturation in a mouse model of CDKL5 deficiency disorder
Nicola Mottolese, Giuseppe Galvani, Laura Gennaccaro, Manuela Loi, Giorgio Medici, Marianna Tassinari, Stefania Trazzi, Elisabetta Ciani

T10-6 Glioblastoma Molecular Subtyping: Four or Three Types?
Giedrius Steponaitis, Vytautas Kucinskas, Ausra Saudargiene, Arimantas Tamasauskas

Reversal of Altered Auditory Reactivity and Filtering in the *Cntnap2* Knock-Out Rat Model for Autism by Selective Activation of $GABA_B$ Receptors with R-Baclofen

Dorit Moehrle[1], Wenxuan Wang[1], Shawn Whitehead[1], Susanne Schmid[1]

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Altered sensory information processing, and auditory processing in particular, is a common impairment in individuals with autism spectrum disorder (ASD). One prominent hypothesis for the etiology of ASD is an imbalance between neuronal excitation and inhibition. The selective $GABA_B$ agonist R-Baclofen previously improved social deficits and repetitive behaviors in several mouse models for neurodevelopmental disorders including ASD, and Arbaclofen has shown to ameliorate social avoidance symptoms in some individuals with ASD.

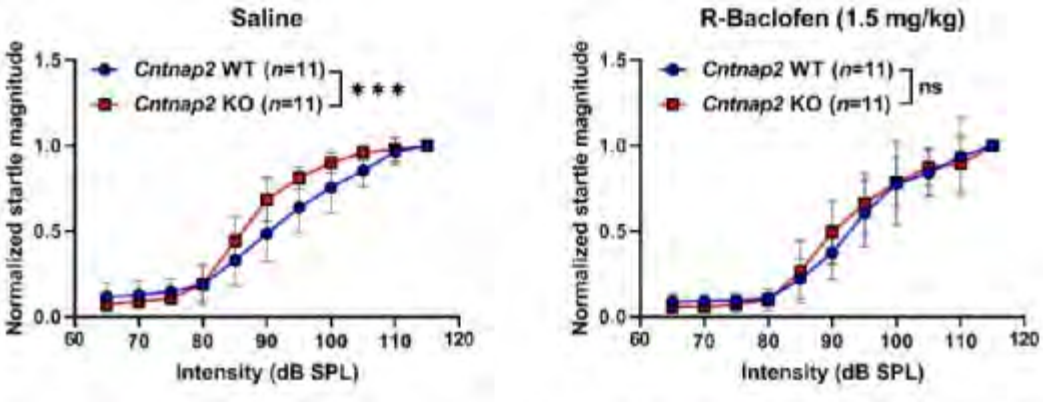
The present study investigated whether selective activation of $GABA_B$ receptors can remediate ASD-related altered sensory processing reliant on excitation/inhibition imbalance in the auditory brainstem.

We tested auditory reactivity, filtering and sensorimotor gating in form of acoustic startle response, habituation and prepulse inhibition after acute administration of R-Baclofen (0.75, 1.5, and 3 mg/kg) in the *Cntnap2* knock-out (KO) rat model. In order to assess a possible excitation/inhibition imbalance in brainstem circuits mediating sensorimotor gating, we also detected and quantified GABA and glutamate neurotransmitter levels in the startle mediating PnC (nucleus reticularis pontis caudalis) as well as in the auditory cortex of *Cntnap2* wild-type (WT) and KO rats using Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI MS).

R-Baclofen treatment suppressed exaggerated auditory startle responses and improved disruptions in habituation in *Cntnap2* KO rats in a dose-dependent fashion, with the higher doses bringing startle responses in *Cntnap2* KO rats close to control animals. Prepulse inhibition of the acoustic startle response (PPI) – an evaluation of sensorimotor gating – increased across various acoustic prepulse conditions after administration of R-Baclofen in *Cntnap2* KO rats, but R-Baclofen did not significantly affect PPI in WT rats. MALDI MS analysis indicated differences in GABA and glutamate signal intensity between the PnC from Cntnap2 WT and KO rats.

Our findings suggest that $GABA_B$ receptor agonists may be useful for pharmacologically targeting sensory processing disruptions involving neuronal excitation/inhibition imbalance in ASD.

We thank SFARI for providing R-Baclofen. This study was supported by CIHR and the Deutsche Forschungsgemeinschaft (MO 3857/2-1)


Saline
Cntnap2 WT (n=11)
Cntnap2 KO (n=11)
***
Normalized startle magnitude
Intensity (dB SPL)
R-Baclofen (1.5 mg/kg)
Cntnap2 WT (n=11)
Cntnap2 KO (n=11)
ns
Normalized startle magnitude
Intensity (dB SPL)

Auditory processing disruptions in Cntnap2 knock-out rats

Susanne Schmid[1], Kaela Scott[1], Rajkamalpreet Mann[1], Dorit Moehrle[1], Brian Allman[1]

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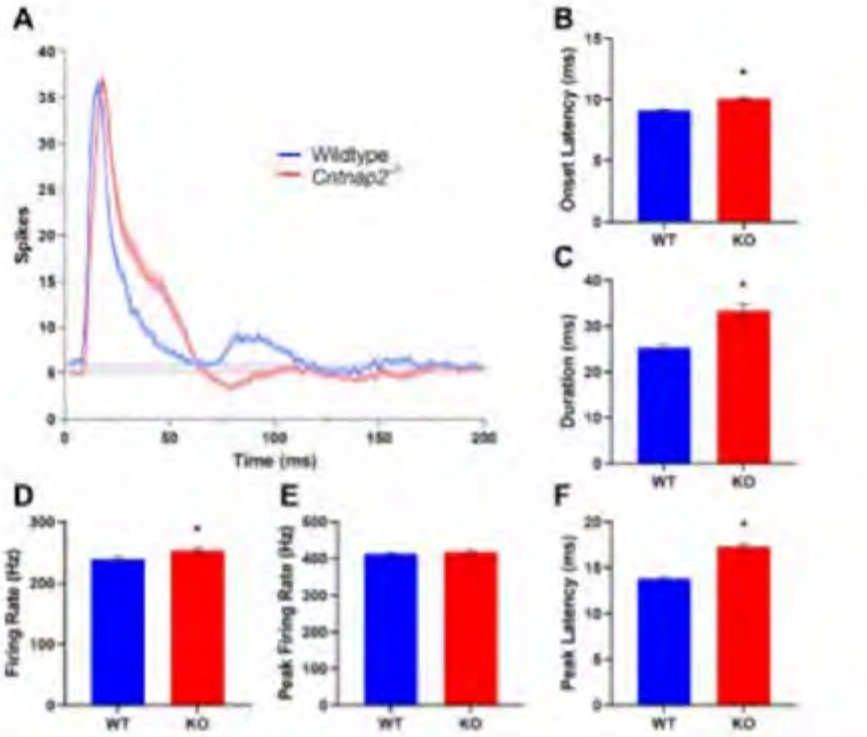
Autism spectrum disorder (ASD) is a pervasive neurodevelopmental disorder that can be caused by a multitude of genetic and/or environmental impacts on the developing brain. It's etiology and the neuronal changes underlying this complex disorder are not well understood. Like most psychiatric disorders, it is difficult to model most ASD core symptoms in an animal model that would allow for invasive approaches to study underlying causes, as these are often intrinsically human in nature and involve higher order cortical processing and language, not possible to observe in common animal models. Changes in sensory processing have been acknowledged as a core ASD symptom with the recent DSM-5. We studied auditory processing in the brainstem and auditory cortex, as well as auditory evoked behaviours in a transgenic rat model, lacking a functional Cnatnap2 gene. Cntnap2 mutations are highly associated with ASD and a lack-of-function of Cntnap2 leads to a developmental disorder with core symptoms of ASD.

Cntnap2 KO rats show an delayed maturation of the auditory brainstem responses (ABRs) resulting in lower and slower wave forms. This phenotype is only observed if they are bred homozygously. Interestingly, brainstem-dependent acoustic startle responses are largely increased and delayed in adolescent and in adult animals, even after the ABR matured to normal levels. Knock-out animals also show disruptions in sensorimotor gating, mirroring the findings in children with ASD. On the cortical level, they seem to perceive sound in a similar way as wild-type littermates, but are more sensitive to sound, as shown by a novel sound avoidance task. Electrophysiological recordings in vivo show that despite mature ABRs in adulthood, cortical auditory evoked responses (AEP) and local multi-unit (MU) responses are slower and loner lasting, indicating immaturity and hyper-excitability, which, again, parallel those reported in ASD. Finally, patch-clamp recordings confirm hyper-excitability on the single neuron level, but also a larger half-width of action potentials impacting the ability of repeated firing at high frequencies. The latter finding indicates that voltage-gated potassium channels might be at least partially responsible for the observed changes.

In summary, our studies of auditory processing in a rat model for ASD allows for behavioural as well as electrophysiological and pharmaceutical approaches geared to decipher changes in synaptic and cellular function underlying sensory ASD symptoms. Future experiments will determine to what extent pharmaceutical approaches that reverse these auditory changes can ameliorate other ASD core symptoms.


A
Wildtype
Cntnap2-/-
Spikes
Time (ms)
B
Onset Latency (ms)
WT
KO
C
Duration (ms)
WT
KO
D
Firing Rate (Hz)
WT
KO
E
Peak Firing Rate (Hz)
WT
KO
F
Peak Latency (ms)
WT
KO

Effects of late-in-life spermidine on mTOR and Ca2+ signalling cascades

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Multiple aspects of physiological ageing has been shown to go along with a decline in autophagy. Caloric restriction and thereby reducing nutrient-signalling followed by an upregulation of autophagy has been identified as major contributors to increased healthy life expectancy across different species. While the level of endogenous polyamines such as spermidine decline with ageing, exogenous polyamine supplementation has been shown to increase autophagy, which might support healthy ageing.
Here we aimed to analyse the effects of the caloric restriction mimetic spermidine using 24 months old mice as a model. We demonstrated that a six month spermidine intake ameliorates ageing-related inflammation within the heart, the liver and the kidney. We also found decreased inflammation within the hippocampus and were interested in the mechanism of action of spermidine. Therefore, we analysed key signalling cascades within the hippocampus using stereotactic injections of Förster resonance energy transfer (FRET)-based biosensors following two-photon excitation. Among others, we studied mTOR kinase signalling using TORCAR biosensor in neurons and characterised calcium waves in astrocytes of young, aged and spermidine fed aged mice using a genetically encoded calcium indicator (GCaMP). Our experiments revealed age-related changes in mTOR and calcium signalling. Moreover, we found that both signalling modules can be modulated by spermidine.

Loss of CtBP1 in mice leads to decline in motor function and skeletal muscle atrophy

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CtBP1 and CtBP2 belong to C-terminal binding protein family of transcriptional co-repressors, which have been shown to be of importance in signalling cascades regulating mice embryonal spinal cord, vasculature and muscle development. Recently, patients harbouring a de novo Arg331Trp mutation in the substrate-binding domain of CtBP1 with weight gain deficiency, hypotonia and defective motor-skill development leading to a later-stage immobility were described. However, a mechanistic linkage between muscle strength, mass, wasting and CtBP1 is still elusive. Using mice behavioural studies, gene-expression profiling, immunohistochemistry and immunoblotting, we aimed to characterize motor-function and skeletal muscle physiology in CtBP1 knock out (KO) mice.

We report motor-movement and limb-strength deficits in adolescent CtBP1 KO mice. Skeletal muscles of these mice have lower weight and display characteristics of muscle atrophy. We also show shifts in signalling cascades, which control anabolic and catabolic processes, in CtBP1-deleted mice skeletal muscle. Interestingly, we detected increased activity of cytochrome c oxidase (COX) in CtBP1 KO muscles, which is in good agreement with an aberrant mitochondrial oxidative capacity described in CtBP1R331W patients.

Overall, we demonstrate compromised motor-function upon loss of CtBP1 in mice, which is in line with reports of degenerative neuromuscular phenotype seen in patients. Dysregulation in growth factor signalling cascades that control skeletal muscle mass might be the underlying mechanism of the behavioural phenotype in CtBP1-deleted mice.

Inhibition of microglia over-activation restores neuronal survival and maturation in a mouse model of CDKL5 deficiency disorder

Nicola Mottolese[1], Giuseppe Galvani[1], Laura Gennaccaro[1], Manuela Loi[1], Giorgio Medici[1], Marianna Tassinari[1], Stefania Trazzi[1], Elisabetta Ciani[1]

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Microglia, the immune cells of the central nervous system (CNS), have recently taken center stage in research due to their role in CNS diseases. An abnormal immune response during critical windows of development and consequent abnormal production of neuro-inflammatory mediators has an impact on the function and structure of the brain and contributes to the pathogenesis of several neurodevelopmental disorders such as Autism Spectrum Disorders. CDKL5 deficiency disorder (CDD) is a rare neurodevelopmental disease caused by de novo mutations in the X-linked CDKL5 gene. The consequent misexpression of the CDKL5 protein in the nervous system leads to a severe phenotype characterized by intellectual disability, autistic features, motor impairment, visual deficits, and early-onset epilepsy. To date, little is known about the etiology of CDD and no therapies are available. Recently, a major cytokine dysregulation proportional to clinical severity, inflammatory status, and redox imbalance was evidenced in plasma from CDD patients, suggesting a subclinical chronic inflammatory status in children affected by this pathology. However, it is still unknown whether such an inflammatory state may even be mirrored at the cerebral level and whether it can contribute to the pathophysiology of CDD. *Cdkl5* knockout (KO) mouse models, recently created to investigate the role of CDKL5 in the etiology of CDD, recapitulate various features of the disorder. Previous studies have shown impaired neuronal maturation and survival in the hippocampus of *Cdkl5* KO mice, but the knowledge of the substrates underlying these alterations is still limited. Interestingly, we found increased microglial activation in the brain of a mouse model of CDD, the *Cdkl5* KO mouse. We found alterations of microglial cell morphology and number, increased levels of AIF-1 and proinflammatory cytokines, and increased STAT3 signaling in the brain of *Cdkl5* KO mice. Remarkably, treatment with Luteolin (a natural anti-inflammatory flavonoid) recovers microglia alterations as well as impaired neuronal survival and maturation in *Cdkl5* KO mice, and prevents increased NMDA-induced cell death in the hippocampus. Our results suggest that neuro-inflammatory processes contribute to the pathogenesis of CDD and imply the potential usefulness of Luteolin as a treatment option in CDD patients.

Glioblastoma Molecular Subtyping: Four or Three Types?

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Background. Glioblastomas (GBM) are the most common intrinsic brain tumors in adults and are nearly uniformly fatal with a patients' median overall survival of 15 months [1]. The assignment of the neoplasia to the GBM class is currently based primarily on histological features; nevertheless, the molecular landscape of GBM shows very high inter- and intra-tumor heterogeneity that indicates the need for more precise and homogeneous classification. A bunch of the research for GBM molecular classification resulted in plenty of publications where different numbers of GBM subtypes were proposed [2–4]. A different number of GBM subtypes causes confusion and interferes with the comparison of data between researchers and is incompatible with research reproducibility. The Aim of this study was to elucidate if the Neural subtype (NE) of GBM proposed by Verhaak et al., 2010 [5] can be described as a distinct group or is a part of the other three (Mesenchymal -MES, Classical –CL and Proneural -PN) subtypes of GBM.
Materials and methods. Affymetrix microarray data (N=528) of GBM mRNA level was downloaded from the Cancer Genome Atlas (TCGA) database. The most informative 300 genes out of the 12000 ones were selected applying the Chi-Square Test feature selection method. The principal component analysis (PCA) and an autoencoder, based on deep feed-forward neural network, were applied for selected 300 genes data compression. The extracted 20 PCA and the autoencoder-compressed three new features we used to compare the NE subtype (Verhaak) with the MES, CL and PN subtypes (Phillips et al., 2006 [6]) applying Hotelling T2 test (HT2). Finally, the Euclidean distance (ED) was calculated to estimate the proximity between subtypes.
Results. Statistically significant differences in the means of the extracted 20 PCA were observed between NE subtype and all three GBM subtypes (MES, CL and PN) defined by Phillips et al., 2006: HT^2=429.95, p<0.001; HT^2=225.22, p<0.001; HT^2= 298.92, p<0.001 accordingly. Similarly, autoencoder revealed significant differences between the NE and MES, CL, PN subtypes (by Phillips): HT^2=238.40, p<0.001; HT^2=94.44, p<0.001; HT^2= 111.91, p<0.001 accordingly. Euclidean distance for the 20 PCAs between the centers of the NE and MES, CL and PN were 2.326, 1.366 and 2.069, respectively. ED for three autoencoder features between the centers of the NE and MES, CL and PN s were accordingly 2.955, 1.140 and 2.822. MES, CL and PN subtypes defined by Phillips et al., 2006 and Verhaak et al., 2010 overlap and do not differ significantly in the means of the extracted PCAs and three compressed features, obtained by the autoencoder.
Conclusions. The study results suggest that the Neural subtype proposed in Verhaak et al., 2010 classification represents a group of the GBM tumors having its unique molecular signature that statistically significantly differs from three subtype model (MES, CL and PN) suggested by Phillips et al., 2006. Very recent research performed by Madurga et al., 2020 revealed that neural subtype is consisted of high content of normal cells [7], therefore neural subtype should be treated as a separate group of GBM indicating that different approaches might be applied for the intended treatment and prognosis. Since the molecular heterogeneity of GBM tumors has an assuredly significant role for the disease initiation, course, and patient outcome [2, 4], the precise subgrouping of GBM to as homogeneous groups as possible is indispensable.

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Poster Topic

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

T11-1 The metabotropic glutamate receptor 5 and deep brain stimulation: expression analyses in a hamster model of dystonia
Stefanie Perl, Anika Lüttig, Rüdiger Köhling, Angelika Richter

T11-2 Expression of c-Fos after short-term deep brain stimulation in the dystonic dt^{sz} hamster
Anika Lüttig, Stefanie Perl, Maria Paap, Denise Franz, Marco Heerdegen, Rüdiger Köhling, Angelika Richter

T11-3 Genetic Polymorphisms in the Renin-Angiotensin System and Cognitive Decline in Parkinson's Disease
Anna Pierzchlinska, Monika Bialecka

T11-4 Modulation of phosphatidylserine externalisation and its effects on morphology, viability and survival in neurons
Alexandra Brigitta Merkel, Daniela Mauceri

T11-5 A modular brain-on-chip for modelling epileptic seizures with functionally connected human neuronal networks
Ropafadzo Roseanne Mzezewa, Anssi Pelkonen, Lassi Sukki, Tomi Ryynänen, Joose Kreutzer, Tanja Hyvärinen, Andrey Vinogradov, Laura Aarnos, Pasi Kallio, Jukka Lekkala, Susanna Narkilahti

T11-6 *In vivo* Imaging with ^{18}F-Florbetaben-PET/MRI to detect Amyloid-β pathology in the Brain of the 5XFAD Mouse Model of Alzheimer's Disease
Franziska Schmitt, Timon N Franke, Caroline Irwin, Nicola Beindorff, Caroline Bouter, Yvonne Bouter

T11-7 Developing 2-D and 3-D models to disentangle the interplay between mitochondrial vulnerability and inflammation in Parkinson's disease
Helena Winterberg, Flora Magno, Jana Heneine, Benjamin Galet, Noemi Asfogo, Julie Smeyers, Morwena Latouche, Aurore Tourville, Patrick P. Michel, Philippe Ravassard, Olga Corti

T11-8 Trimethyltin induces calcium dysregulation and inflammatory phenotype in astrocytes in vitro
Katarina Milicevic, Milorad Dragic, Marija Adzic, Ivana Grkovic, Pavle Andjus, Nadezda Nedeljkovic

T11-9 Toxic Metamorphosis - How Changes from Lysosomal to Cytosolic pH Modify the Alpha-Synuclein Aggregation Pattern
Alice Drobny, Bisher Eymsh, Timon R. Heyn, Wei Xiang, Ralph Lucuis, Karin Schwarz, Julia K. Keppler, Friederike Zunke, Philipp Arnold

T11-10 Deep brain stimulation in the inferior colliculus reduces motor deficits in 6-OHDA lesioned hemiparkinsonian rats
Nicole Richter, Camila Pasquini de Souza, Davi Felipe de Almeida, Silvana Regina de Melo, Catarina Raposo, Claudio da Cunha, Rainer Schwarting, Liana Melo-Thomas

T11-11 Targeting the serotonin receptor 7 ameliorates Tau pathology and memory deficits
Josephine Labus, Kathrin Jahreis, Shaobo Jia, Alexander Dityatev, Evgeni Ponimaskin

T11-12 Pathophysiological *in vitro* profile of neuronal differentiated cells derived from Niemann-Pick disease Type C1 patient-specific iPSCs carrying the heterozygous mutation p.V1023Sfs*15/p.G992R
Christin Völkner, Maik Liedtke, Luise Arndt, Andreas Hermann, Moritz J. Frech

T11-13 Hippocampal low-frequency stimulation prevents seizure generation in a mouse model of mesial temporal lobe epilepsy
Enya Paschen, Piret Kleis, Jessica Link, Diego M Vieira , Katharina Heining, Philipp Janz, Ulrich Egert, Ute Häussler, Carola A Haas

T11-14 Sphingolipids and Parkinson's Disease
Razvan-Marius Brazdis, Roberto Damián Bilbao Canalejas, Iryna Prots, Beate Winner, Christiane Mühle, Johannes Kornhuber

T11-15 Defining the role of $p75^{NTR}$ in mediating neuroinflammation and cognitive decline in Alzheimer's disease
Hendrik Demuth, Marta Zagrebelsky, Andreas Holz, Martin Korte

T11-16 Amyloid-like aggregates cause lysosomal defects in neurons by impairing the AP-3 adaptor complex
Irina Dudanova, Irene Riera-Tur, Tillman Schaefer, Daniel Hornburg, Archana Mishra, Lorena Fernández-Mosquera, Dennis Feigenbutz, Patrick Auer, Matthias Mann, Wolfgang Baumeister, Rüdiger Klein, Felix Meissner, Nuno Raimundo, Rubén Fernández-Busnadiego

T11-17 OXIDATIVE STRESS AND IRON METABOLISM IN ACQUIRED EPILEPSY: ROLE FOR ASTROCYTES
Till S. Zimmer, Diede W.M. Broekaart, James D. Mills, Erwin A. van Vliet, Eleonora Aronica

T11-18 Scavenging β-amyloid monomers prevents early neuronal dysfunction in Alzheimer's disease models
Benedikt Zott, Lea Nästle, Christine Grienberger, Manuel M Simon, Felix Unger, Aylin D Keskin-Dargin, Arne Skerra, Arthur Konnerth

T11-19 Pathophysiological in vitro profile of neuronal differentiated cells derived from Niemann-Pick disease Type C2 patient-specific iPSCs carrying the NPC2 mutations c.58G>T/c.140G>T
Maik Liedtke, Christin Völkner, Alexandra Jürs, Andreas Hermann, Moritz J Frech

T11-20 The Role of CTSD in the Degradation of alpha-Synuclein
Susy Prieto Huarcaya, Josina Bunk, Alice Drobny, Jan Philipp Dobert, Philipp Arnold, Friederike Zunke

T11-21 Identification and characterization of Parkinson´s Disease progression markers in the human gastrointestinal tract
Annika Kluge, Eva Schaeffer, Alice Drobny, Mark Ellrichmann, Philipp Arnold, Francois Cossais, Daniela Berg, Friederike Zunke

T11-22 Astrocyte-mediated glutamate toxicity in a mouse model of adult spinal muscular atrophy
Linda-Isabell Schmitt, Markus Leo, Andrea Kutritz, Christoph Kleinschnitz, Tim Hagenacker

T11-23 The extracellular matrix proteins TnC and TnR modulate regenerating myelin sheath thickness and OPC maturation in a long-term Cuprizone-induced demyelination animal model
Juliane Bauch, Andreas Faissner

T11-24 Functional analysis of central and peripheral synapses in severe and intermediate mouse models for spinal muscular atrophy
Florian Gerstner, Jannik M. Büttner, Josiane K. Longang, Katharina Apel, Christian M. Simon

T11-25 Dysregulated mitochondrial sodium/calcium/lithium exchanger (NCLX) expression sensitizes neurons to excitotoxic stimuli and renders synaptic activity neurotoxic
Anna M. Hagenston, Jing Yan, Carlos Bas-Orth, Yanwei Tan, Marko Kostic, Israel Sekler, Hilmar Bading

T11-26 Increased LRRK2 kinase activity alters neuronal autophagy by disrupting the axonal transport of autophagosomes.
Clemens Alexander Boecker, Juliet Goldsmith, Dan Dou, Erika L.F. Holzbaur

The metabotropic glutamate receptor 5 and deep brain stimulation: expression analyses in a hamster model of dystonia

Stefanie Perl[1], Anika Lüttig[1], Rüdiger Köhling[2], Angelika Richter[1]

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Question: Dystonia is a severe movement disorder characterized by involuntary muscle contractions leading to abnormal movements and postures. As the pathophysiology is still poorly understood, the availability of effective therapeutics is very limited. Deep brain stimulation (DBS) of the globus pallidus internus (GPi; entopeduncular nucleus, EPN, in rodents) has become an important alternative treatment option for dystonia during the last years. In human patients, the improvement of symptoms usually occurs after a latency of several weeks, leading to the assumption that changes in the neuronal network such as synaptic plasticity seem to play a major role in the underlying mechanisms of DBS. For further examination of these still largely unknown mechanisms, we analyzed the expression of the metabotropic glutamate receptor 5 (mGlu5 receptor), a G-protein coupled receptor which is involved in neuronal excitability and synaptic plasticity. Previous investigations revealed a higher mGlu5 receptor expression in the striatum and cortex of 33 day old dtsz hamsters, a phenotypic animal model of inherited paroxysmal dystonia, compared to age-related control animals. Therefore, in the present study, mGlu5 receptor expression should be analyzed in the same model after short-term EPN-DBS.

Material and methods: As stress-inducible dystonic episodes in the dtsz model are age-dependent, reaching a maximum between day 30 and 42 of life, hamsters underwent the bilateral implantation of stimulation electrodes in the EPN before this maximum period. Using an external stimulator, sham-DBS (stimulator connected, but turned off) and DBS with 130 Hz, 40 Hz or 15 Hz (amplitude 50 µA and pulse width 60 µs) over a three-hour period followed. At the end of the in vivo experiments, hamsters were euthanized and transcardially perfused with 4 % paraformaldehyde. Brains were removed and post-fixed for molecular-biological examinations. Expression analyses of mGlu5 receptors were performed on 40 µm thick striatal brain sections (3 per animal) using immunohistochemistry (IHC). For evaluation, measurements of the fluorescence intensity (ImageJ) were conducted.

Results and conclusions: In IHC analyses, the mGlu5 receptor expression in the striatum and motor cortex of all examined groups (including sham-stimulated, naiv and non-dystonic control animals) appeared comparable. These findings suggest, that EPN-DBS for three hours was not sufficient to cause critical changes in (mGlu5 receptor-dependent) synaptic plasticity. In ongoing studies, effects and underlying mechanisms of long-term EPN-DBS (10-14 days) in dtsz hamsters will be investigated, using a fully implantable stimulator device. In this context, analyses of mGlu5 receptors will be interesting again, because changes at cellular level including plasticity changes seem to occur only after a longer stimulation period, as the time delayed improvement of dystonic symptoms in human indicates.

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Expression of c-Fos after short-term deep brain stimulation in the dystonic dt^{sz} hamster

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Deep brain stimulation (DBS) is an important therapeutic option for patients with dystonia, Parkinson disease and other neurological disorders. While the molecular effects of DBS are nearly unknown, it has been hypothesized that beneficial effects of DBS of the globus pallidus internus (entopedundular nucleus, EPN, in rodents) in patients with dystonia could be based on slow normalization of synaptic plasticity or re-organization of the basal ganglia network in addition to immediate effects on neuronal activity.

In the dt^{sz} mutant hamster, a model of paroxysmal dystonia, we recently found that the severity of dystonia was significantly reduced during short-term (3 h, 50 µA, 60 µs) EPN-DBS with 130 Hz EPN-DBS, while 40 Hz were less effective. 130 Hz stimulations of the subthalamic nucleus (STN) failed to exert beneficial effects as also observed in sham-stimulated groups with implanted electrodes into the EPN or STN (Paap et al., 2020, Neurobiol. Dis.). Immediately (15 min) after DBS or sham-stimulation, the hamsters were transcardially perfused and brains were collected for fluorescence immunohistochemistry (IHC). In order to examine the neuronal activity, we used c-Fos as an indirect marker for neuronal activity. C-Fos-reactive cells were counted in regions of the cortico-basal ganglia network (striatum, STN, EPN, substantia nigra, ventromedial thalamic nuclei and motor cortex) as well as in the lateral habenula and deep cerebellar nuclei. The number of c-Fos reactive cells in stimulated groups was compared with those in age-matched sham-stimulated as well as naïve dtsz hamsters. Furthermore, we performed intensity measurement 100 µm around the electrode location.

As expected, the intensity measurement revealed a higher c-Fos expression around the electrodes in stimulated groups in comparison to sham groups (EPN 130 Hz vs. sham, $p < 0.05$). We found a tendency towards decreased neuronal activity in the deep cerebellar nuclei after 130 Hz EPN DBS, but there were no significant differences within all investigated brain regions between EPN stimulated groups (130 Hz, 40 Hz) or in STN (130 Hz) stimulated hamsters vs. control groups. This unexpected finding does not exclude changes of specific cell types. With regard to recent electrophysiological data after DBS in mutant hamsters, double labeling of c-Fos and GABAergic neurons are currently performed. Furthermore, ongoing long-term DBS over ten days by novel implantable stimulators should clarify the mechanisms of slow normalization of synaptic plasticity. probably more suitable to give insights into the mechanisms of DBS.

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Genetic Polymorphisms in the Renin-Angiotensin System and Cognitive Decline in Parkinson's Disease

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Renin-angiotensin system (RAS) influences the central nervous system not only through its peripheral impact – the brain possesses its own local RAS. Studies showed altered RAS components in Parkinson's disease (PD), and their association with oxidative stress which may be linked to neurodegeneration and dementia. Moreover, the protective functions of RAS blockade antagonists against cognitive decline and dementia have been suggested.

The presented study aimed to examine whether genetic variability in RAS's genes correlates with cognitive decline in PD.

Subjects with idiopathic late-onset Parkinson's disease (n = 256) were divided into three groups based on the neuropsychological assessment: patients without cognitive decline, with mild cognitive decline (MCI) and with PD dementia. We analysed single nucleotide polymorphisms (SNPs) in angiotensin (*AGT*: rs699, rs4762), angiotensin II receptors (*AGTR1*: rs5186 and *AGTR2*: rs5194, rs1403543) genes, as well as insertion/deletion polymorphism in the angiotensin-converting enzyme gene (*ACE* I/D).

We did not find any significant differences in the frequencies of the examined polymorphisms in any of the groups.

Despite no direct correlation between the polymorphisms in RAS's genes and cognitive decline in PD, it is plausible that the impact of those genotypes may be indirect, affecting RAS blockade treatment for instance.

Modulation of phosphatidylserine externalisation and its effects on morphology, viability and survival in neurons

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The plasma membrane of eukaryotic cells is characterised by an asymmetric distribution of lipids within the phospholipid bilayer. Phosphotidylserine (PS) is under normal circumstances confined to the inner leaflet. This asymmetry is maintained by P4 ATPases, which catalyse the energy-dependent inward movement of phospholipids (flippases), by floppases with opposite function and by scramblases that catalyse bi-directional movement. Transport of PS toward the outer cell membrane and its recognition are primarily linked to apoptosis in most cell types. In neuronal cells, however, this phenomenon is less clear as PS exposure has been additionally detected under non-apoptotic conditions. Further, neuronal PS exposure can be transient in stressed but viable cells. Thus, interference with PS-triggered phagocytosis of neurons has been suggested to be beneficial in central nervous system disorders.

Here, we found by time-lapse microscopy that PS gets exposed on the surface of cultured primary hippocampal neurons after NMDA-mediated excitotoxicity and we characterized the expression of several flippases, floppases and scramblases. We observed that NMDA treatment, which triggers PS exposure, is specifically accompanied by a reduction in the expression level of the flippase Atp8a2. We designed and generated recombinant adeno-associated viruses (rAAVs) targeting expression of Atp8a2. Interfering with Atp8a2 expression in neurons caused a significant increase in PS exposure at the surface and triggered prominent cleavage of Caspase 3, typically associated with apoptosis. Morphometric analysis revealed that reduction of Atp8a2 expression caused a simplification of neuronal dendritic architecture. Time-lapse experiments showed that a loss of Atp8a2 causes neuronal death and impaired synaptic activity-dependent neuroprotection over time.

Mutations and translocations of the atp8a2 gene have been linked to mental retardation, motor deficits and optic atrophy in humans. Thus, future work will investigate the role of Atp8a2 in vivo in the hippocampus and retina.

A modular brain-on-chip for modelling epileptic seizures with functionally connected human neuronal networks

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Epilepsy is a complex neurological disorder that affects over 50 million people worldwide. This disease of neuronal networks consists of seizures that affect few networks of the brain (focal seizures) or the entire brain circuitry (generalized seizures). For several epilepsy forms, there is no permanent cure and with the current antiepileptic drugs available, about a third of the patients do not benefit and are left with persisting symptoms, which raises the need for improved human specific pre-clinical disease models. The use of human pluripotent stem cells (hPSCs) are advantageous for modelling epilepsy, but alone they cannot model interconnected neuronal networks. Our approach is therefore to create a novel brain-on-chip platform that can monitor the event of seizure like activity in hPSC-derived cortical neuronal networks. The Modular platform for epilepsy modelling in vitro (MEMO) was thus developed. Cortical neuronal cultures are compartmentalized in a custom-made microfluidic device and functionally interconnected through the microtunnels. The event of electrophysiological cell activity is monitored on a custom-made microelectrode array (MEA), assembled beneath the microfluidic device. We show that in MEMO neuronal networks develop well and are able to be cultured for up to 98 days. To create a phenotype that models seizure or epileptic-like behaviour, cells were treated with a seizure inducing substance, kainic acid, and counteracted with an anti-seizure inducing substance, phenytoin. The kainic acid treated networks increased bursting activity and remained localized to that region and similarly with phenytoin. MEMO is thus a suitable platform for modelling focal seizure behaviour in vitro and pharmacological responses.

In vivo Imaging with ^{18}F-Florbetaben-PET/MRI to detect Amyloid-β pathology in the Brain of the 5XFAD Mouse Model of Alzheimer's Disease

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The only method for a final diagnosis of Alzheimer's Disease is the post mortem detection of amyloid-β plaques and neurofibrillary tangles. However, the non-invasive positron-emission tomography (PET) with amyloid-tracers such as ^{18}F-Florbetaben is a well-established clinical diagnostics tool. Therefore we investigated the amyloid tracers applicability in the commonly used model of Alzheimer's Disease, 5XFAD. These mice carry five mutations affecting amyloid processing, which lead to age-dependent amyloid-β aggregations, intraneuronal amyloid-β, neuronal loss, and behavioral deficits in 5XFAD mice.

We analyzed the uptake of ^{18}F-Florbetaben and amyloid deposition in comparison to neurological deficits and neuropathological changes. To this end, seven- and 12-month-old male 5XFAD mice, as well as age-matched wild type littermates, were analyzed. Mice were fasted overnight and ^{18}F-Florbetaben was injected into a tail vein. Mice were scanned for 30 min using a small animal 1 Tesla nanoScan PET/MRT. For comparison, we additionally evaluated immunohistochemical stainings of 4 µm paraffin sections for pan-Abeta (2431-1 antibody) and analyzed the spatial reference memory using the Morris Water Maze test.

Our results show that seven- and 12-month-old 5XFAD mice showed a significant increase in ^{18}F-Florbetaben uptake and increased cerebral amyloid deposition, except for the olfactory bulb which did not show amyloid deposition also seen in the staining.

Our findings reinforce that the 5XFAD mouse model is a reliable model for familial Alzheimer's Disease and that the ^{18}F-Florbetaben-PET is a well-suited marker for early detection of amyloid pathology even before the onset of memory deficits. Therefore, ^{18}F-Florbetaben can be used to analyze the treatment effects of new therapeutic approaches in 5XFAD mice.

Developing 2-D and 3-D models to disentangle the interplay between mitochondrial vulnerability and inflammation in Parkinson's disease

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Neuroinflammation and mitochondrial dysfunction play an increasingly important role in the susceptibility and progression of Parkinson's disease (PD), emerging as promising targets for the development of therapies aimed at halting neurodegeneration. Two major regulators of mitochondrial quality control and mitophagy, PINK1 and Parkin, are associated with early onset familial PD. Recent observations of our team have highlighted a hyperresponsiveness of microglial cells from Parkin-deficient mice *(Park2-/-)* and macrophages from patients with *PARK2*/Parkin mutations to activators of the NLRP3 inflammasome, suggesting an involvement of this proinflammatory pathway in this familial form of PD.

To date, there has been little research on the interplay between neuronal and microglia cells in the context of *PINK1* and *PARK2*/Parkin mutations. Our objective is to explore whether and how the hyperactivation of the NLRP3 inflammasome pathway in a Parkin-deficient context affects the dopaminergic neurons that degenerate in PD, taking advantage of 2-D and 3-D culture mesencephalic models. Different types of primary mouse 2-D cultures are being explored: in a first paradigm, conditioned medium from microglia activated in vitro by specific proinflammatory stimuli known to induce NLRP3 inflammasome-dependent responses is transferred to purely neuronal E13/14 mesencephalic cultures. Our preliminary experiments demonstrated that priming of microglia with the bacterial inflammogen LPS and treatment with 2'(3')-O-(4-benzoylbenzoyl)-adenosine-5'-triphosphate (BzATP), a ligand of the P2X7 ion channel and activator the NLRP3 inflammasome pathway, leads to the expected release of the inflammasome-dependent cytokine IL-1ß. As a complementary strategy, we are studying the direct impact of microglial cells layered on top of the mesencephalic neurons, following addition of the NLRP3 inflammasome activators to the co-culture. In our first experiments, LPS and BzATP did not have direct deleterious effects when applied to neurons cultured alone. In current work, we are investigating the functional integrity and survival of dopaminergic neurons incubated with microglia-conditioned medium or co-cultured with microglia isolated from *Park2-/-* or wildtype mice.

In parallel, we are developing a 3-D model to study neuro-immune interactions in a context similar to the human brain. We have adapted a robust protocol for the generation of brain organoids with midbrain identity (hMO). These organoids showed a progressive increase in the expression of dopaminergic markers, including the rate-limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase (TH), and the FOXA2 transcription factor. In ongoing work, human microglia-like cells differentiated from peripheral blood-derived monocytes have been incorporated into 30 day-old hMOs, and we are investigating their long-term

integration, and their impact on dopamine neuron maturation and organization within the hMO.
As a perspective, these studies will be extended to integrate into hMO microglia-like cells from PD patients carrying *PARK2* gene mutations. Altogether, these approaches should provide novel insight into the complex interplay between neuroinflammation and mitochondrial vulnerability in *PARK2*-linked PD.

Trimethyltin induces calcium dysregulation and inflammatory phenotype in astrocytes in vitro

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Astrocytes are amongst the first responders to noxious stimuli by undergoing cellular and functional transition referred as reactive gliosis. Their response may be detrimental (A1) or beneficial (A2) for nervous tissue. Change of Ca2+ homeostasis is involved in modifying glia-dependent neuroprotection, reactivity, as well as glia-derived neurotoxicity. Trimethyltin (TMT) is a tri-substituted organotin neurotoxicant, which selectively targets hippocampus. Data suggest that TMT lesions share molecular hallmarks seen in Alzheimer's disease which makes TMT intoxication a useful model of AD-like pathology. However, the role of astrocytes in TMT-induced degeneration is not completely understood. Accordingly, the aim of this study was to elucidate the role of TMT on astrocyte activation in vitro. For this purpose, primary astrocytes were isolated from cortices of male Wistar rat pups. Ca2+ dynamics and mitochondrial membrane potential were monitored in Fluo-4- and Rhodamine 123-labeled astrocytes, respectively, following brief bath application of TMT alone or with different blockers. In order to measure gene and protein expression, astrocytes were treated with TMT for 24 hours and qPCR, immunoblot and immunocytochemistry were performed. Bath applied TMT induced Ca2+ increase in astrocytes which was attenuated by nifedipine block of L-type voltage gated Ca2+ channels. Interestingly, brief application of TMT induced gradual mitochondrial depolarization that was partially reversible and independent of extracellular Ca2+. Furthermore, TMT intoxication of astrocytes for 24 hours in vitro lead to induction of inflammatory phenotype and increased expression of pro-inflammatory cytokines and A1 markers. Our data indicate that TMT induce Ca2+ and mitochondrial dysregulation in astrocytes and promote inflammatory phenotype which underlies an important role of astrocytes in TMT-induced pathology.

Toxic Metamorphosis - How Changes from Lysosomal to Cytosolic pH Modify the Alpha-Synuclein Aggregation Pattern

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Alpha-synuclein (aSyn) is a cytosolic, aggregation-prone protein that is associated with neurodegenerative disorders like Parkinson's disease. Interestingly, the protein can appear in different conformations, including monomeric and oligomeric forms as well as insoluble beta-sheet rich fibrils. Its aggregation properties seem to be dependent on various factors and the composition of the respective cellular environment. Although under physiological conditions, most aSyn is found in the cytosol and synapses of neurons, aSyn can also be found in lysosomal compartments, where it gets degraded. We here compare the assembly speed, morphology, folding state, and spreading of aSyn at cytosolic pH (pH 7.4) and lysosomal pH (pH 5) using Thioflavin T, transmission electron microscopy, circular dichroism, and Fourier transform infrared spectroscopy. Interestingly, we found substantial differences between aSyn aggregation under neutral and acidic pH conditions, like those present in cytosolic and lysosomal cellular compartments. Also, lysosomal aSyn enriched from an aSyn-overexpressing cell line was able to seed aggregation in a concentration-dependent manner. Moreover, we observed that aSyn aggregates formed under in vitro lysosomal pH (pH 5) conditions were not stable at neutral pH and collapsed into partly soluble aggregates with changed structural characteristics. Our findings implicate meaningful intracellular molecular and structural changes of aSyn under different intracellular conditions which ware followed by intracellular toxicity.

Deep brain stimulation in the inferior colliculus reduces motor deficits in 6-OHDA lesioned hemiparkinsonian rats

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Deep brain stimulation (DBS) is a commonly used treatment option for Parkinson's disease (PD). Since DBS targeting classical areas to treat PD usually can induce side effects, an urgent need of alternative brain targets exists. Here, we have recently reported that the inferior colliculus (IC), part of the auditory and brain aversive system, might serve as a target region for DBS since it successfully reduces catalepsy in haloperidol treated rats, which reproduces a Parkinson-like immobility state in rats. In the present study, we investigated whether IC DBS in either of two different frequencies (30 or 130 Hz) applied in the dorsal or ventral part of the IC could reduce motor impairments in rats with unilateral 6-OHDA lesions of the nigro-striatal dopamine system, i.e. a chronic and neurodegenerative animal model of Parkinsonism. Each rat received DBS or sham-stimulation in a counterbalanced way and drug-induced turning behavior test was monitored after administration of the dopamine D2-receptor agonist apomorphine. IC DBS ipsilateral to the lesion with 30 or 130 Hz reduced contraversive turning in rats with chronic unilateral lesions. We also found that the motor benefits of intracollicular DBS depends on the targeted IC region, since only DBS in the ventral part of the IC caused a reduction of rotations. This part of the IC plays an important role in the induction of movement in contrast to the dorsal part that does not seem to substantially affect motor circuits. No aversive side effects, such as jumping or freezing, during ventral IC low frequency DBS in the chronic Parkinson model was observed. However, high frequency DBS induced aversive effects, reproducing previous data showing that the brain aversive system is activated in a frequency-sensitive manner. The present study suggest that the IC can be an alternative DBS target to treat Parkinsonian motor disturbances. DBS in two different frequencies successfully improved this motor behavior in a chronic animal model of Parkinsonism. Importantly, low frequency DBS in the IC induced motor benefits without causing any aversive side effects, which might be clinically relevant.

Targeting the serotonin receptor 7 ameliorates Tau pathology and memory deficits

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Tauopathies comprise a heterogeneous family of neurodegenerative diseases characterized by intracellular deposition of hyperphosphorylated Tau protein. Due to the lack of causative treatments, it is necessary to understand the pathological mechanisms involved in Tau hyperphosphorylation. During the last decade, the serotonergic system gained attention as a potential target for the treatment of neurodegenerative diseases, but the underlying mechanisms remains poorly understood.
To study the role of the serotonin receptor 7 (5-HT7R), we used a model of tauopathy induced by overexpressing the human Tau[R406W] mutant associated with inherited forms of frontotemporal dementia. We demonstrated that 5-HT7R signaling induces Tau hyperphosphorlyation, aggregation as well as the formation of neurofibrillary tangles in neuroblastoma cells, primary neuronal cultures and cortical neurons in vivo. Blocking the constitutive receptor activity by inverse agonists was found to attenuate these effects. Among different drugs approved for the treatment of depression or schizophrenia, Amisulpride was identified to possess a potent inverse agonism towards the 5 HT7R. Accordingly, learning and memory impairment induced by overexpression of Tau[R406W] in the prefrontal cortex of mice were ameliorated by intraperitoneal injections of Amisulpride. In light of these findings, blocking the constitutive activity of 5 HT7R emerged as a new, promising strategy for the treatment of tauopathies.

Pathophysiological *in vitro* profile of neuronal differentiated cells derived from Niemann-Pick disease Type C1 patient-specific iPSCs carrying the heterozygous mutation p.V1023Sfs*15/p.G992R

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Niemann–Pick Type C1 disease is an autosomal recessive neurodegenerative disorder caused by mutations in the *NPC1* gene, which encodes for a lysosomal membrane-bound protein involved in the cholesterol transport. A mutation in the *NPC1* gene disrupts efflux of cholesterol from late endosomes and lysosomes, leading to a clinically heterogeneous phenotype that includes neurological and systemic manifestations. Since iPSCs are able to differentiate into every cell type of the human body, they display an excellent tool for studying multisystemic diseases. Here, we describe the generation of a hiPSC line from an NPC1 patient with the compound heterozygous mutation p.Val1023Serfs*15/p.Gly992Arg and the characterization of their neuronal and hepatic derivatives regarding the NPC1 phenotype.

Dermal fibroblasts were reprogrammed into iPSCs by retroviral transduction with SOX2, OCT4, C-MYC and KLF4. IPSCs displayed a stem cell-like morphology including cells with high nucleus-to-cytoplasm ratio. Pluripotency of the iPSC line was validated by high levels of alkaline phosphatase activity and expression of pluripotency markers NANOG, OCT4, SSEA4, TRA-1-60 and TRA-1-81 as shown by immunocytochemistry and flow cytometry analysis. Furthermore, RT-PCR for pluripotency-related genes was performed wherein the expression of OCT4, NANOG, SOX2, KLF4, C-MYC, hTERT, ZFP296, FGF4 and ESG1 was shown. The differentiation potential of the iPSC lines into three embryonic germ layers was tested by spontaneous *in vitro* differentiation of embryoid bodies. Immunocytochemistry confirmed expression of α-fetoprotein (α-FP, endoderm), nestin (ectoderm) and muscle actin (MA, mesoderm). Chromosome analysis revealed a normal karyotype (46, XX) for both iPSC lines.

The new generated cell line carrying the mutation p.Val1023Serfs*15/p.Gly992Arg is of special interest, as the patient suffered from hepatosplenomegaly. More over the patient developed severe neurological symptoms. Therefore, fully characterized iPSCs were subsequently differentiated into NPC1 disease-affected cell types: neuronal differentiated cells and hepatocyte-like cells.

Fibroblasts, neuronal differentiated cells and hepatocyte-like cells were used for identifying NPC1 disease-associated phenotypes. Interestingly, cells did not show a typical NPC1 phenotype. Filipin stained fibroblasts did not show a significant cholesterol accumulation, which is the hallmark of the disease. However, neuronal differentiated cells and hepatocyte-like cells showed slightly increased cholesterol levels compared to controls. The investigation of NPC1 protein levels showed a nearly normal amount of NPC1 protein level compared to controls. The majority of the protein was present as Endoglycosidase H resistant form, indicating that the protein was not retained in the ER but advanced beyond the ER in the secretory pathway, suggesting a correct localization in the lysosomal membrane. To verify this, we performed quantitative colocalization analysis of lysosomal marker LAMP2 and NPC1. Analysis revealed almost wild-type like colocalization, proving the correct subcellular localization of the NPC1 protein.

Taken together, the mutant was identified as a biochemical “variant”. Consequently, this cell line is of special

interest, because despite the normal phenotype that is seen in vitro, the patient suffers from severe systemic and neurological symptoms.

Hippocampal low-frequency stimulation prevents seizure generation in a mouse model of mesial temporal lobe epilepsy

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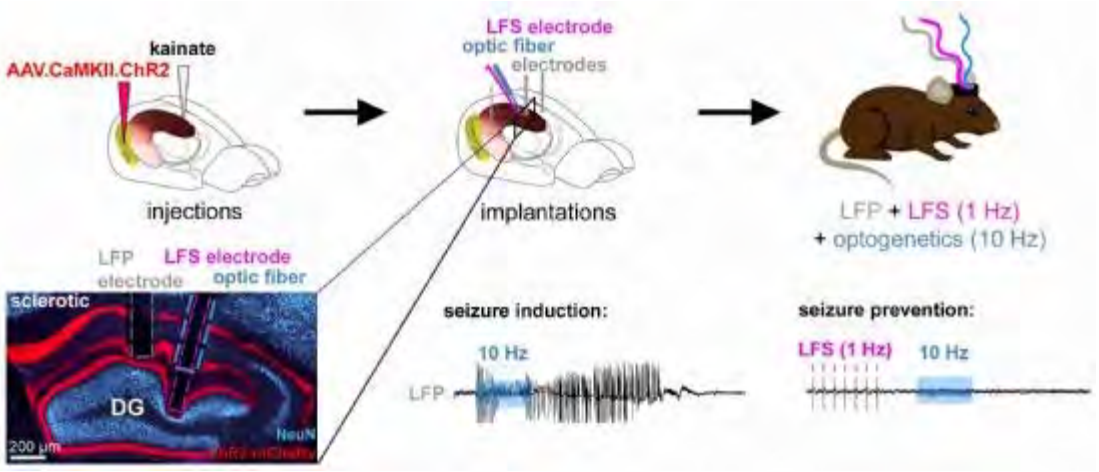
Mesial temporal lobe epilepsy (MTLE) is the most common form of focal, pharmacoresistant epilepsy in adults and is often associated with hippocampal sclerosis. For MTLE patients, one alternative to surgical resection of the epileptic focus is electrical deep brain stimulation in the hippocampus. Due to the low duty cycle, low-frequency stimulation (LFS) may have favorable clinical implementation over high-frequency stimulation as it requires less electric current injection, thus allowing for a longer battery life.

In an in vivo mouse model of MTLE, we showed previously that optogenetic LFS (0.5 and 1 Hz) in the hippocampus interfered with the generation of spontaneous epileptiform activity. Stimulation at higher frequencies (5–20 Hz) often resulted in the induction of behavioral seizures. Here, we aimed at identifying parameters for successful seizure control by the application of electrical LFS in the sclerotic dentate gyrus. We injected kainate (KA) unilaterally into the hippocampus and a channelrhodopsin2-encoding viral vector into the entorhinal cortex of C57BL/6 mice. Two weeks later, these animals were implanted with one recording electrode in each hippocampus and an optic fiber in the sclerotic hippocampus at a 30° angle to optogenetically induce behavioral seizures. An additional stimulation electrode was placed in parallel to the optic fiber. To assess seizure-suppressive effects of electrical LFS, we applied unilateral 1 Hz pulses one to three hours daily for three weeks and recorded local field potentials (LFPs) in both hippocampi.

We found that (i) 1 Hz LFS interfered with both spontaneous epileptiform activity and evoked behavioral seizures originating from the KA-injected hippocampus; (ii) suppression of epileptiform activity during LFS was effective over several weeks without desensitization; (iii) initial 1 Hz stimulation pulses can elicit behavioral seizures, which was avoided with a stepwise increase of the stimulation current. Since continuous LFS could potentially disrupt hippocampal function and shorten the battery life of implanted devices, we applied a discontinuous stimulation protocol which was also highly effective. Our current aim is to assess whether electrical LFS impairs hippocampal function in stimulated KA- and control-mice using the object location memory and Barnes maze tests.

Our results indicate that hippocampal LFS reliably suppresses the generation of spontaneous epileptiform activity and the generalization into behavioral seizures without losing effectiveness over prolonged treatment. Thus, LFS at 1 Hz may constitute a promising approach for seizure control in MTLE.


kainate
AAV.CaMKII.ChR2
injections
LFS electrode
optic fiber
electrodes
implantations
LFP + LFS (1 Hz)
+ optogenetics (10 Hz)
LFP
electrode
LFS electrode
optic fiber
sclerotic
DG
NeuN
200 µm
seizure induction:
10 Hz
LFP
seizure prevention:
LFS (1 Hz)
10 Hz

Sphingolipids and Parkinson's Disease

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Synucleinopathies including Parkinson's disease (PD) are strongly associated with the aggregation of the small presynaptic protein α-synuclein. Recent studies indicate a role of sphingolipids as constituents of biomembranes and as bioactive molecules in PD as well as their metabolizing enzymes such as glucocerebrosidase or acid sphingomyelinase.
We have analyzed the activity of enzymes involved in synthesizing or degrading ceramide as a central sphingolipid molecule in midbrain dopaminergic neurons differentiated from human-induced pluripotent stem cell (iPSCs) derived from PD patients carrying a duplication of the α-synuclein encoding gene compared to healthy controls. Preliminary data from four cell lines of two PD patients and two cell lines of two healthy controls indicate a significantly two-fold increased activity of sphingomyelin synthase whereas the activity of acid and neutral sphingomyelinase was highly variable and unaltered. Neither acid nor neutral ceramidase activities were detectable in these cells. Treatment with the oligomer reducing agent NPT100-18A did not significantly change the measured enzyme activities in any cell line.
Further studies are aimed at elucidating the effect of altered sphingolipid enzymes in mouse or cell culture models on α-synuclein aggregation as well as the effect of a cell culture α-synuclein overexpression and aggregation model on the activity and expression of sphingolipid enzymes in order to improve the understanding of underlying disease mechanisms and to improve diagnostic and therapeutic methods.

Defining the role of p75^{NTR} in mediating neuroinflammation and cognitive decline in Alzheimer's disease

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The p75 neurotrophin receptor (p75^{NTR}) is expressed in neurons of the central nervous system (CNS) and is involved in the regulation of several crucial cellular functions, including neuronal proliferation, differentiation, survival and activity-dependent plasticity. Recently, p75^{NTR} has also been found to be expressed in microglia, the tissue resident macrophages of the CNS, where its expression levels are significantly upregulated under pathological conditions, i.e. during Alzheimer disease (AD). AD is the most frequent form of dementia characterized by a gradual cognitive decline associated to the accumulation of Aβ-oligomers, chronic neuroinflammation and progressive neuronal degeneration. Interestingly, amongst other cell-surface proteins, p75^{NTR} has been shown to bind soluble Aβ-oligomers. This observation indicates p75^{NTR} as a possible candidate for mediating the Aβ-induced phenotypes. Here we use APP/PS1 transgenic mice, a model for AD to address a possible role of p75^{NTR} in mediating the Aβ-induced alterations in chronic immune activation as well as their cognitive consequences by comparing these parameters in wild type (WT), p75^{NTR} exon IV knockout (p75^{NTR} KO), APP/PS1 transgenic as well as APP/PS1-p75^{NTR} KO mice at 10 and 18 months of age.

To assess the degree of neuroinflammation the morphological activation of microglia was analyzed using Sholl analysis in an IMARIS® based approach. This analysis is dependent on the knowledge that upon activation microglia retract their processes and transform into an amoeboid shape. The results reveal a significantly lower microglial complexity in APP/PS1 animals compared to WT controls. APP/PS1 p75^{NTR}KO mice showed no differences compared to APP/PS1 mice. However, the morphology of microglia gives only a broad information about its activation status. Therefore, to get a more detailed insight of the ongoing chronic inflammation, cytokine levels and activation markers are currently being analyzed. Preliminary results indicate decreased levels of the proinflammatory cytokine IL-6 in brain homogenates of APP/PS1 p75^{NTR} KO mice compared to APP/PS1 mice.

For the cognitive performance, our results show that regardless of age WT and p75^{NTR} KO are able to successfully learn a spatial task tested using the Barnes maze. While APP/PS1 transgenic mice still learn the task at 10 months, albeit with a longer latency, they mostly fail this task at 18 months of age. APP/PS1 p75^{NTR} KO mice show no improvement of the spatial learning abilities, however they display increased and more frequent periods of immobility during the test. To address this interesting result, we are currently investigating exploratory and fear related behavior.

Taken together out results indicate that p75^{NTR} is involved in mediating some of the behavioral and

inflammatory alterations observed in a mouse model of AD.

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Amyloid-like aggregates cause lysosomal defects in neurons by impairing the AP-3 adaptor complex

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Impairment of the autophagy-lysosomal pathway is a crucial common pathogenic mechanism in diseases characterized by protein aggregation and neurodegeneration. However, the link between aggregation and lysosomal dysfunction remains poorly understood. Here, we used rationally designed amyloid-like β-sheet proteins (β proteins) to investigate the effects of protein aggregation in primary neurons. β proteins are prone to aggregation, but do not have an evolved biological function, and are therefore ideally suited to study the gain-of-function aspect of aggregation. When expressed in primary neurons, β proteins formed aggregates, impaired cellular morphology and caused toxicity. High-resolution cryo-electron tomography and light microscopy experiments revealed accumulation of enlarged, cargo-rich autophagolysosomes, alterations reminiscent of those observed in neurodegenerative and lysosomal storage disorders. Moreover, biochemical analyses pointed to a partial block at a late stage of the autophagy-lysosomal pathway. To search for the molecular causes of lysosomal alterations, we characterized the interactome of β proteins using quantitative label-free mass spectrometry, and found that β proteins sequester AP-3µ1, the medium subunit of the AP-3 adaptor complex involved in protein trafficking to lysosomal organelles. AP-3 subunits have been previously detected within aggregates of natural disease-related proteins, and mutations in AP-3 subunits are known to cause defects of lysosomal organelles in mice and in human patients. Consistent with these previous results, we found that knockout of AP-3 subunits resulted in lysosomal impairments. Importantly, restoring AP-3µ1 expression was sufficient to ameliorate neurotoxicity caused by β proteins. We propose that impaired trafficking of lysosomal proteins due to an insufficient cellular pool of intact AP-3 complex may contribute to lysosomal defects and neurotoxicity caused by protein misfolding. Our results emphasize the toxic gain-of-function role of protein aggregates in lysosomal dysfunction, and uncover a new molecular link between protein aggregation and lysosomal defects in neurons.

OXIDATIVE STRESS AND IRON METABOLISM IN ACQUIRED EPILEPSY: ROLE FOR ASTROCYTES

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Oxidative stress (OS) is a major pathogenic mechanism in the development of epilepsy. Evidence from congenital epilepsies implies a role for iron in potentiating the effects of OS. Therefore, we investigated iron-related neuropathology in acquired epilepsies. Furthermore, we aimed to examine the potential of iron to serve as a pathogenic cue in conjunction with OS. Using immunohistochemistry, we assessed 4-hydroxynonenal (4-HNE), heme oxygenase 1 (HO-1), ferritin and iron in surgically resected tissue of patients suffering from temporal lobe epilepsy with hippocampal sclerosis as well as post mortem tissue from patients that died after status epilepticus (SE) compared to autoptic control tissue. Quantitative real-time PCR and Western blotting were used to assess anti-oxidant and iron metabolism in TLE-HS tissue. Moreover, we analyzed anti-oxidant and iron metabolism during the acute, latent and chronic stage of epileptogenesis in the electrically induced SE rat model of acquired epilepsy. Finally, we investigated the effect of OS and iron overload in vitro using acute hippocampal slice preparations and human fetal astrocytes by stimulating them with glucose oxidase and/or ferric ammonium citrate. 4-HNE and HO-1 expression were consistently higher in neurons in epileptogenic tissue as compared to control tissue. Ferritin expression and iron accumulation was detected specifically in astrocytes in epileptogenic tissue. RNA and protein analysis revealed elevated anti-oxidant metabolism, lower iron import and retention as well as higher iron export. In vitro, astrocytes appear to display resistance to OS and iron by upregulating their anti-oxidant and iron capacity. However, specifically when OS is paired with iron stimulation astrocytes seem to acquire a pro-inflammatory phenotype. Electrically induced SE rats display higher anti-oxidant and iron metabolism during the latent phase, but have consistently higher tissue iron during the chronic phase when they suffer from recurrent spontaneous seizures. We found evidence for oxidative damage and iron-mediated cell death, termed ferroptosis, primarily in neurons in epileptogenic tissue. Additionally, astrocytes acquire a role in iron sequestration specifically in chronic epilepsy. This function might transform astrocytes into a highly resistant, pro-inflammatory phenotype that potentially contributes to inflammatory processes during epileptogenesis.

Scavenging β-amyloid monomers prevents early neuronal dysfunction in Alzheimer's disease models

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Accumulating evidence from observations in mouse models and humans indicates that one of the earliest brain changes in Alzheimer's disease (AD) is an amyloid-β (Aβ)-dependent neuronal dysfunction such as excessive activity (i.e. hyperactivity) in a subset of neurons. In mouse models of AD, hyperactive neurons appear before the formation of plaques when only soluble Aβ concentrations are elevated in the brain. Conversely, the application of soluble Aβ in wild type mice is sufficient to sustain this neuronal dysfunction. As neuronal hyperactivity is implicated in brain circuit impairment and cognitive decline, its treatment could have beneficial effects in AD. Here, we introduced a novel approach of direct application of an Anticalin protein to the brain, which, by binding to Aβ monomers, prevented neuronal hyperactivity in young AD mice in vivo.

We used the Aβ-specific Anticalin H1GA, a small engineered antibody-like protein, to scavenge Aβ in the APP23/PS45 mouse model of AD and evaluated its effect on neuronal activity using in vivo two-photon calcium imaging. We demonstrated that the application of H1GA to the hippocampal CA1 area stopped neuronal hyperactivity in young pre-depositing APP/PS1 mice but had no effect in wild-type mice. We thus hypothesized that the removable of soluble Aβ was sufficient to prevent neuronal hyperactivity. To determine, which Aβ species were responsible for this effect, we next applied synthetic Aβ directly in the hippocampal CA1 area of wildtype mice. In line with our previous findings, the application of Aβ dimers, but not monomers, reliably induced neuronal hyperactivity. Remarkably, the addition of Anticalin did not prevent the hyperactivating effects of Aβ dimers. Using size exclusion chromatography, we next determined that H1GA binds Aβ monomers but not dimers or larger aggregates. We thus concluded that the hyperactivity-preventing effects of H1GA in vivo might rely on a prevention of the formation of larger Aβ aggregates such as dimers and oligomers by binding Aβ monomers. To investigate this, we designed a combined in vitro-in vivo assay. We incubated Aβ monomers for 1-2 hours in vitro and confirmed the emergence of aggregates using a Thioflavin T fluorescence assay. The application these aggregates, containing Aβ dimers and oligomers, to hippocampal CA1 neurons reliably induced neuronal hyperactivity in vivo and in vitro. Incubation of Aβ monomers in H1GA-containing medium, on the other hand, prevented the hyperactivating Aβ effect. Together, this data indicates that the removal of Aβ monomers from the brain is sufficient to prevent neuronal hyperactivity, possibly trough the prevention of Aβ aggregation.

In summary, in this study we established that scavenging of functionally inert Aβ monomers can prevent early neuronal dysfunction in mouse models of AD in vivo, most likely through reducing the formation of toxic Aβ dimers and oligomers. These results identify Aβ monomer scavenging proteins, such as the Anticalin H1GA, as promising therapeutic agents at early stages of AD.

Pathophysiological in vitro profile of neuronal differentiated cells derived from Niemann-Pick disease Type C2 patient-specific iPSCs carrying the NPC2 mutations c.58G>T/c.140G>T

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Niemann-Pick type C2 (NPC2) disease is a rare hereditary disease caused by mutation in the NPC2 gene. NPC2 is a small, soluble protein mainly expressed in late endosomes and lysosomes (LE/L). Its main function is the exclusion of cholesterol from these organelles in collaboration with the transmembrane protein NPC1 and to maintain cellular cholesterol homeostasis. Independent which one is affected a mutation in NPC1 (95% of the cases) or NPC2 (5%) results in a pathophysiological accumulation of cholesterol and other sphingolipids in LE/L making both diseases appear to be biochemically indistinguishable from each other. Studies of the pathological mechanisms underlying NPC2 are mostly based on NPC2 animal models and NPC2 patient derived fibroblasts. Recently, we established induced pluripotent stem cells (iPSCs), derived from a donor carrying the NPC2 mutations c.58G>T/c.140G>T. Here, we present a profile of pathophysiological in vitro features, shared by NPC1 and NPC2, of neural differentiated cells obtained from the patient specific iPSCs. Profiling comprised a determination of NPC2 protein level, detection of cholesterol accumulation by Filipin staining, analysis of oxidative stress, and determination of autophagy, as well as organelle transport. Expectably, the NPC2 deficient cells display a significantly reduced amount of NPC2 protein and accordingly a significantly increased amount of cholesterol was observed. They show only a slight increase of reactive oxygen species without any further signs of oxidative stress. These results differ from the phenotype observed in NPC1, as cells with mutation in the NPC1 gene have a massive lack of catalase and accordingly an increased level of ROS. Live cell imaging experiments revealed an affected transport of mitochondria, in accordance with results observed in NPC1 deficient cells. Regarding autophagy, the LC3BII/LC3BI ratio of NPC2 deficient cells show no differences to control cell lines and induction of autophagy by starvation do not lead to a higher LC3BI to LC3BII conversion. Furthermore, inhibition of the clearance of autophagosomes by Bafilomycin A1 leads only to a minor increase of the LC3BII/LC3BI ratio pointing at an impaired autophagy induction machinery and a defective organelle fusion. As a proper transport and localization of autophagosomes and lysosomes is necessary for their fusion during autophagy we evaluate the transport efficiency of both organelles. As expected, we found a reduced track displacement and mean speed of both lysosomes and autophagosomes providing a possible cause for a defective clearance of autophagic vesicles. Taken together the results differ from the ones observed in NPC1 regarding antioxidative capacity and autophagic activity whereas the phenotype of a defective overall transport system seems to be a comparable mechanism. Generally, the here described cell line provides a valuable tool to gain deeper understanding, not only of the pathogenic mechanism of NPC2, but also of NPC1 and the differences between the two diseases.

The Role of CTSD in the Degradation of alpha-Synuclein

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Cathepsin D (CTSD) is a lysosomal protease that has an essential role in the degradation of several substrates, in particular aggregation-prone proteins such as alpha-synuclein (α-syn) (Sevlever et al. 2008). Moreover, CTSD is ubiquitously present in all mammalian cells and it is remarkably abundant in the brain (Vidoni et al. 2016). Although mutations within the CTSD gene have been linked to neurodegenerative disorders such as Parkinson's disease (PD) (Robak et al. 2017), the exact role of CTSD dysfunction in disease development and potential therapeutic properties is not well understood. Here, four PD-associated CTSD variants found in genomic sequencing data of patients were analyzed for cellular localization, maturation, enzymatic activity and their effect on α-syn degradation to understand their role in disease. Additionally, we examined whether enhancing CTSD protein levels can decrease α-syn in synucleinopathy cell models.
Our findings suggest that PD-associated variants do not impair CTSD localization or maturation. Surprisingly, CTSD variant A239V showed increased enzymatic activity and enhanced α-syn degradation, which could be due to a structural change within a loop adjacent to the catalytic center of the enzyme. Taken together, our data contribute to a better understanding of CTSD role in neurodegeneration and might provide novel therapeutic strategies for PD.

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Identification and characterization of Parkinson´s Disease progression markers in the human gastrointestinal tract

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Parkinson´s disease (PD) is the second most common neurodegenerative disease, characterized by motor and non-motor symptoms including hyposmia, sleep disturbances and constipation (Dorsey et al., 2007; Schaeffer & Berg, 2017). The onset of those non-motor symptoms starts often years before the typical motor symptoms (Schaeffer et al., 2020). Therefore the beginning of PD is supposed to be outside the substantia nigra, often in the peripheral nervous system.
The molecular key hallmark of PD is the aggregation of synaptic α-synuclein (a-syn), which is able to form pathological oligomers and amyloid fibrils (Riederer et al., 2019). The occurrence of a-syn has been shown in the peripheral nervous system like the gastrointestinal tract, the skin or the submandibular gland of PD patients. However, so far a-syn detection in these tissues was neither 100% sensitive nor 100% specific and therefore has not qualified for a reliable biomarker for PD, yet (Manne et al., 2020).
Our study set out to identify specific proteins such as different a-syn species in the rectum, qualifying as specific diagnostic and progression marker in PD.
We obtained and homogenized submucosal rectal biopsies from 24 patients and 24 healthy controls and performed various biochemical assays as well as ultrastructural imaging such as transmission electron microscopy. Through immunoreactivity assays, we could show different levels and conformations of selected a-syn forms and amyloid structures in the two groups. Next, after establishment of RTQuiC (Real-time quaking-induced conversion) assays, we could detect and amplify specific protein aggregates in the biopsies of PD patients, which had the potential to induce spreading of pathological protein forms.
In summary, our data indicate that pathology-associated protein aggregates can be found in the gut and that those aggregates can be amplified by RTQuiC in order to perform in-depth structural analyses of the ENS-derived a-syn species.
This emphasizes an important role of the gut in PD pathology and underlines its crucial role for identification and characterization of potential biomarkers of PD. Hence, our next steps are in detail structural analyses of protein aggregates as well as to analyze cellular disease mechanisms.

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Astrocyte-mediated glutamate toxicity in a mouse model of adult spinal muscular atrophy

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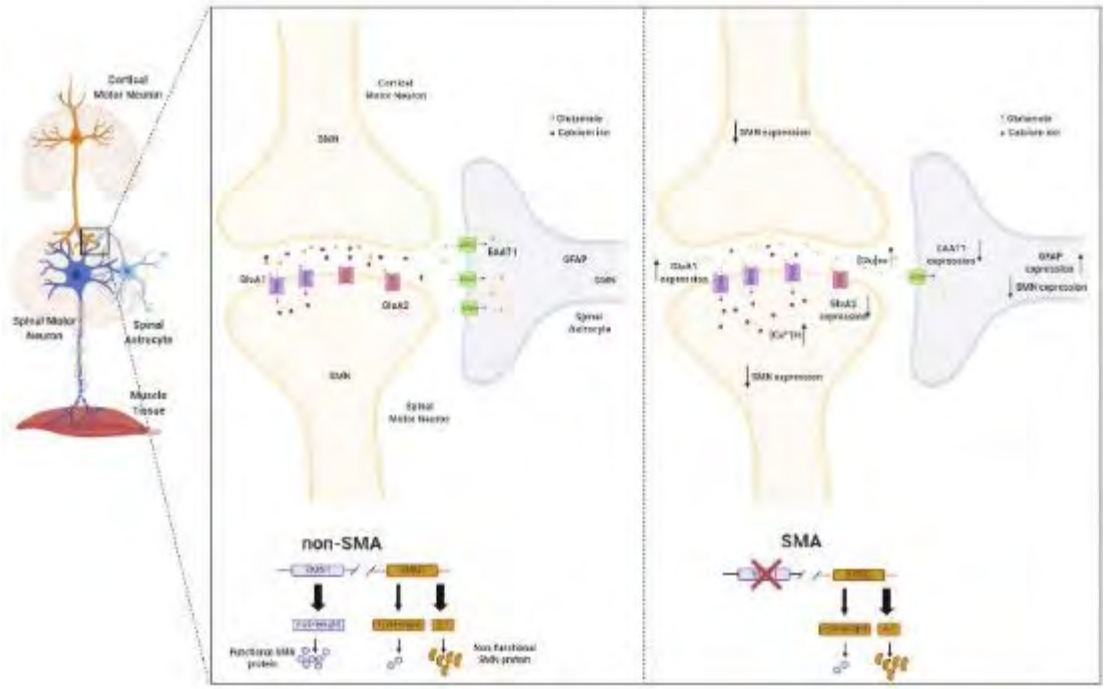
Spinal muscular atrophy (SMA) is a hereditary neuromuscular disorder leading to motor neuron loss and muscle wasting caused by a deficiency in SMN (survival of motor neuron) protein and deletion of the SMN1 gene. The disease can be classified severity and onset (type 1-4), where type 1 is the most severe and type 4 the less severe. The different types are defined by the number of copies of the SMN2 gene, which can only produce a fraction of the functional SMN protein.
The here used mouse model (FVB.Cg-Smn1tm1Hung Tg(SMN2)2Hung/J) reflects SMA's adult form. In contrast to humans, rodents do not have an SMN2 gene, so the model uses a deletion of the SMN1 and the insert of the human SMN2 gene (4 copies). For investigating the mechanisms in adult SMA pathogenesis, immunohistochemical (IHC) staining of the spinal cord, western blot analysis, and patch-clamp recordings were performed.
In IHC of astrocytes in SMA mice's spinal cord, an overexpression of glial fibrillary acidic protein (GFAP) at different time points could be observed. Simultaneously, the excitatory amino acid transporter 1 (EAAT1, GLAST) protein, a glutamate transporter responsible for glutamate uptake from the synaptic cleft, is reduced, explaining the increased extracellular glutamate concentration and a direct influence on the motor neurons. An increase in the expression of neuronal-pentraxin 2 (NPTX2) could be measured for P42 and P70. The SMA mice model also reflects motor neuron loss, which could be measured starting after P28. Especially in the range from P28-P42, most motor neurons are lost, but no further loss occurs afterward. There seems to be a correlation between the reduced motor neuron loss and the overexpression of NPTX2. Studies could link the upregulation of NPTX2 to glutamate toxicity. The NPTX2 overexpression function is to withdraw AMPA receptors from the neuronal membrane due to excess glutamate. There is an increased expression of the glutamate ionotropic receptor AMPA type subunit 1 (GluA1) at P28 before the loss of motor neurons and NPTX2 overexpression occurs. At the same time, the GluA2 subunit is reduced. The GluA1 subunit initially favors the loss since the subunit is particularly calcium-permeable, indicating an increased calcium influx and possible calcium toxicity. The reduction in GluA1 expression correlates with NPTX2 overexpression and the upregulation of the GluA2 subunit. Patch-clamp measurements could show differences in the electrophysiological properties of SMA mice (Fig.1).
In further experiments, the measurement of free glutamate, in vivo NPTX2 knockdown, and in vivo pharmacological inhibition of AMPA receptors will be performed.


Cortical Motor Neuron
Spinal Motor Neuron
Spinal Astrocyte
Muscle Tissue
SMN
GLAST
GFAP
SMN
Spinal Astrocyte
SMN
Spinal Motor Neuron
SMN expression
GLAST expression
GFAP expression
SMN expression
SMN expression
non-SMA
SMA

The extracellular matrix proteins TnC and TnR modulate regenerating myelin sheath thickness and OPC maturation in a long-term Cuprizone-induced demyelination animal model

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Oligodendrocytes are the myelinating cells of the central nervous system. The physiological importance of oligodendrocytes is highlighted by diseases such as multiple sclerosis, where axonal signal transmission is compromised. During the course of the disease, oligodendrocytes are attacked by cells of the own immune system and the myelin sheath is degraded. In a healthy organism spontaneous remyelination follows, in which oligodendrocyte precursor cells (OPCs) migrate to the lesions and differentiate to mature oligodendrocytes. These regenerating oligodendrocytes eventually form new myelin sheaths. However, the newly formed myelin sheaths are thinner and shorter than the former ones. The processes of migration and differentiation is influenced by proteins of the extracellular matrix (ECM), which consists of a network of glycoproteins and proteoglycans. In particular, the glycoprotein Tenascin C (TnC) has an inhibitory effect on the differentiation of OPCs and on the remyelination efficiency of oligodendrocytes. The structurally similar tenascin R (TnR) exerts an inhibiting influence on the formation of myelin membranes in vitro. (Czopka et al., 2009; Czopka et al., 2010). To induce demyelination, we have used the cuprizone animal model. Therefore 8-week old male mice were used and received a special Cuprizone replenished diet for ten weeks, which is sufficient to induce CNS demyelination. Thereafter, a subgroup received a normal diet for 2, 4 or 6 weeks to allow for remyelination. After the end of the incubation period, we have analyzed the myelin sheath thickness as well as the oligodendrocyte development using electron microscopy, RT-PCR analysis and immunohistochemical stainings with antibodies which detect different development stages of oligodendrocytes (Ulc et al., 2019).
When examining the functions of tenascins in vivo, we could show that mouse knockout lines for Tnc-/- and Tnr-/- displayed a significant increase of regenerating myelin sheath thickness after Cuprizone exposure. Furthermore, we observed that in the absence of either tenascin the number of OPCs was also increased. Thereby, we were also able to confirm results from previous studies in vitro, in which purified TnC displayed an inhibitory effect on the differentiation of Oligodendrocytes, whereas purified TnR seemed to be important for oligodendroglia differentiation (Czopka et al., 2009). Moreover, when Tnc knockout oligodendrocytes were applied to an in vitro myelination assay using artifical fibers, a higher number of sheaths per single cell were obtained, compared to the wildtype control. These results clearly indicate that the fine tuning of myelin regeneration is regulated by the principal Tn proteins of the CNS.

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Functional analysis of central and peripheral synapses in severe and intermediate mouse models for spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a neurodegenerative disease which affects mostly newborn and young children with an incidence of 1:10.000. The disease causing mutation in the *survival of motor neuron (SMN)* gene leads to a reduction of the SMN protein that results in an axial to distal progression of muscle atrophy, motor neuron death and motor circuit dysfunction in SMA patients. The amount of SMN protein defines the onset and severity of SMA. SMA Type I patients have the lowest SMN level and exhibit an onset at birth, whereas Type III patients have higher SMN levels with a later onset after 18 months of life. These severity forms of SMA can be reliably modeled by different mouse models. Previous work of the severe *SMA-Delta7* mouse model has shown that degeneration of central proprioceptive synapses and peripheral neuromuscular junctions (NMJs) contribute heavily to the SMA motor phenotype. The functional impairment of central and peripheral synapses in milder forms, however, have not been investigated.

Here, we compare the degree of loss and dysfunction of proprioceptive synapses onto motor neurons and NMJs at the muscle between a severe and milder form of SMA which are modelled by *SMA-Delta7* and *Smn(2b/-)*, respectively. To examine the function of the proprioceptive synapses, we applied a repetitive 10 Hz suprathreshold stimulation to the proprioceptive axons in the dorsal root of the lumbar L1 spinal cord segment in an *ex vivo* spinal cord preparation. Simultaneously, L1 ventral root response were recorded as a functional readout of L1 vulnerable motor neurons axons, which mostly innervate proximal muscles. Both, the *SMA-Delta7* and *Smn2b-* mouse model showed a significant higher depression of the ventral root response compared to control littermates, with a lower amplitude in the severe model, which is an indication of proprioceptive synaptic dysfunction. Parallel confocal analysis identified proprioceptive synaptic degeneration presymptomatically prior to motor neuron loss in both SMA mouse models.

To test the functionality of the NMJ, the compound muscle action potential of the proximal muscle quadratus lumborum was recorded following L1 ventral root stimulation at different frequencies. These experiments revealed that the NMJ function is severely impaired in amplitude size and depression in both SMA models. Morphological analysis revealed that NMJ dysfunction is accompanied by a high degree of denervation.

In summary, we show that dysfunction and degeneration of the NMJs and proprioceptive synapses proceed the death of motor neurons in severe and mild SMA mouse models underlining the importance of synaptic vulnerability in SMA. This suggests that central synaptic loss might be a key component for motor circuit pathology in SMA mice and patients. Since both, the proprioceptive synapse and the NMJ contribute to the clinical H-reflex analysis, this finding could be the foundation for novel diagnostic tools for SMA patients.

Dysregulated mitochondrial sodium/calcium/lithium exchanger (NCLX) expression sensitizes neurons to excitotoxic stimuli and renders synaptic activity neurotoxic

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In this study, we tested the hypothesis that physiological expression levels of the mitochondrial sodium/calcium/lithium exchanger (NCLX) are essential for maintaining neuronal resilience in the face of excitotoxic challenge. Using an shRNA-mediated approach, we showed that reduced NCLX expression exacerbates neuronal mitochondrial calcium dysregulation, mitochondrial membrane potential breakdown, and reactive oxygen species generation during excitotoxic stimulation of primary hippocampal cultures with NMDA. Moreover, NCLX knockdown—which in our hands affected both neurons and glia—results not only in enhanced neurodegeneration following an excitotoxic insult, but also in neuronal and astrocytic cell death under basal conditions. Surprisingly, our data also revealed that synaptic activity, which is widely considered to be neuroprotective, can be made deadly by NCLX depletion: expression of NCLX-targeted shRNA impaired the recovery of mitochondrial calcium levels following action potential bursts and was associated both with mitochondrial membrane potential breakdown and substantial neurodegeneration in hippocampal cultures undergoing ongoing synaptic activity. Finally, we showed that NCLX knockdown within the hippocampal CA1 region in vivo causes widespread neuro- and astrodegeneration. In summary, we demonstrated that dysregulated NCLX expression not only sensitizes neuroglial networks to excitotoxic stimuli but notably also renders otherwise neuroprotective synaptic activity toxic. These findings may explain the emergence of neuro- and astrodegeneration in patients having disorders with disrupted NCLX expression or function, and suggest that treatments aimed at enhancing or restoring NCLX function may prevent central nervous system damage in a broad range of disease states.

Increased LRRK2 kinase activity alters neuronal autophagy by disrupting the axonal transport of autophagosomes.

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Parkinson's disease-causing mutations in the LRRK2 gene hyperactivate LRRK2 kinase activity and lead to increased phosphorylation of Rab proteins, which are master regulators of intracellular trafficking. Here, we investigated the effect of the hyperactivating G2019S mutation in LRRK2 on endolysosomal organelle transport in the axon. LRRK2-G2019S did not affect the axonal motility of LAMP1-positive late endosomes/lysosomes nor did the mutation perturb microtubule dynamics. In contrast, we found that LRRK2-G2019S dramatically decreased the processivity of axonal autophagosome transport in a kinase-dependent manner. This effect was consistent across multiple models, including LRRK2-G2019S overexpressing rat hippocampal neurons, cortical neurons from a G2019S knock-in (KI) mouse, and gene-edited human iPSC-derived G2019S KI neurons. Further, we found Rab29, a known activator of LRRK2 genetically linked to Parkinson's disease, to be associated with axonal autophagosomes. LRRK2 hyperactivation induced by Rab29 overexpression decreased the processivity of autophagosome transport to a similar extent as expression of LRRK2-G2019S. Downstream of LRRK2 kinase, we found that hyperactive LRRK2 recruits JIP4, a motor adaptor known to bind to LRRK2-phosphorylated Rab proteins, to the autophagosomal membrane. We found that increased JIP4 levels induce abnormal activation of kinesin-1, resulting in an unregulated tug-of-war between anterograde and retrograde motors and thereby disrupting the normal pattern of highly processive retrograde autophagosome motility along the axon. Disruption of autophagosome transport was accompanied by defective autophagosome acidification, suggesting that the observed transport deficit impairs effective degradation of autophagosomal cargo. Autophagosome transport and maturation were rescued by pharmacological LRRK2 kinase inhibition. Together, our work provides robust evidence for a detrimental effect of LRRK2 hyperactivation on autophagosome transport and maturation in neurons, further establishing the role of LRRK2 as a regulator of intracellular trafficking in autophagy, a pathway that has long been implicated in the pathogenesis of Parkinson's disease.
Supported by the German Research Foundation (DFG; BO 5434/1-1) and the Michael J. Fox Foundation (Grant #15100).

Poster Topic

T12: Neuroimmunology, Inflammation and Neuroprotection

T12-1 Neural progenitor cell derived extracellular vesicles enhance blood-brain barrier integrity by NF-κB dependent regulation of ABCB1 in stroke mice
Lin Zhang, Irina Graf, Yaoyun Kuang, Xuan Zheng, Matteo Haupt, Arshad Majid, Ertugrul Kilic, Dirk M Hermann, Marios-Nikos Psychogios, Martin S Weber, Jasmin Ochs, Mathias Bähr, Thorsten R Doeppner

T12-2 IL-37 reduces neuroinflammatory response in primary microglia
Melanie Ohm, Niklas Lonnemann, Charles A Dinarello, Wei He, Karsten Hiller, Martin Korte

T12-3 The effects of two novel radioprotectors in the rat brainstem of gamma-irradiated rats followed by Synchrotron FTIR spectroscopy
Dušica M. Kocovic, Andrej Korenic, Pavle R. Andjus, Tanja Ducic

T12-4 Valproic acid treatment after experimental traumatic brain injury in mice
Regina Hummel, Sonja Zander, Christina Goelz, Michael K. E. Schaefer

T12-5 Dynamical systems model explains the role of neuroimmune interactions in epilepsy development
Danylo Batulin, Fereshteh Lagzi, Peter Jedlicka, Jochen Triesch

T12-6 CIDP antibodies target junction proteins and identify patient subgroups: an autoantigenomic approach
Christian P. Moritz, Yannick Tholance, Oda Stoevesandt, Karine Ferraud, Jean-Philippe Camdessanché, Jean-Christophe Antoine

T12-7 Neutralization of glycine receptor autoantibodies from patients' serum samples
Inken Piro, Vera Rauschenberger, Vikram Kasaragod, Anna-Lena Eckes, Claudia Sommer, Carmen Villmann

T12-8 Reciprocal short- and long-term effects of traumatic brain injury and femoral fracture in a murine polytrauma model
Katharina Ritter, Markus Baalmann, Kirsten Jung, Christopher Dolderer, Dominik Appel, Ulrike Ritz, Michael Schäfer

T12-9 Multimodal imaging in experimental cerebral malaria reveals early-stage accumulation of infected erythrocytes and altered brain perfusion.
Rituparna Bhattacharjee, Patricia Wenk, Anja M. Oelschlegel, Eike Budinger, Kai Matuschewski, Dirk Schlüter, Gopala Nishanth, Jürgen Goldschmidt

T12-10 Cognitive decline is associated with a wide spectrum of serum and cerebrospinal fluid neuronal autoantibodies

Niels Hansen, Berend Malchow, Inga Zerr, Winfried Stöcker, Jens Wiltfang, Charles Timäus

T12-11 Intra-ganglionic delivery of Iba1 siRNA alters macrophages perineuronal ring formation in SNL neuropathic pain model
Andreea-Violeta Grosu, Roxana-Olimpia Gheorghe, Alexandru Filippi, Gisela Gaina, Violeta Ristoiu

T12-12 Exploiting benign MS: Identification of astrocyte-specific endogenous neuroprotective and neuroregenerative mechanisms to stop progressive MS

Janis Kerkering, Marlen Alisch, Volker Siffrin

T12-13 Neuroprotection in insects: Roles of ancestral erythropoietin-like proteins and acetylcholinesterase
Debbra Yasemin Knorr, Denise Hartung, Kristin Schneider, Luca Büschgens, Jan Förster, Nadine Georges, Luzia Hintz, Hanna Sophie Pies, Sonja Pripecic, Ralf Heinrich

T12-14 Influence of antisera against the intracellular parasite Toxoplasma gondii on Complexin 2-regulates exocytosis in SH-SY5Y neuroblastoma and RBL-2H3 granulocyte cell lines
Aaron David Kleine, Bernhard Reuss

Neural progenitor cell derived extracellular vesicles enhance blood-brain barrier integrity by NF-κB dependent regulation of ABCB1 in stroke mice

Lin Zhang[1], Irina Graf[1], Yaoyun Kuang[1], Xuan Zheng[1], Matteo Haupt[1], Arshad Majid[2], Ertugrul Kilic[3], Dirk M Hermann[4], Marios-Nikos Psychogios[5], Martin S Weber[1,6], Jasmin Ochs[6], Mathias Bähr[1], Thorsten R Doeppner[1,3]

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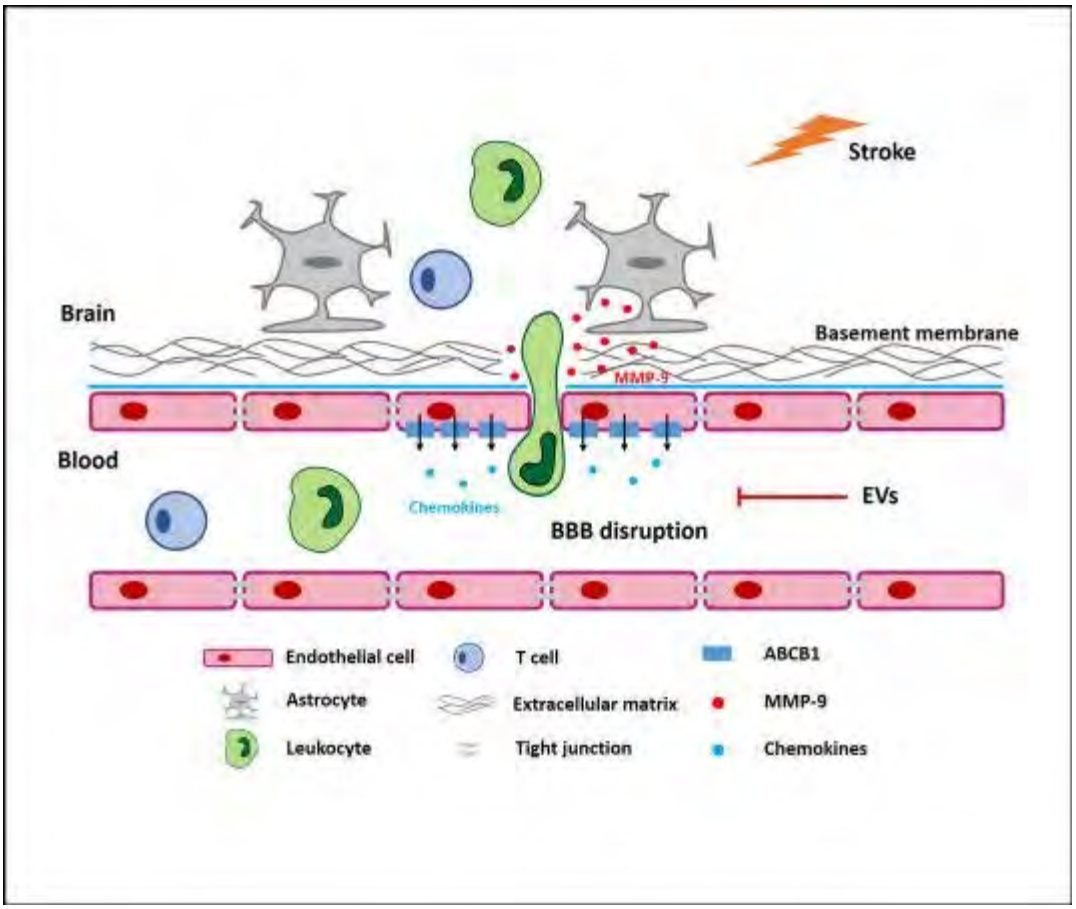
Objective Extracellular vesicles (EVs) derived from neural progenitor cells (NPCs) enhance post-stroke neurological recovery, albeit the underlying mechanisms remain elusive. Since previous research described an enhanced post-stroke integrity of the blood-brain barrier (BBB) upon systemic transplantation of NPCs, we examined if NPC-derived EVs affect BBB integrity and which cellular mechanisms are involved in the process. **Approach and Results** Using in vitro models of primary brain endothelial cell (EC) cultures as well as co-cultures of brain endothelial cells (ECs) and astrocytes exposed to oxygen-glucose-deprivation (OGD), we examined the effects of EVs or vehicle on microvascular integrity. In vitro data were confirmed using a mouse transient middle cerebral artery occlusion model. Cultured ECs displayed increased ATP-binding cassette transporter B1 (ABCB1) levels when exposed to OGD, which was reversed by treatment with EVs. The latter was due to an EV-induced inhibition of the NF-KB pathway. Using a BBB co-culture model of ECs and astrocytes exposed to OGD, EVs stabilized the BBB and ABCB1 levels without affecting the transcellular electrical resistance (TER) of ECs. Likewise, EVs yielded reduced Evans blue extravasation, decreased ABCB1 expression as well as an inhibition of the NF-KB pathway and downstream matrix metalloprotease-9 activity in stroke mice. The EV-induced inhibition of the NF-KB pathway resulted in a post-stroke modulation of immune responses. **Conclusions** Our findings suggest that EVs enhance post-stroke BBB integrity via ABCB1 and MMP-9 regulation, attenuating inflammatory cell recruitment by inhibition of the NF-KB pathway.


Stroke
Brain
Basement membrane
MMP-9
Blood
Chemokines
EVs
BBB disruption
Endothelial cell
T cell
ABCB1
Astrocyte
Extracellular matrix
MMP-9
Leukocyte
Tight junction
Chemokines

IL-37 reduces neuroinflammatory response in primary microglia

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The anti-inflammatory cytokine Interleukin-37 (IL-37) is a member of the Interleukin-1 (IL-1) family. A transgenic mouse model (hIL-37tg) has been described that expresses human IL-37. Previous studies suggest an immune modulatory role of IL-37 and demonstrate that this cytokine is able to reduce inflammation. Hence, IL-37 may act as an important suppressor of innate immunity. Recently, it was shown that IL-37 also reduces neuroinflammation in the spinal cord after spinal cord injury leading to a reduction in local tissue damage.

In this study, we investigated the function of IL-37 in the immune cells of the central nervous system. Using the hIL-37tg mouse strain and wildtype littermates, primary microglia cultures were prepared. We explored the effect of IL-37 on the metabolism of microglia, phagocytic activity and the gene expression of pro-inflammatory cytokines. Preliminary results showed reduced levels of itaconate and succinate, which are markers associated with inflammation. In addition, the treatment of recombinant IL-37b (recIL-37b) could reduce the inflammatory response in wildtype microglia. After LPS stimulation, IL-6 and IL-1β levels were decreased in microglia pre-treated with recIL-37b.

Concluding so far, our results show a reduction in pro-inflammatory response in microglia, the brain resident immune cells. In ongoing studies, we are analyzing if alterations identified in the metabolome of APP/PS1 animals compared to wildtype littermates can be rescued by crossing hIL-37tg into the Alzheimer´s Disease model.

The effects of two novel radioprotectors in the rat brainstem of gamma-irradiated rats followed by Synchrotron FTIR spectroscopy

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Despite decades of investigations and testing of different natural and synthetic radioprotective compounds, the search for an effective and non-toxic radioprotector is still ongoing. Therefore, in this study, we employed high-resolution synchrotron radiation-based Fourier Transform Infrared (SR FTIR) spectroscopy to investigated the radioprotective potential of anisomycin and of the natural aminothiol-based radioprotector GL2011 through the assessment of molecular changes in the brainstem tissue slices of irradiated and radio-protected rats. We tested one dose of intraperitoneally applied radioprotector 30 min before, and 3h, or 6h after irradiating the two months old male Albino Wistar rats with a mild sublethal dose of gamma rays (6,7 Gy). We collected measurements from tissue samples from two animals. As controls pairs of animals were also treated with radiation only or with radioprotector only. The spectral analysis was powered by the multivariate Principal Component Analysis (PCA). In this way, we were able to identify the main sources of variation in the SR-FTIR spectra through the reduction of the dimensionality of the original data sets. This gave us criteria to estimate the effect of radioprotectors on specific molecular biomarkers (bringing the value of a PC closer to the non-irradiated control value). If the results in animal pairs were not congruent or if the irradiation alone did not cause a significant difference from the non-irradiated animal these animal pairs were discarded from the results. Thus, the integrated proteins and ester (carbonyl) band (1480-1800 cm-1) showed a significant effect for both radioprotectors, particularly for applications after radiation. Extracting data just for the Amid II band (~ 1540 cm-1) for proteins showed a significant effect for both radioprotectors albeit for all three timings. Similar has been seen for the band at ~1232 cm-1 (PO2- asymmetric stretching), corresponding mostly to DNA.

These results are in congruence with our previous proteomic and Raman microspectroscopy data and thus reveal novel FTIR spectral biomarkers to follow the effect of radioprotectors anisomycin and GL2011, both showing a stabilizing effect on the genetic material as well as on the protein content in the brainstem tissue of irradiated animals.

Valproic acid treatment after experimental traumatic brain injury in mice

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Traumatic brain injury (TBI) represents the most common cause of death and disability in adults under 45 years. Treatment options are limited and restricted to surgical intervention and supportive therapies but do not address delayed brain damage [1]. Secondary processes in TBI include the activation of CNS-resident microglia and astrocytes and the release of pro- and anti-inflammatory cytokines and chemokines which are major modulators of the immune response following TBI [2]. Therefore, anti-inflammatory and neuroprotective drugs may alleviate secondary consequences of TBI.

Here, we investigated the role of the widely used anti-epileptic drug valproic acid (VPA) with a neuroprotective role in cerebrovascular disease. VPA exerts its activity through multiple signaling pathways e.g. inhibition of sodium/ calcium channels, histone deacetylase (HDAC) inhibition and many more [3].

Adult C57Bl/6 mice were subjected to the controlled cortical impact (CCI) model of TBI and received VPA directly after CCI via intraperitoneal injection for three consecutive days after injury at a dose of 400 mg/ kg body weight. To elucidate the potential benefit of VPA we performed (immuno-) histology, gene and protein expression analyses 7 days after TBI. Behavioral assessment using a neurological severity score was conducted at 1, 3, 5 and 7 days after TBI.

The pharmacological effect of VPA was demonstrated by increased Acetyl-Histone H3 levels in perilesional brain samples. To reveal possible gene transcription changes triggered by the administration of VPA, we investigated a panel of relevant marker genes by qPCR in sham and ipsi-lesional brain samples at 7 days post injury. Among them the TBI-induced mRNA expression of IL-1β and iNOS was significantly attenuated by VPA treatment.

VPA treatment shows subtle beneficial effects in a clinically relevant model of TBI. Our findings suggest that these beneficial effects could be attributed to anti-inflammatory processes in the injured brain.

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Dynamical systems model explains the role of neuroimmune interactions in epilepsy development

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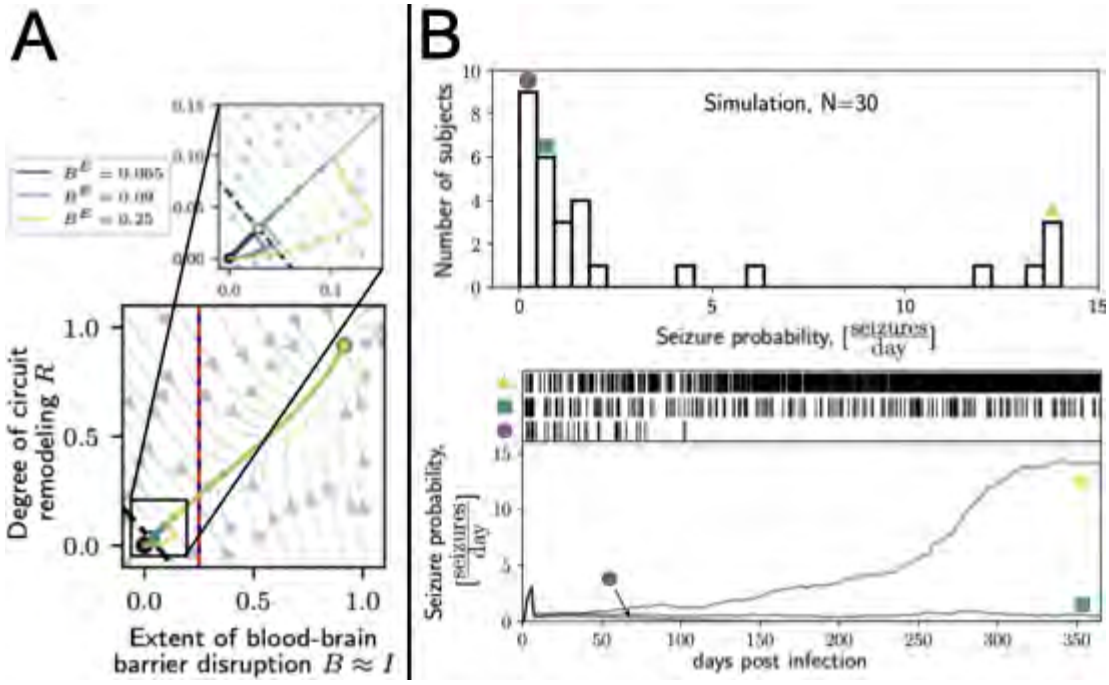
Much progress has been made in understanding the dynamics of individual seizures, while an understanding of principles of epilepsy development remains elusive. Multiple lines of evidence from clinical and experimental studies in epilepsy research highlight the importance of neuroimmune interactions and their dysfunction for disease development (epileptogenesis). Here, we present here a first-of-its-kind phenomenological model of epileptogenesis describing interactions between neuroinflammation *(I)*, blood-brain barrier disruption *(B)*, neuronal death *(D)*, circuit remodeling *(R)* in conditions of injury-related stress. These dynamical variables are coupled in a set of nonlinear stochastic ODEs. The occurrence of spontaneous recurrent seizures is modeled with a Poisson process, the probability density function of which dependents on the intensity of the neuroinflammation *(I)* and the extent of the circuit remodeling *(R)*. Seizures are assumed to induce leakiness of the blood-brain barrier, which was confirmed in human and animal model studies. The model captures how very different types of neurological injuries can all initiate the development of epilepsy. Mathematically, the model is characterized by two stable fixed points corresponding to healthy and epileptic steady states divided by a separatrix. The model is tested with data from 3 animal models of epilepsy, mimicking epileptogenesis caused by neural infection, chemically-induced status epilepticus (prolonged seizure), and blood-brain barrier leakage. The model captures long timescales of disease development (~years) after a transient injury (~days). The surprisingly slow progress of epileptogenesis is explained as a slowing of dynamics near an unstable fixed point (Fig. 1A). The dose-dependence of epileptogenesis risk on injury intensity is explained by different injury intensities pushing the system into different basins of attraction (Fig. 1A). Furthermore, our results suggest an explanation for the variability of epileptogenesis outcomes in subjects exposed to identical injury (Fig. 1B). This variability originates from the noise in the stochastic process of spontaneous seizures generation and complex *(I-B-D-R)*response to injury captured by our mathematical model. Moreover, modeling results show that neuronal death is not a necessary condition for epileptogenesis. However, neuronal loss alone may be sufficient to cause epileptogenesis, which suggests degeneracy in mechanisms of disease development. Our framework may be used for in-silico testing of therapeutic strategies, which will guide the design of further experimental and clinical studies.

Figure 1: A. Central nervous system response to injuries of 3 different intensities illustrated in the state space plot. **B.** Seizure probability one year post infection follow-up (top panel) and characteristic epileptogenesis courses (bottom panel): resolved (circle), developing (square) and progressed (triangle).

Vertical line sequences indicate seizure occurrence in time.



CIDP antibodies target junction proteins and identify patient subgroups: an autoantigenomic approach

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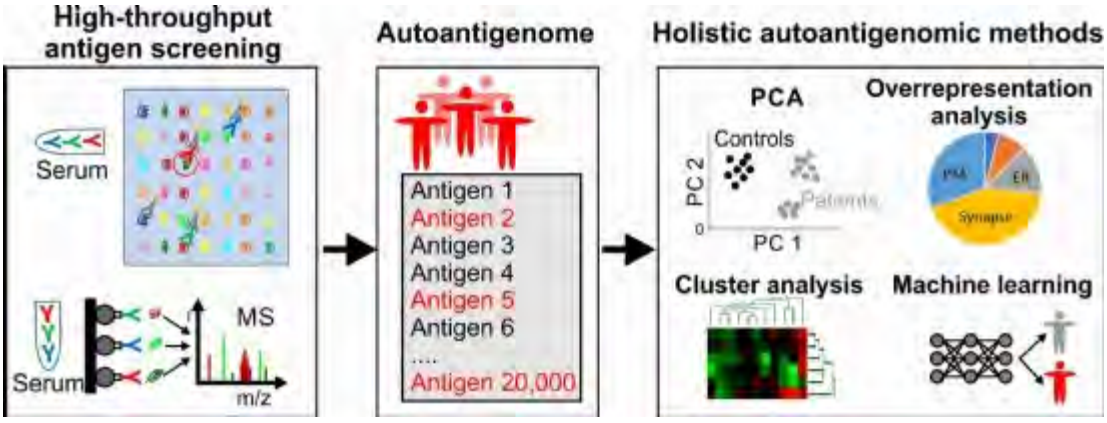
Objective: In order to discover systemic characteristics in the repertoires of targeted autoantigens in chronic inflammatory demyelinating polyneuropathy (CIDP), we detected the entire autoantigen repertoire of patients and controls and analyzed them systematically.
Methods: We screened 43 human sera, comprising 22 CIDP patients, 12 patients with other neuropathies (ONP), and 9 healthy controls (HC) via HuProtTM Human Proteome microarrays testing about 16,000 distinct human bait proteins. Autoantigen repertoires were analyzed via bioinformatical autoantigenomic approaches: principal component analysis, analysis of the repertoire sizes in disease groups and clinical subgroups, and overrepresentation analyses using Gene Ontology and PantherDB.
Results: 1) The autoantigen repertoires enabled the identification of a subgroup of 10/22 CIDP patients with a younger age of onset and a higher frequency of mixed motor and sensory CIDP. 2) Intravenous immunoglobulin therapy responders targeted three times more autoantigens than non-responders. 3) There is no CIDP-specific autoantibody that is present in all patients; but 4) anchoring junction components were significantly targeted by 86.4% of the CIDP patients. 5) There are potential novel CIDP-specific autoantigens such as the myelination- or axo-glial structure-related proteins Actin-related protein 2/3 complex subunit 1B (ARPC1B), Band 4.1-like protein 2 (EPB41L2), Cadherin-15 (CDH15), Cytohesin-1 (CYTH1), Epidermal growth factor receptor (EGFR), Ezrin (EZR), and Radixin (RDX).
Conclusions: The repertoire of targeted autoantigens of CIDP patients differs in a systematic degree from those of controls. Systematic autoantigenomic approaches can help to understand the disease and to discover novel bioinformatical tools and novel autoantigen panels to improve diagnosis, treatment, prognosis, or patient stratification.


High-throughput antigen screening
Serum
Serum
MS
m/z
Autoantigenome
Antigen 1
Antigen 2
Antigen 3
Antigen 4
Antigen 5
Antigen 6
....
Antigen 20,000
Holistic autoantigenomic methods
PCA
Controls
Patients
PC 2
0
PC 1
Overrepresentation analysis
PKA
ER
Synapse
Cluster analysis
Machine learning

Neutralization of glycine receptor autoantibodies from patients' serum samples

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Stiff-Person Syndrome (SPS) is a neurological disease caused by autoantibodies against various synaptic target molecules. One of these targets is the glycine receptor (GlyR). Binding of autoantibodies to its extracellular domain leads to reduced inhibitory neurotransmission. Patients suffer from stiffness and spasms of abdominal and limb muscles. A more severe form of the disease is progressive encephalomyelitis with rigidity and myoclonus (PERM). Patients with PERM display sensory problems and brainstem dysfunction in addition to muscle stiffness and spasms. Patients can be treated with intravenous immunoglobulins, GABAergic drugs or plasmapheresis. However, patients respond differently to those forms of therapy, and relapses with high antibody titers are not uncommon.

For the glycine receptor, different subunits (α1, α2, α3, β) have been identified. In the adult, extrasynaptic α-homomeric and synaptic α-β-heteromeric configurations of the receptor have been described. GlyR autoantibodies have been determined to bind to a common epitope located in the far N-terminal region of the GlyRα-subunits.

Two approaches have been used to neutralize GlyR autoantibodies from serum samples. Living GlyRα1 transfected HEK293 cells were incubated with patients' sera. This approach ensures the native configuration of the receptor. The supernatant was transferred from one cover slip to a next of seeded HEK293 cells expressing the GlyRα1. Finally, the supernatants were characterized by immunocytochemical staining. The patient serum sample exhibited either a reduced staining of the GlyR or the detection of the GlyR was completely abolished.

The determined N-terminal region of the receptor harboring a common epitope for GlyR autoantibody binding is close to a glycosylation site in the receptor. Glycosylation as a perquisite for autoantibody binding has been studied for other autoantibody-mediated diseases, such as NMDA receptor encephalitis. We investigated a mutant GlyR variant lacking the glycosylation in cell-based assays and determined that binding of GlyR autoantibodies to the receptor was still present. Moreover, the variant was transported to the cell surface and formed functional ion channels with a slight rightward shift in the glycine dose-response curve. Homology modeling using the GlyRα3 subunit as a target verified that the overall structure was not changed upon deletion of the glycosylation site. Therefore, we used recombinant expression in Escherichia coli to purify the extracellular domains of the receptor. Purified receptor domains were then used to neutralize GlyR autoantibodies. Following neutralization, immunocytochemical staining of transfected HEK293 cells and primary motoneurons were performed. Our data show efficient neutralization of the GlyR autoantibodies following preincubation with the purified receptor domains leading to reduced labeling or absent detection of the GlyR in cell lines and primary neurons.

To summarize, we were able to show that GlyR autoantibody binding is independent of receptor glycosylation and that purified receptor domains can be used to efficiently deplete patient autoantibodies from the serum.

Reciprocal short- and long-term effects of traumatic brain injury and femoral fracture in a murine polytrauma model

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Background: Traumatic brain injury (TBI) is a major cause of death in early adulthood or lifelong disabilities. Long bone fracture is a frequent concomitant trauma in TBI patients, yet the reciprocal effects between both injuries remain poorly understood.
Material and Methods: After approval of the Landesuntersuchungsamt Rheinland-Pfalz two cohorts (A, B) of C57BL/6N mice (n(A)=43, n(B)=57) received either controlled cortical impact (CCI, n(A)=12, n(B)=15) or fracture of the left femur (FF, n(A)=12, n(B)=15) as a single trauma and were compared to a group of combined injury (CCI+FF, n(A)=11, n(B)=15) and sham (n(A)=8, n(B)=12). Behavioral impairment was assessed by neurological severity score (NSS), leg performance test (LPT) and open field test (OFT) as well as elevated plus maze (B). At 5 (A) and 42 (B) days post injury (dpi) brains were evaluated by histological and immunohistochemical (IHC) analyses, and gene expression was determined by quantitative real-time PCR in brain and bone tissue. Plasma concentrations of progranulin (PRGN) and osteopontin (OPN) were quantified by enzyme-linked immunosorbent assay (ELISA). Statistical analysis: Grubbs‘ test, Shapiro-Wilk test, Student`s t-test/Mann-Whitney-test, ANOVA, $p<0.05$.
Results: CCI+FF mice demonstrated increased neuromotor impairment in NSS compared to all other groups up to 14dpi ($p<0.01$) and reduced expression of anxiety behaviour, assessed by counts of rearing and stretch attend posture in the OFT 5dpi ($p<0.05$). Histological analyses revealed a substance loss of the dentate gyrus suprapyramidal blade in CCI+FF mice compared to isolated CCI at 5dpi as well as an increased ratio of hemispheric volumes at 42dpi ($p<0.05$). Perilesional astrogliosis showed an increased ipsi- to contralateral ratio in animals with combined injury at 5dpi while opposite effects could be observed at 42dpi ($p<0.05$) caused by an augmented contralateral astrogliosis. At 5dpi, mRNA expression of runt-related transcription factor 2 (Runx2), bone sialoprotein (BSP), alkaline phosphatase (ALP) and osteocalcin (OC) were decreased in fractured bone tissue of CCI+FF mice compared to isolated FF. Plasma concentrations of PRGN and OPN were increased compared to all other groups.
Discussion: A concomitant femoral fracture aggravated neuromotor impairment and structural damage of brain tissue after TBI. In bone tissue, mRNA expression of bone-healing associated genes was suppressed by TBI. Combined injury of TBI and FF further leads to increased plasma levels of the neuroprotective and inflammatory mediators OPN and PRGN suggesting a role in reciprocal interactions between brain and bone in our murine polytrauma model (1, 2).
References:
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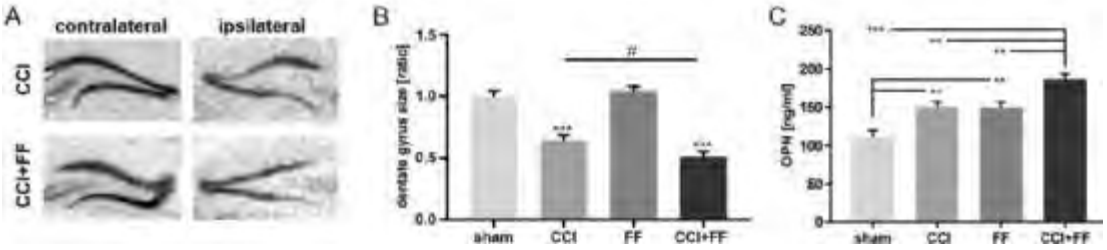
A
contralateral
ipsilateral
CCI
CCI+FF
B
dentate gyrus size [ratio]
1.5
1.0
0.5
0.0
#
***
***
sham
CCI
FF
CCI+FF
C
OPN [ng/ml]
250
200
150
100
50
0
***
**
**
**
**
sham
CCI
FF
CCI+FF

Multimodal imaging in experimental cerebral malaria reveals early-stage accumulation of infected erythrocytes and altered brain perfusion.

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Introduction: Cerebral malaria (CM), one of the most common neurological diseases, is a severe complication of human malaria caused by *Plasmodium falciparum*. It has been hypothesized that intravascular sequestration and congestion of infected-RBCs (iRBCs) play a key role in a cascade of events leading to a prothrombotic state, brain edema and death. Direct evidence for this is lacking in humans as well as in animal models. We here study, in *P. berghei* ANKA (*Pb*A)-infected mice as a model of experimental CM (ECM), brain iRBC accumulation and its potential consequences for brain perfusion using SPECT and MR imaging techniques.

Methods: Mice were infected intraperitoneally with *Pb*A-iRBCs. For whole body and brain SPECT-imaging of iRBC-distributions, iRBCs enriched from blood of day 7 infected mice, were labelled with ^{99m}Technetium. ^{99m}Tc-labelled iRBCs were injected intravenously into infected mice (n=8) and non-infected controls (n=8) at day 5 post infection (p.i.). Brain perfusion was studied using ^{99m}Tc-HMPAO-SPECT at day 5 and day 7 p.i. and with MR angiography (MRA). Flow-compensated FLASH-based TOF images were acquired in combination with velocity maps on day 5 and day 7 p.i. T2-weighted RARE images were used as anatomical reference and for edema detection.

Results: SPECT-imaging of ^{99m}Tc-labelled iRBCs showed substantial and highly significant increases of brain iRBC-contents in infected as compared to control mice. Labeling was most intense in areas of high blood volume as the venous sinuses. SPECT-perfusion experiments showed areas of decreased cerebral blood flow in territories of large draining veins at day 5 p.i., increasing in extent at day 7 p.i. Velocity maps indicated reductions in flow velocity increasing in severity from day 5 to day 7 p.i. Concordant with severe clinical symptoms, T2-MRI revealed brain edema on day 7 p.i. TOF-angiographies showed reduced flow in large draining veins and sinuses at day 5 p.i. Flow decreased to almost undetectable levels in severe cases at day 7 p.i. To the best of our knowledge, our data provide the first *in vivo* evidence for brain-wide early stage intracerebrovascular iRBC accumulation in experimental CM and in CM in general. Our SPECT and MR-findings of reduced cerebral flow suggest that flow deficits can precede MR-detectable edema.

Conclusion: In humans, brain edema is a hallmark of CM and fatal outcomes are related to edema severity. Controversies exist about the underlying pathophysiology and the validity of animal models. Our data argue for similar pathologies in mouse and man and are in line with early theories on CM pathology in humans

proposing that intravascular iRBC sequestration and congestion might lead to reduced venous efflux that in turn could contribute to brain edema.

Cognitive decline is associated with a wide spectrum of serum and cerebrospinal fluid neuronal autoantibodies

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Background: The immune system is attracting growing attention in disorders associated with cognitive decline. Autoantibody diagnostics are a field of major importance in memory-clinic patients with cognitive decline and who present indicators suggesting autoimmunity, such as atypical fluctuating psychopathology or neurological deficits.

Methods: In our retrospective and observatory study, we enrolled 154 patients during one year (2019-2020) who presented for their first time in a memory clinic in the Department of Psychiatry and Psychotherapy of the UMG. We have analyzed different parameters in their patient files concerning their neuropsychology, psychopathology, autoantibody occurrence in serum and cerebrospinal fluid (CSF), and molecular markers in serum and CSF.

Results: We looked for serum and CSF autoantibodies in 26 of 154 patients, as those patients had revealed one or more “yellow flags” or “red flags” indicating possible autoimmunity. Our search strategy revealed serum and CSF autoantibodies in 58% of them. We found a positive CSF autoantibody in 15/154 (9.7%), and a positive serum autoantibody in 4/154 (2.6%) of patients. Specific cell-surface (serum: n=1 anti-CASPR2, n=2 anti-Glycin receptor, n= 1 anti-IgLON5, n=1 anti-KCNA2, n=1 anti-myelin; CSF: n=1 anti-IgLON5, n=1 anti-MOG) and intracellular autoantibodies (serum: n=1 anti-CV2, n=1 anti-ITPR1, n=2 anti-Recoverin, n=1 anti-Titin, n=1 anti-Yo; CSF n=2 anti-Yo) were each detected in 4.5% of 154 patients. We detected no differences in psychopathology, CSF parameters, or neuropsychological profiles in autoantibody-positive patients with cognitive impairment (n=14) compared to those without detected autoantibodies and cognitive impairment (n=8) and patients with biomarker-based Alzheimer´s disease (n=9). The Aß42/40 ratio in CSF was significantly lower in patients with Alzheimer´s disease than in the two other patient groups.

Conclusions: Autoantibody-associated cognitive impairment is a frequent phenomenon in a memory clinic in those patients presenting with additional clinical features indicative for autoimmunity. These findings indicate the need to optimize our diagnostic approach to be better able to identify those patients who might profit from an early, rapid immunotherapy intervention. The sheer variety of mostly serum autoantibodies involved in cognitive decline is evidence of their importance for research, although their significance remains enigmatic.

Intra-ganglionic delivery of Iba1 siRNA alters macrophages perineuronal ring formation in SNL neuropathic pain model

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Introduction: Iba1 (Ionized calcium-binding adapter molecule 1) is a macrophage-specific cytoskeleton protein involved in migration, phagocytosis, cell adhesion and proliferation. After spinal nerve ligation (SNL), Iba1 (+) macrophages cluster around DRG neurons, forming tight perineuronal rings mainly around large NF200 (+) neurons and, to a lesser extent, around small CGRP (+) neurons. The aim of this study was to investigate if the intra-ganglionic delivery of the Iba1 siRNA alters the perineuronal ring formation and Iba1 (+) macrophage's morphology, besides Iba1 expression.

Materials and methods: Male Sprague-Dawley rats were assigned to 4 groups: Control (rats did not undergo any kind of surgery), Sham (rats were injected with 4 µl of PBS 1x or scramble Iba1 siRNA), SNL (L5 spinal nerve was ligated and cut) and SNL+Iba1 siRNA (after ligation and cutting of the L5 spinal nerve, 4 µl of Iba1 siRNA were injected in the L5 DRG). 5 days after surgery, L5 DRGs were collected, fixed, sectioned and double immunostained using antibodies against Iba1, NF200 and CGRP. To quantify the ring area of Iba1 (+) macrophages around NF200 (+) and CGRP (+) DRG neurons we used a plugin for ImageJ 1.37v (NIH, USA) to measure mean fluorescence intensity/area ratio (MF/A ratio) around and on top of DRG neurons.

Results: Our results showed that intra-ganglionic delivery of Iba1 siRNA significantly reduced Iba1 fluorescence intensity, and therefore Iba1 expression. In addition, we observed that Iba1 siRNA administration altered the formation of perineuronal rings after SNL. Although they were not completely disorganized, they became looser and macrophages were no longer located in the immediate vicinity of the neurons. Last but not least, treatment administration did not cause any major changes in morphology: in SNL+Iba1 siRNA group, macrophages had enlarged cell bodies with thick processes, which is characteristic for this peripheral neuropathic pain model.

Conclusions: Intra-ganglionic delivery of Iba1 siRNA after SNL decreased Iba1 expression, altered the formation of perineuronal rings, which became looser and did not have any major effect on Iba1 (+) macrophage's morphology.

Exploiting benign MS: Identification of astrocyte-specific endogenous neuroprotective and neuroregenerative mechanisms to stop progressive MS

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Multiple sclerosis (MS) is the most frequent chronic inflammatory disease of the central nervous system. Due to a highly heterogeneous disease course, the prognosis ranges between a mild type without MS related disability to a severe, ultimately heavily disabling form of the disease. We propose that CNS endogenous cells contribute to neuroprotection and –regeneration, which might explain clinically diverse disease courses. The mechanisms and the extent of neuroprotection and –regeneration in particular performed by CNS endogenous cells are largely unknown. We focused here on astrocytes and their potential to protect neurons from inflammation-mediated damage.

To study the role of astrocytes in these processes, we generated induced pluripotent stem cell (iPSC) lines from 6 individual MS patients (3 patients with a benign disease course, i.e. EDSS <3 and 3 patients with a disabling disease course, i.e. EDSS >6).

By differentiating these iPSCs into astrocytes, we established a co-culture model of patient-derived astrocytes together with a healthy control of an inducible neuronal cell line (neurogenin2, NGN2) to study inflammation related neuronal damage and protection in MS. To evaluate the impact of (pro)-inflammatory stress on neurons, we performed immunofluorescence staining against SMI-32, an antibody recognizing an epitope of a non-phosphorylated Neurofilament H (NF-H) variant associated with neuronal damage. We show that neurons in co-cultures with astrocytes derived from benign patients were protected against damage-inducing pro-inflammatory cytokines TNFα and IL17. In contrast, co-cultures of neurons with astrocytes from disabling patients were not protected against these cytokines, shown by significantly increased proportions of SMI-32 positive neurons. Direct cytokine treatment of monocultures of neurons derived from the different patient subsets did not show clear differences.

These results highlight, that astrocytes from benign MS patients effectively contribute to prevent signs of neurodegeneration in a novel preclinical model for immune mediated neurodegeneration. We plan to further exploit the model to investigate other mechanism of astrocyte driven neuroprotection. Our model holds great potential to explain the rare phenomenon of benign MS and the ultimate goal of this work is to identify drug targetable effector mechanisms that support neuroregeneration and -protection in MS patients.

Neuroprotection in insects: Roles of ancestral erythropoietin-like proteins and acetylcholinesterase

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The cytokine erythropoietin (Epo) initiates cell-protective mechanisms in various tissues of vertebrates. While erythropoietic functions of circulating Epo are understood with considerable detail, neuroprotective functions of both hormonal and locally released Epo seem to be more diverse. In addition to the widely expressed “classical” homodimeric Epo-receptor, other “alternative” Epo receptors increase survival of neurons under challenging physiological and pathological conditions. Understanding the mode of action of Epo in neuroprotection and regeneration will allow the design of novel neuroprotective agents for specific treatment of degenerative diseases.

Invertebrates including insects neither express Epo nor any known Epo receptor. Our studies however, have unraveled neuroprotective and regenerative properties of recombinant human Epo in insect primary neuron cultures. The cytokine receptor-like factor 3 (CRLF3) was identified as an Epo-responsive receptor mediating Epo’s beneficial effects in insects by activating JAK/STAT transduction pathways. CRLF3 is highly conserved and regarded as an “orphan receptor”, since neither its ligand nor its particular functions have been identified so far. We hypothesize, that the endogenous ligand of insect CRLF3 is an ancestral cytokine that shares structural similarity with vertebrate Epo. Our studies aim to characterize the neuroprotective and neuroregenerative mechanisms regulated by CRLF3 functions in insect neurons.

We report that cell-free hemolymph from locusts (*Locusta migratoria*) protects both locust and beetle (*Tribolium castaneum*) neurons from hypoxia-induced apoptosis. Hemolymph-mediated neuroprotection is dose-dependent and is abolished following RNAi-induced knockdown of *crlf3*. The characteristics and extend of neuroprotection are very similar to those described for Epo. We fractioned locust hemolymph by size exclusion chromatography into ~40 subfractions, to reduce the numbers of proteins tested *in vitro*. We identified few fractions with pronounced neuroprotective effects while most hemolymph fractions were unable to protect neurons from hypoxia-induced apoptosis. Our data indicate that an intermediate-sized protein activates CRLF3 to mediate the anti-apoptotic effects of locust hemolymph.

We recently reported that acetylcholinesterase (AChE) promotes apoptosis in insect neurons, suggesting a conserved role of AChE in apoptosome formation and/or DNA degradation among insects and vertebrates. New data indicate that apoptosis-inducing physiological challenges increase *ache*-expression in locust primary neuron cultures. Moreover, increased *ache*-expression in hypoxia-exposed primary neurons is suppressed by application of Epo. This indicates that CRLF3 activation mediates anti-apoptotic mechanisms by prevention of pro-apoptotic AChE accumulation. If the activity of the esterase is also affected by Epo-induced intracellular pathways, is currently under investigation.

Taken together, our data suggest the presence of an evolutionary ancient endogenous ligand for CRLF3 in insects that shares functional characteristics with vertebrate Epo. Neuroprotective effects of CRLF3-activation seem to be mediated by interference with pro-apoptotic AChE expression, which is elevated under stressful conditions. Understanding these neuroprotective mechanisms and exploring their potential conservation in vertebrates may foster the development of specific and safe neuroprotective agents for the clinics.

Influence of antisera against the intracellular parasite Toxoplasma gondii on Complexin 2- regulates exocytosis in SH-SY5Y neuroblastoma and RBL-2H3 granulocyte cell lines

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Previous studies revealed an association between maternal prenatal serum IgG antibodies to the neuro-invasive parasite Toxoplasma gondii (T. gondii) and an increased risk for the fetus to develop neuropsychiatric illness i.e. schizophrenia later in life (Brown et al. 2005, Am J Psychiatry 162, 767–773; Mortensen et al. 2007, Biological Psychiatry 61, 688–693; Blomström et al. 2012, Schizophrenia Research 140, 25–30). Since maternal IgG are known to cross the placental barrier into the fetal bloodstream (Simister 2003, Vaccine 21, 3365–3369), they could affect early brain development due to molecular mimicry (Birner et al. 2000, Journal of Infection 41, 32–38). We investigated here interactions of a polyclonal anti-T. gondii antiserum with a human fetal brain multiprotein array, demonstrating epitopes of Complexin 2 (CPLX2), a cytosolic protein able to synchronize and accelerate SNARE complex-mediated exocytosis, to bind parasite specific antibodies. On the functional level, a ß-hexosaminidase assay, revealed exocytosis of a basophilic granulocyte cell line (RBL-2H3) incubated with anti- T. gondii antibodies, to be significantly reduced ($p<0.05$), and this reduction is also accompanied by lower CPLX2 immunofluorescence. In contrast, in SH-SY5Y neuroblastoma cells, vesicle recycling as revealed by FM1-43 fluorescence was not significantly changed. However, these cells if pretrated with anti-T. gondii antibodies revealed significantly reduced mitochondrial activity, as shown by an MTT assay ($p<0.01$). Together these results demonstrate that antisera against T. gondii are able to impair exocytosis either by direct interaction with CPLX2 or indirectly by changing mitochondrial energy metabolism. Together with previous findings that expression of CPLX2, is decreased in the brains of schizophrenia patients (Sawada et al. 2005, Arch Gen Psychiatry 62, 263–272), and that this decrease correlates to disease severity (Hass et al. 2015, Eur Arch Psychiatry Clin Neurosci 265, 137–145), our findings open the possibility that both mechanisms could play a role in schizophrenia pathogenesis.

Poster Topic

T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

T13-1 Sphingomyelin synthases in depression and antidepressant treatment
Roberto Damián Bilbao Canalejas, Claudia von Zimmermann, Tanja Richter-Schmidinger, Bernd Lenz, Johannes Kornhuber, Christiane Mühle

T13-2 Effect of salicylate-induced tinnitus on hearing thresholds
Veralice Lanaia, Konstantin Tziridis, Holger Schulze

T13-3 The Neuromodulator Octopamine Mediates Sugar Relief from a Chronic Stress-Induced Depression-Like State (DLS) in *Drosophila*
Sonja Boxhorn, Tim Herrmanns, Burkhard Poeck, Roland Strauss

T13-4 Sex differences in emotional information processing in the GAD65 knock out model of heightened stress susceptibility
Anne Albrecht, Michelle Ulrich, Evangelia Pollali, Oliver Stork, Gürsel Çaliskan

T13-5 Common and unique pathways confer vulnerability versus resilience to food and cocaine addiction-like behavior: role of PFC-NAc dopamine D2 receptor
Inigo Ruiz de Azua, Mohit Navandar, Elena Martin-Garcia, Rafael Maldonado, Beat Lutz, Gerber Susanne

T13-6 Peripheral lysosomal acid sphingomyelinase in major depressive disorder
Christiane Mühle, Roberto Damián Bilbao Canalejas, Claudia Johanna von Zimmermann, Tanja Richter-Schmidinger, Bernd Lenz, Johannes Kornhuber

T13-7 Chemogenetic modulation of the rhesus amygdala as a translational model for human anxiety disorders
Sascha A. L. Mueller, Patrick H. Roseboom, Jonathan A. Oler, Marissa K. Riedel, Victoria R. Elam, Miles E. Olsen, Alexandra H. Difilippo, Bradley T. Christian, Juan Gomez, Mike Michaelides, Ned Kalin

T13-8 Multimodal analysis of structural plasticity of the prefrontal cortex and changes in behaviour in a mouse model of chronic pain
Jennifer John, Amrita Das Gupta, Livia Asan, Carlo Beretta, Ariel Iporre, Claudia Falfan-Melgoza, Felix Hörner, Wolfgang Weber-Fahr, Thomas Kuner, Johannes Knabbe

T13-9 Impaired cognitive flexibility, fear and safety learning in Shank2-deficient mice
Markus Fendt, Nora Broda, Iman Hassan, Judith C. Kreutzmann, Elaheh Soleimanpour, Ceylan Steinecke, Pei-Ling Tsai, Daniela C. Dieterich

T13-10 Role of orexin in cognitive flexibility, sensorimotor gating and impulsivity
Archana Durairaja, Alexandrina Demidova, Markus Fendt

T13-11 The impact of a plasmalogen-deficiency in the modulation of the axon initial segment position
Tiago Ferreira Silva, Luis Granadeiro, Diogo Bessa-Neto, Pedro Brites

T13-12 Unawareness and Volition in Alcohol Consumption: A Study using Functional Magnetic Resonance Imaging
Iasmina Livia Hornoiu, Haoye Tan, Alycia Lee, Helmut Nakovics, Sabine Vollstaedt-Klein

Sphingomyelin synthases in depression and antidepressant treatment

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Major depressive disorder is a severe and increasingly common psychiatric disease. Recent studies in mice have revealed that pharmacological inhibition of a group of enzymes known as sphingomyelin synthases (SMS) induces neurogenesis and rapidly improves depression-like behaviour by activating autophagy. However, whether these effects are due to the inhibition of one or both SMS isoforms (SMS1 and SMS2) remains unknown. We aim to elucidate the involvement of SMS1 and SMS2 in depression and to identify their role in the autophagy-dependent antidepressant effect. To do this, we will compare the activity and expression of SMS under healthy and pathological conditions, both in humans and in mice. First data indicate a sex-independent higher expression of SMS1 and SMS2 genes in peripheral blood cells of patients with a current major depressive episode compared to healthy controls. Although we could not detect SMS activity in serum and plasma samples, we were able to optimize an SMS activity assay in human peripheral blood cells. An increase of SMS activity was observed in female but not male depressed patients compared to controls. Overall, these results fit with the hypothesis of increased activity of SMS in depression. By treating cellular and animal models with specific SMS inhibitors, we will determine which isoform mediates the antidepressant effect, thereby identifying a potential pharmacological target for the design of fast-acting antidepressants.

Effect of salicylate-induced tinnitus on hearing thresholds

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Diseases affecting the inner ear resulting in hearing loss may lead to subjective tinnitus. Tinnitus is an auditory phantom percept without an external sound source and a symptom of many pathological conditions. It occurs with a surprisingly high prevalence affecting about 35% of the general population, with 10%-15% of individuals experiencing prolonged tinnitus requiring medical evaluation. For 10% of the population, tinnitus has a significant impact on their quality of life. Despite this high prevalence and the tinnitus-associated distress of affected patients, which in severe cases may experience insomnia, psychological disorders like depression, the inability to work, or even commit suicide, the pathophysiology of tinnitus remains largely unknown, making prevention and treatments difficult to develop. In recent studies, we suggest a central origin of tinnitus-related development of neuronal hyperactivity based on stochastic resonance (SR). SR refers to the physiological phenomenon that weak sub-threshold signals for a given sensor (or synapse) can still be detected and transmitted if appropriate noise is added to the sensor's input. The SR neuronal noise is the neurophysiological correlate of tinnitus-related enhanced neuronal activity. In this view, tinnitus is a side effect of a physiological adaptive mechanism within the auditory system whose main purpose is to compensate for hearing loss by constantly optimizing information transmission from the cochlea into the central auditory system.

The main objective of this study was to characterize the effect of salicylate, as a model of tinnitus induction, on Auditory Brainstem Responses (ABR) and the possible perception of tinnitus as assessed by behavioural testing (gap-prepulse inhibition of the acoustic startle reflex, GPIAS) in Mongolian gerbils. Frequency-specific differences in the startle responses due to salicylate were analysed and correlated with possible differences in the ABR at different time points after the treatment in the same animals.

Ours data show, in line with the pharmacokinetics, that ABR thresholds generally increase 2 h after salicylate injections. This increase is significantly stronger at the region of best hearing (around 4 kHz) compared to other frequency regions. In the GPIAS data, the salicylate animals showed the presence of tinnitus. Again, the general effect of salicylate on the behaviour was significant after 2h, but already after 20 min first effects could be detected at 4 kHz. We found no correlation of behaviour and ABR data in the salicylate animals.

Comparing these data with noise trauma induced tinnitus effects, the lack of correlation between hearing threshold and behavioural signs of tinnitus indicate that the development of tinnitus after salicylate injection is not based on the proposed SR model. Hence trauma and salicylate induced tinnitus seem to result from different neurophysiological mechanisms and are not comparable. Specifically, salicylate induced tinnitus is not a good model for most forms of clinically relevant tinnitus.

The Neuromodulator Octopamine Mediates Sugar Relief from a Chronic Stress-Induced Depression-Like State (DLS) in *Drosophila*

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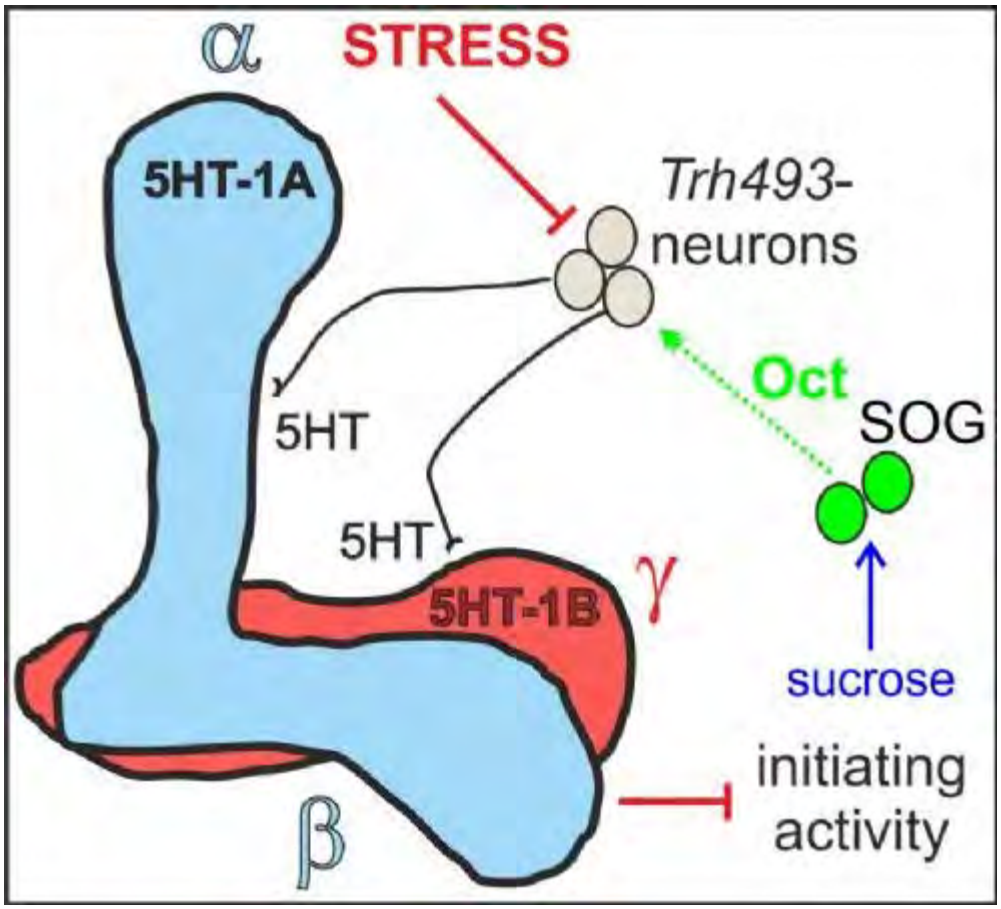
Chronic, uncontrollable, stress can result in psychiatric syndromes including anxiety and major depressive disorder (MDD). However, the underlying pathophysiology mechanisms are far from understood. Rodent models have been developed using chronic mild stress or unavoidable punishment (learned helplessness) to induce features of depression, like general inactivity and anhedonia. We have developed a chronic stress protocol for *Drosophila* flies (three days of vibrational stress and food deprivation) that reduces voluntary behavioural activity (Ries et al. 2017). In particular, the motivation to climb a gap that exceeds their body size (risk taking) and the voluntary locomotor activity are significantly reduced. Moreover, stressed males show a longer courtship latency when confronted with an unreceptive female. As a measure of anhedonia, we have developed the a Stop-for-Sweet paradigm where flies, while performing negative geotaxis, cross a sweet-tasting stripe. Stressed and food deprived flies showed a reduced number of stops in comparison to hungry controls. The reduced motivation to perform voluntary behaviours (whereby more reactive behaviours like escape running are unchanged) suggests that chronic stress results in a depression-like state (DLS) in flies comparable to rodent models of depression. Notably, the observed behavioural changes correlate with reduced serotonin (5-HT) release at the mushroom body and can be relieved by feeding the antidepressant 5-hydroxy-L-tryptophan or the selective serotonin reuptake inhibitor fluoxetine. Relief is mediated by 5-HT-1A receptors expressed in the α-/β-lobes of the mushroom body. Notably, feeding 5% sucrose for a couple of hours results in elevated 5-HT levels in the brain and can ameliorate the DLS.
Here we show, that the sugar relief is mediated through octopamine signalling to serotonergic neurons, expressing the octopamine β 1 receptor, which innervate the mushroom body. Null mutants for tyramine β hydroxylase ($T\beta^{hnM18}$), the key limiting enzyme in octopamine synthesis, cannot be relieved from the DLS by sugar treatment; however, feeding octopamine ameliorates the DLS phenotype. Neuronal silencing experiments revealed that octopaminergic neurons of the suboesophageal ganglion are required for the sugar relief and feeding octopamine can compensate this defect. We like to suggest that octopamine functions as a neurohormone to signal the sweet sensation via serotonergic interneurons to the α-/β-lobes of the mushroom body, which modulate the motivation to perform innate behaviours.

Ries et al. (2017). Serotonin modulates a depression-like state in *Drosophila* responsive to lithium treatment. *Nature Commun*. 8:15738. doi: 10.1038/ncomms15738


α
STRESS
5HT-1A
Trh493-
neurons
Oct
SOG
5HT
5HT
5HT-1B
γ
sucrose
initiating
activity
β

Sex differences in emotional information processing in the GAD65 knock out model of heightened stress susceptibility

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Recent studies suggest altered Ɣ-amino butyric acid (GABA) neurotransmission within the anterior cingulate cortex (ACC) as a neuronal correlate for gender differences in anxiety. Due to their lowered postnatal GABA levels, mice bearing a null mutation of the GABA synthetizing enzyme glutamate decarboxylase 65 (GAD65-/-) constitute excellent models to study the neurobiological basis of GABA-related gender differences. However, studies in female GAD65-/- mice are sparse. We therefore compared different aspects of emotional information processing in male vs. female GAD65-/- mice and their wildtype littermates. Our behavioral test battery revealed complex interactions of sex and genotype in open field habituating as well as sex differences in the exploration of novel objects, in social interaction and in active avoidance learning. Together, our data suggests an increased adaptation to aversive situations in female mice compared to male mice. However, in both sexes a GAD65 null mutation dampened adaptive responses. Assessing interneuron integration into local networks by measuring oscillatory activity in slice preparations, demonstrated enhanced beta-gamma (15-40 Hz) oscillations in the ACC of GAD65-/- mice of both sexes, which was paralleled by an increased number of parvalbumin-positive interneurons in this region as assessed by immunohistochemical analysis. In addition, somatostatin-positive interneurons were decreased especially in the dorsal dentate gyrus and basolateral amygdala of male GAD65-/- mice. These results highlight the importance to study interneuron function in female vs. male individuals to gain insights into gender differences in anxiety disorders and the role of GABAergic neurotransmission therein.

Common and unique pathways confer vulnerability versus resilience to food and cocaine addiction-like behavior: role of PFC-NAc dopamine D2 receptor

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Addiction is a chronic relapsing brain disorder characterized by compulsive reward-seeking despite harmful consequences. The mechanisms underlying addiction are orchestrated by transcriptional reprogramming in the reward system of vulnerable subjects. By computational analysis of RNA-seq data from two independent studies, we found common and unique genes in the PFC associated with palatable food and cocaine addiction. Gene ontology and protein-protein association analysis of common genes identified several G protein-coupled receptors and the cAMP signaling pathway as a core network in addiction. As a proof-of concept, the upregulation of dopamine D2 receptor in PFC-NAc pathway by viral gene delivery promoted compulsive behavior to palatable food. Our study unravels a new neurobiological mechanism underlying resilience and vulnerability to develop food addiction, which is expected to pave ways for novel and efficient interventions to battle other types of addiction.

Peripheral lysosomal acid sphingomyelinase in major depressive disorder

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Major depressive disorder (MDD) is a highly prevalent and devastating psychiatric illness with a strong individual and societal burden. There is an urgent need for biomarkers to improve an objective diagnosis and for a better understanding of underlying mechanisms. Evidence from clinical and animal studies suggests a pathophysiological role of sphingolipids particularly increased levels of ceramides and of the enzyme acid sphingomyelinase hydrolyzing sphingomyelin to ceramide.

We analyzed routine blood parameters and quantified the activity of the lysosomal acid sphingomyelinase (L-ASM) in peripheral blood mononuclear cells of patients with current (unmedicated n=58, medicated n=58) and remitted (n=39) MDD and healthy subjects (n=55) using fluorescently labelled substrates. Depression severity and anxiety and their 3-weeks prospective course of treatment as usual were assessed by psychometric scales.

Similarly to the secretory form of ASM in serum, L-ASM activity was independent of sex, not different between the four groups and did not decrease during treatment. In contrast to patients' cultured peripheral blood mononuclear cells or cell lines treated with antidepressant functionally inhibiting L-ASM, the enzyme's activity was not lower in directly isolated and lysed peripheral blood cells from patients taking these drugs. Whereas we did not observe the expected positive correlation between L-ASM activity and severity of depression, lower L-ASM activity at recruitment was predictive of a stronger improvement of depression severity as assessed by clinicians (HAM-D and MADRS scales) in female patients. Interestingly, predominantly in healthy females, there was a strong correlation between the activities of L-ASM and the enzyme sphingomyelin synthase in peripheral blood mononuclear cells catalyzing the reverse reaction. Only the male subgroup showed negative associations of L-ASM activity with C reactive protein as an inflammatory marker as well as with blood zinc ions, a known cofactor of the enzyme.

These findings aim to contribute to a better understanding of sphingolipid mechanisms in health and disease and to the development of novel diagnostic biomarkers and therapeutic targets for MDD.

Chemogenetic modulation of the rhesus amygdala as a translational model for human anxiety disorders

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Developing chemogenetic strategies to reversibly modulate the neural circuits underlying psychopathology has the potential to not only advance our understanding of anxiety disorders, but also to develop targeted treatment strategies. To investigate the feasibility of translating DREADDs into a tool for treating refractory psychiatric illness, we utilized the inhibitory hM4Di-DREADD in a nonhuman primate model, with the intent of reducing anxiety-related behaviors. Complementary to this approach, we also utilized the excitatory hM3Dq-DREADD to establish a model of pathological anxiety in nonhuman primates, with the intent of increasing anxiety-related behaviors. In both experiments, young rhesus monkeys underwent intraoperative MRI surgery to bilaterally infect the amygdala with a DREADDs viral construct. Because of its high affinity for DREADD receptors, as well as its long-standing clinical use in humans, low dose clozapine was chosen as the ligand to facilitate DREADD-activation. Using brain tissue from hM4Di subjects, autoradiographic studies validated that clozapine binds to the hM4Di receptors expressed in the amygdala, and immunohistochemical methods determined that neurons expressing hM4Di-DREADDs were most prominently located in the dorsal regions of the basolateral nuclei. In vivo µPET imaging with [11C]-deschloroclozapine further demonstrated selective binding in the amygdala of both hM4Di and hM3Dq subjects. To assess their behavior during stress-related contexts, subjects were tested in the human intruder paradigm. Compared to their respective control groups, clozapine selectively decreased anxiety-related freezing behavior in hM4Di subjects and increased anxiety-related freezing behavior in hM3Dq subjects. Together, these results successfully demonstrate the implementation of viral vector methods to express DREADDs in the rhesus amygdala, the utility of using low-dose clozapine as an exogenous ligand, and importantly, the ability to use this chemogenetic method to alter threat-related anxiety.

Multimodal analysis of structural plasticity of the prefrontal cortex and changes in behaviour in a mouse model of chronic pain

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Pain is a basic and life-supporting perceptions of every organism. It functions as an immediate warning system and is crucial for preventing greater damage. However, if acute pain outlasts the healing of the original damage, it may become chronic. It is currently the leading cause of disability and disease burden globally and occurs in connection with many illnesses and even promotes further comorbidities such as depression and anxiety. Persistent chronic pain is shown to be accompanied by a reversible decrease of grey matter volume in pain-associated areas of the brain, which can also directly be transferred to rodent models of chronic pain.

The first aim of this study is the longitudinal characterizations of chronic pain-associated volume changes of cortical grey matter and their cellular basis by using correlative magnetic resonance imaging (MRI) in combination with in vivo 2-photon fluorescence imaging in mice. The second aim is the investigation of a possible correlation between cellular and behavioural changes after induction of chronic pain in mice.

For the longitudinal analysis of all cells in the brain, transgenic mice expressing Histone-GFP in every nucleus were used for the study. Nuclear morphology is distinct for each cell type, allowing to indirectly infer changes in cellular composition and spatial arrangement of cortical cells.

Chronic cranial window implantation was performed on 8 to 10 weeks old mice and four weeks later, microscopic imaging using 2-photon microscopy and macroscopic imaging by MRI were carried out. Furthermore, Von-Frey and Cold Plate (behaviour tests) were performed. In the MRI structural images were acquired using T2-weighted RARE sequences and Diffusion Tensor Imaging. Voxel-Based Morphometry (VBM) calculations were performed on the MRI data for estimating volume changes longitudinally. For 2-photon imaging, two large 3D stacks were imaged on each hemisphere in the anterior cingulate cortex and neighbouring areas. Cell counting and nuclear shape analysis were achieved using machine-learning based algorithms for automated image segmentation. The neuropathic pain model of spared nerve injury (SNI) and its corresponding Sham surgery were induced after the baseline measurements. Behavioural tests and imaging were repeated at 1 week and 12 weeks after the surgery. Two further behavioural tests allowing the measurement of the emotional aspect of chronic pain were performed 8 weeks after the SNI/ sham surgeries, the Open Field and Place Escape Avoidance Paradigm tests.

Preliminary data analysis demonstrate that SNI mice may exhibit an increased depressive behaviour, which also plays an important role in the treatment of chronic pain in humans. First results from the fully automated analysis of the imaging data show that the total cell count, nuclear volume and the nearest neighbour distance between cells change in SNI compared to Sham mice, especially in the first week after SNI. Further analyses correlating behavioural tests with MRI data will complete these results.

We present a new technique to correlate MRI and cellular changes of cortical grey matter using a chronic

pain model in mice. Fully automated analysis will make it possible to use this approach in different disease models in the future. For the study of chronic pain, the results may give a new insight into the transition from acute to chronic pain, possibly leading to novel strategies preventing this transformation in human patients.

Impaired cognitive flexibility, fear and safety learning in Shank2-deficient mice

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Shank2 is an excitatory postsynaptic scaffold that is important for synaptic protein assembly and NMDA receptor related signaling. In humans, Shank2 mutations are associated with neuropsychiatric diseases including autism spectrum disorders, schizophrenia and bipolar disorder. Shank2-deficiency in mice results in behavioral endophenotypes of neuropsychiatric diseases such as social deficits, repetitive behavior, and anxiety-like behavior. Of note, these mice also show a NMDA receptor hypofunction, and pharmacologically restoring NMDA receptor function improves some of the behavioral deficits. In the present study, female and male Shank2-deficient mice were tested in the attentional set shifting task (ASST) and in a fear/safety learning task. We found a sex-specific deficit in the reversal phase of the ASST (pilot data) and impaired contextual fear and safety learning in homozygous Shank2-deficient mice that was independent of sex. Treatment with the partial NMDA receptor agonist D-cycloserine rescued the ASST deficit. In addition to the behavioral experiments, we started to analyze the expression pattern of components of NMDA receptor related signaling pathway –such as different subunits of the NMDA receptor, serine racemase, and phosphoglycerate dehydrogenase (PHGDH)– in different subregions of the frontal cortex which are critical for the ASST or fear/safety learning. These analyses are performed in the different sexes and genotypes – in naïve, trained, and trained & treated mice. Taken together, our data demonstrate an important role of Shank2 in cognitive flexibility measured by the ASST and in contextual fear and safety learning. Furthermore, first data from molecular analyses as well as the rescuing effects of D-cycloserine indicate that the observed effects of Shank2-deficiency are associated by changes in NMDA receptor signaling.

Role of orexin in cognitive flexibility, sensorimotor gating and impulsivity

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Deficits in cognitive flexibility, impaired sensorimotor gating and increased impulsivity are behavioral endophenotypes of several neuropsychiatric disorders including schizophrenia and attention-deficit/hyperactivity disorder (ADHD). Several classical transmitter systems including dopamine and noradrenaline are shown to be involved in these behavioural endophenotypes, however, there is not much known about the role of neuropeptides. The orexin neuropeptides, which are brain-widely released by neurons of the lateral hypothalamus, are major players in maintaining sleep/wake cycle, feeding behavior, arousal, and motivational behavior. Disruption of orexin signaling in basal forebrain impairs attention and cognition. In addition, blocking orexin-1 receptor (OX1R) decreases impulsivity in rats. Recently, we showed a sex-dependent modulation of cognitive flexibility in homozygous orexin-deficient mice. To further investigate the role of orexin in cognitive flexibility, we acutely blocked OX1R brain wide in male and female C57BL/6J mice and tested them in the attentional set shifting task (ASST). Furthermore, we tested attention and impulsivity in male and female orexin-deficient mice in the 5-choice serial reaction time task (5-CSRTT) using an automated touch screen set-up during both light and dark phase. In addition, we also performed cliff avoidance reaction (CAR) paradigm to assess impulsivity and pre-pulse inhibition (PPI) of the startle response to assess sensorimotor gating. Our preliminary results show sex differences in impulsivity in 5-CSRTT. Lack of orexin neuropeptide in male homozygous orexin-deficient mice seems to decrease premature responses indicating decreased impulsivity compared to wildtypes. Whereas, we observed an increase in premature responses in female homozygous orexin-deficient mice compared to wildtypes. This indicates a sex-dependent role of orexin in impulsivity and urges the need for more sex-specific research and treatment strategies for the symptoms of neuropsychiatric disorders.

The impact of a plasmalogen-deficiency in the modulation of the axon initial segment position

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The axon initial segment (AIS) is a specialized region within axons that contains an enrichment in ion channels and an intricate cytoskeletal organization. These features provide the optimal conditions for the generation of the action potentials and the selective filtering of axonal components. Neurons are known for their ability to modulate the AIS's position and length to accommodate the fine-tuning of their excitability. However, the complexity of these systems and cellular processes hinders identifying the molecular and/or cellular determinants. Studies on rare monogenic disorders have been of added value since they provide models for identifying key molecules in specific cellular processes. Our past and recent research on Rhizomelic Chondrodysplasia Punctata (RCDP) highlights that acquiring insight on the pathology and disease mechanism has a great impact and significance on understanding key cellular processes. RCDP is caused by a defect in the biosynthesis of plasmalogens (PL), a class of ether-phospholipids highly enriched in nervous tissue. From the different features of RCDP presentation, the neurologic presentation, including seizures, epilepsy, and autistic-like presentation, is extremely relevant but often neglected.

Using the Gnpat knockout (KO) mouse as a mouse model for RCDP, we set out to investigate the relevance of plasmalogen defects in the AIS assembly and function. Our results show that plasmalogens are not essential for the formation or composition of the AIS in a wide variety of neurons. However, we unraveled that a deficiency in plasmalogens affects the positioning of the AIS. Under basal culture conditions, neurons from Gnpat KO neurons had their AIS located at more distal positions from the cell body. To characterize the mechanism behind the observed changes, we discovered that the repositioning of the AIS caused by the lack of plasmalogens is not mediated by dysregulation of L-type voltage-gated calcium channels activation. Instead, we unraveled that the positioning of the AIS is regulated by a signaling cascade involving AKT. Our results contribute to the understanding of mechanisms by which neurons regulate changes in their excitability and provide important aspects of neuron function in RCDP.

Unawareness and Volition in Alcohol Consumption: A Study using Functional Magnetic Resonance Imaging

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According to the incentive salience theory (Robinson & Berridge, 1993), consumption is guided by the initial hedonic effects of the substance of abuse (“liking“). During the course of the development of dependence the increased salience (“wanting”) becomes the primary motivation for substance consumption via a sensitization of the mesolimbic system. Everitt and Robbins (2016) have expanded the incentive sensitization theory by the importance of habitual acts and a state of “must do!“. According to this model, which was mainly derived from preclinical studies and for cocaine, habitual consumption and automatisms can play a prominent role in later stages of dependence. In the present study we examined the association between cue-induced brain activation and automated alcohol consumption to (a) further validate this model for alcohol addiction, (b) to study the role of unawareness and volition in alcohol dependence, (c) to examine the neural basis of motivational versus motor habits in maladaptive alcohol consumption, and finally (d) to validate our questionnaire for automated craving (Vollstaedt-Klein et al., 2015) on a neural basis.

Poster Topic

T14: Vision: Invertebrates

T14-1 Influence of idiothetic cues on sun compass neurons in monarch butterflies
Christian Michael Kraus, M. Jerome Beetz, Basil el Jundi

T14-2 The organization of the central and lateral complex in the brain of the cockroach *Rhyparobia maderae*
Vanessa Althaus, Stefanie Jahn, Uwe Homberg

T14-3 Visually-guided proboscis control in the hummingbird hawkmoth
Anna Stöckl, Christian Drerup, Nadine Kraft, Alexa Haan

T14-4 3D-Reconstruction of a hummingbird hawkmoth lamina cartridge at electron microscopic resolution
Ronja Bigge, Kentaro Arikawa, Anna Stöckl

T14-5 Sex- and caste-specific brain structures in desert ants (Hymenoptera: Formicidae)
Pauline Nikola Fleischmann, Robin Grob, Niklas Heinig, Wolfgang Rössler

T14-6 Dynamic properties of directional coding in compass neurons of the bumblebee
Lisa Rother, Keram Pfeiffer

T14-7 Into the light: the orientation strategies of beetles faced with light pollution
James Foster, Marie Dacke

T14-8 The spatial frequency tuning of translational optic-flow responses in hawkmoths of varying body size
Rebecca Grittner, Keram Pfeiffer, Anna Stöckl

T14-9 A combination of skylight cues is required to set the migratory direction in monarch butterflies
Myriam Franzke, Tu Anh Thi Nguyen, Christine Merlin, Basil el Jundi

T14-10 The visual system of locusts as an inspiration for an anti-collision sensor for drones
Konstantinos Kostarakos, Jan Wille, Manfred Hartbauer

T14-11 Sun compass neurons are tuned to migratory orientation in monarch butterflies
Tu Anh Thi Nguyen, M. Jerome Beetz, Christine Merlin, Basil Jundi

T14-12 Activity of compass neurons in the brain of the desert locust exposed to the full natural sky

Erich, M. Staudacher, Keram Pfeiffer, Uwe Homberg

Influence of idiothetic cues on sun compass neurons in monarch butterflies

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Each fall millions of monarch butterflies (Danaus plexippus) migrate thousands of kilometers from North America southwards to their overwintering habitat in Central Mexico. To maintain their migratory direction over this enormous distance, these butterflies rely on a sun compass for orientation [Mouritsen and Frost, PNAS (2002)]. This information is processed in the central complex, an insect brain region that is innervated by sun-compass neurons that encode the position of a simulated sun (Nguyen et al., submitted) and acts as internal compass [Heinze and Reppert, Neuron (2011)]. Traditionally, electrophysiological recordings in monarchs have been performed in restrained animals. However, there is a growing amount of evidence from Drosophila that neuronal activity is modulated by the animal's behavioral state [e.g. quiescent vs. flight; Weir and Dickinson, PNAS (2015)]. Our recent data in tethered flying monarch butterflies have shown that the receptive fields of some sun-compass neurons shift dramatically when the animal is allowed to set its desired heading (Beetz et al., in preparation). We investigated whether idiothetic cues, derived from angular movements, underly these tuning shifts. Therefore, we restrained monarch butterflies on a rotation stage and recorded the responses of central-complex neurons to a sun stimulus and a passive rotation in darkness using tetrode recordings. We found that sun-compass neurons encoded the animal's heading in darkness. Thus, compass neurons integrate both, allothetic (e.g. sun position) and idiothetic information in order to encode the animal's heading, reliably. To understand this integration, we designed an experiment in which we set allothetic and idiothetic cues in conflict while recording from sun-compass neurons in butterflies that were either rotated on the stage or that could freely rotate around the sun stimulus. We set the conflict by displacing the sun stimulus by 180°. If receptive fields shift by 180° after sun displacement, then sun-compass neurons mostly rely on allothetic cues while neurons that do not shift their receptive fields mostly rely on idiothetic cues. Our conflict experiments revealed that allothetic cues mainly influence the receptive field in restrained butterflies, while idiothetic cues dominate the compass in flying animals.

The organization of the central and lateral complex in the brain of the cockroach *Rhyparobia maderae*

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Many insects use the position of the sun or the polarization pattern of the sky to navigate in diverse environments. The central complex (CX), a group of neuropils in the center of the insect brain, plays a key role in these navigational tasks. The CX consists of the protocerebral bridge (PB) and the upper (CBU) and lower division of the central body (CBL), each consisting of arrays of 16-18 columns, and a pair of globular-shaped noduli. The most prominent input and output area of the CX is the lateral complex (LX). These structures have been analyzed in several insect species including a locust, bees, beetles, the fruit fly, and the monarch butterfly (Honkanen et al 2019, J Exp Biol 222:jeb188854).

Three major categories of CX neurons can be distinguished: tangential, columnar and pontine neurons. Tangential neurons are the main input elements of the CX targeting particular layers across most or all columns. Columnar neurons connect individual columns of different neuropils of the CX in a topographic manner, and pontine neurons, present only in the CBU, connect columns across the brain midline (Pfeiffer and Homberg 2014, Annu Rev Entomol 59:165).

To investigate the organization of the CX in an insect that, in addition to visual cues, strongly relies on antennal information for spatial orientation, we investigated the structure of the CX in the nocturnal cockroach *Rhyparobia maderae* at the level of individual dye-filled neurons that were reconstructed in two or three dimension. In addition, subcompartments of CX neuropiles were reconstructed through immunostainings and arborization patterns of CX neurons.

The neuronal organization of the CX in *R. maderae* differs in several aspects from that in other well-studied insects. Most strikingly, the CBL consists of intercalated systems of 8 cones and 9 wedges, which is not known from other insects. The anterior lip, a brain area closely associated with the CX, is particularly large in the cockroach, considerably smaller in the locust, and completely absent in flies. The LX of the cockroach is highly compartmentalized. In addition to a large bulb, housing the input terminals of tangential neurons to the CBL, small distinct bulb-like regions receive axonal terminals of columnar neurons from the CBL and CBU.

To date, seven different types of tangential neurons of the CBL (TL neurons) could be distinguished. They differ considerably from TL neurons of other insect species both in dendritic arborizations in the bulb and in their innervation of the CBL. Two major categories of TL neurons, each with several subtypes, could be distinguished that either innervate the wedges or the cones. Four different classes of columnar neurons were found, again composed of several subtypes. Several types of columnar neurons of the CBU and PB show small arborizations in ventral parts of the CBL, which was never seen in other insects. Taken together the anatomical organization of the CX in the Madeira cockroach shows several specialties not found in other insects. Physiological studies planned in the future will reveal whether these features correspond to changes in sensory input related to the nocturnal lifestyle of the animal.

Visually-guided proboscis control in the hummingbird hawkmoth

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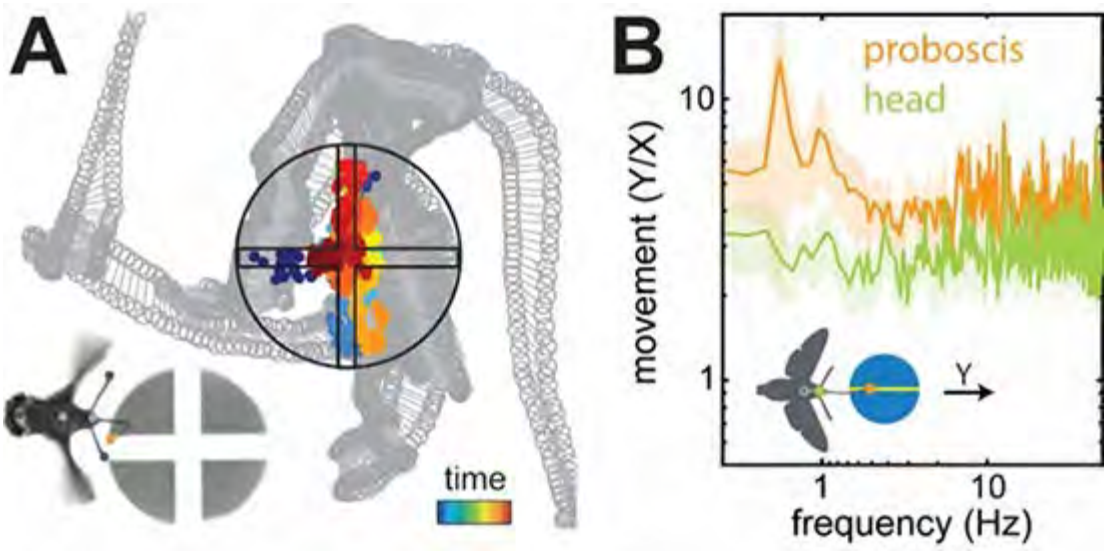
Visually-guided reaching is a central feature of mammalian behaviour. However, in insects, limb movements often follow a stereotyped motor programme, and only a few instances of visual appendage guidance are known. Forelimb reaching movements during crossing gap-crossing in horse-headed grasshoppers and Drosophila are one example. Here we provide the first evidence for visually guided movements of a different appendage: the targeted movements of the hummingbird hawkmoth's (*M. stellatarum*) proboscis on artificial flower patterns. While merely pleasant to us, flower patterns have been shown to provide important signals to insects. Also known as "nectar guides", they improve the efficiency of nectar foraging in a diverse range of pollinating insects.

To investigate the cause for this increased efficiency, we filmed hawkmoths while approaching and searching for the nectaries of artificial flowers with prominent patterns. We demonstrate that the hawkmoths aligned their approach flight with the prominent pattern axes and probed the patterns with their proboscis in a targeted manner while hovering around the flowers. To dissect the underlying control strategies of this behaviour, we quantified the visual and motor precision of probing, by varying the width of the patterns. Furthermore, we dissected the different stages of probing, from approach flight, body alignment, first contact and continued scanning, to separate the contributions of flight control and active proboscis movements. Together, we provide a comprehensive model of visual probing via proboscis and flight movements in hawkmoths.



3D-Reconstruction of a hummingbird hawkmoth lamina cartridge at electron microscopic resolution

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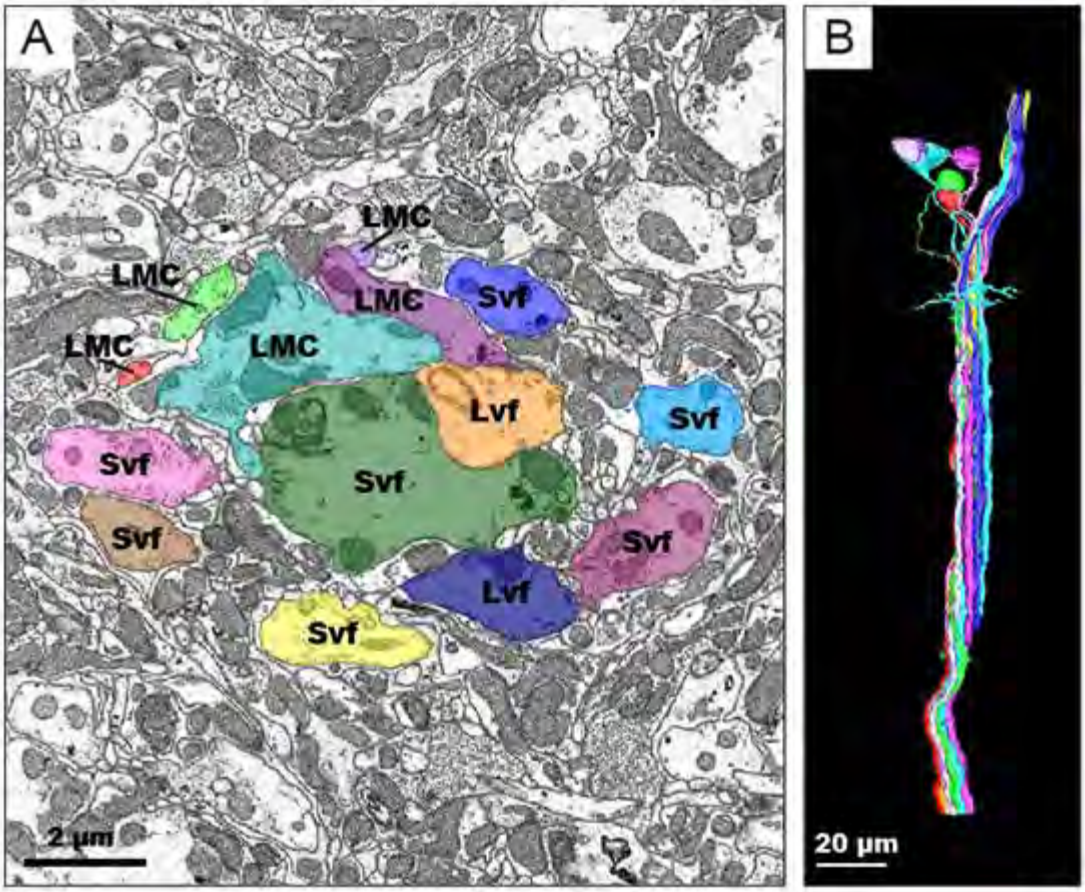
Many animals strongly rely on their visual sense, as it provides information about the natural world with particularly high dimensionality. But this complex sensory input also increases the need to filter and streamline the visual signals. Especially animals with limited neural capacities, like insects, rely on such mechanisms to extract relevant features out of the breadth of information. An important neuropil for such filtering in the insect visual system is the lamina. It is the first visual processing stage and receives information directly from the photoreceptors. Different contrast, luminance, color or temporal properties are transmitted downstream by the lamina's main relay neurons: lamina monopolar cells (LMCs). Recently, we have shown that one type of LMCs performs spatial summation in the hummingbird hawkmoth *Macroglossum stellatarum*, by integrating visual information via its lateral dendrites that reach into neighboring visual processing units or cartridges.

To characterize the dendritic profiles and the potential for spatial integration of the other LMC types in the hummingbird hawkmoth, we used serial block-face scanning electron microscopy (SBF-SEM) to obtain a full 3D-scan of one lamina cartridge. We here present our first results from identifying and reconstructing the different cell types: surprisingly, our reconstructions yielded a different number of LMCs than previously described in Golgi stainings. We identified 5 LMCs, 7 short visual fibers (Svf) terminating in the lamina and 2 long visual fibers (Lvf), which extend their axons through the lamina into the medulla, together with the LMCs. Based on the anatomical locations of the neurons in the cartridge, and their lateral extensions, we suggest a preliminary classification of the different LMCs, based on homologies with other insect groups.


A
LMC
LMC
LMC
LMC
LMC
Svf
Lvf
Svf
Svf
Svf
Svf
Svf
Lvf
Svf
2 µm
B
20 µm

Sex- and caste-specific brain structures in desert ants (Hymenoptera: Formicidae)

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The eusocial insects' lifestyle is characterized by cooperative brood care, overlapping generations and division of labor. This brings along special challenges, e.g. individual workers have to forage to provide food for the colony that consists of their mother (queen), their sisters (infertile female workers) and the brood (queen's offspring of which workers and alates will eclose). Desert ants (*Cataglyphis nodus*) are prime examples of solitary central place foragers. First, the individual workers perform indoor duties within the dark nest for the first four weeks of their adult lives. Then they become foragers outside the nest and henceforth pursue extensive foraging trips while navigating mainly visually. The desert ants' main navigational strategy is path integration, i.e. they combine information about directions and distances travelled so that after finding a food item, they can return to the nest, the central place, in a straight line following their home vectors. In addition, the ants use any cue available for navigation, e.g. visual and olfactory landmarks, wind direction, ground structure and the geomagnetic field. In order to process, memorize, integrate, access and use these multimodal pieces of information, the ants need a powerful brain. Recently, the brain of *Cataglyphis* has been described in detail offering a standard brain atlas for desert ant workers (https://www.insectbraindb.org). Here, we aim to shed light on the differences in brain structures of different sexes and castes in *C. nodus*. In contrast to workers, *Cataglyphis* alates, i.e. winged female (queen) and male ants, do not spend long times outside the nest to forage. Instead, they have to disperse, mate, and – in the case of the queens – found a new colony. Our findings show that there are substantial differences between female castes and males. In our analysis, we focus on the two main input regions, the optic lobes receiving visual input and the antennal lobes receiving olfactory input. Males have the largest optic lobes when compared to the female castes (queens and workers). Furthermore, only the antennal lobes of males contain macroglomeruli. In the central brain, we measured the volumes of the mushroom bodies, centers for sensory integration, learning and memory, and the central complex that is important for spatial orientation and high-order motor control. Here, males possess both smaller mushroom bodies and central complexes. These differences likely reflect adaptations to sex and caste-specific behavioral challenges executed by *Cataglyphis*' workers, queens and males. Desert ants are recognized experimental models to study insect navigation. However, as eusocial insects, they offer also the opportunity to analyze the neuronal basis underlying different behavioral repertoires across sexes and castes.

This study was supported by the German Research Foundation (projects: FL1060/1-1 to PNF and RO1177/7-1 to WR) and the Klaus Tschira Foundation gGmbH (GSO/KT 16 to PNF).

Dynamic properties of directional coding in compass neurons of the bumblebee

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Bees show remarkable orientation abilities when searching for food or homing after a foraging trip, using celestial cues like the position of the sun or the polarization pattern of the sky. These cues are processed in the sky-compass pathway, which receives light information from the compound eye and relays it to neurons in the medulla, the anterior optic tubercle and eventually to the core compass network in the central complex.
The neural tuning of compass neurons has traditionally been characterized with respect to simulated skylight cues that rotate at a slow, constant velocity. However, nothing is known about their dynamic properties and their coding properties in response to naturalistic stimulus dynamics as they occur during flight. Freely flying bumblebees usually make rapid saccadic turns interspersed with segments of translational flight (Boedekker et al., 2015). During saccades, they rotate around their yaw axis at angular velocities of up to 2000 °/s, which leads to a highly dynamic input into the visual system, with spatiotemporal properties that differ fundamentally from those of the sky-compass stimuli used in previous studies. Here we asked how the preferred angle of polarization changes at different rotation velocities.
We recorded intracellularly from compass neurons in the central complex of bumblebees during stimulation with a rotatable polarizer backlit by an ultraviolet LED (365 nm). The polarizer was rotated continuously at discrete velocities between 30°/s to 1920 °/s either clockwise or counter-clockwise. We found neuronal responses that were significantly phase locked to the stimuli, at all velocities, even though, at the highest velocities, not every individual revolution of the polarizer lead to action potentials. The preferred angle of polarization, i.e. the phase of the response, was strongly dependent on both rotation direction and stimulus velocity. With increasing rotation velocity, the activity peak occurred with increasing angular phase-delay. Since the phase-delay angle scaled linearly with rotation velocity it is most likely a consequence of a constant time-delay in the photoreceptors and the synaptic transmission. A different effect was observed at low rotation velocities. Here, the neurons activity was phase-advanced, i.e. the responses showed a negative time-delay, which was more pronounced, the slower the stimulus rotated. This effect at low rotation velocities cannot be readily explained, mechanistically. However, it is possible that photoreceptor adaptation and intrinsic properties of the neurons and the network play a role in this process. Future experiments will be done to investigate the mechanisms underlying phase advances in these neurons.

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Into the light: the orientation strategies of beetles faced with light pollution

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As cities grow, nocturnal animals are exposed to light pollution both directly, from streetlights and illuminated buildings, and indirectly, in the form of skyglow. The mechanisms nocturnal animals use for orientation may prove increasingly important to their continued survival. Here we present the results of behavioural experiments at light-polluted and dark-sky sites, paired with photographic measurements of the visual environment at each. While our measurements showed that vital natural cues were obscured and degraded by skyglow, we observed a change in behaviour that allowed nocturnal dung beetles to remain oriented at the light-polluted site.

We investigated orientation in the ball-rolling African dung beetle *Scarabaeus satyrus*. This nocturnal species performs a well-described orientation behaviour (Dacke *et al*., 2011, 2013; el Jundi *et al*., 2015; Smolka *et al*., 2016; Foster *et al*., 2017), typically relying on the lunar polarization pattern (Dacke *et al*., 2011; el Jundi *et al*., 2015; Smolka *et al*., 2016) and the Milky Way (Dacke *et al*., 2011, 2013; Smolka *et al*., 2016) to orient itself when rolling its dung ball away from the dung pile. We now present data showing that these beetles remained well oriented under light-polluted skies, when both the Milky Way and the lunar polarization pattern are obscured by skyglow. We further demonstrated that these beetles were unable to orient using the sky when terrestrial cues are blocked from view, suggesting that they relied on terrestrial cues amplified by direct light pollution. We propose that this change in orientation behaviour would have population-level consequences for fitness that may extend to other nocturnal species (Foster *et al*., 2018), and should inform future efforts at mitigating light pollution.

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Foster JJ, el Jundi B, Smolka J, Khaldy L, Nilsson D-E, Byrne MJ & Dacke M (2017) *Phil. Trans. R. Soc. B* 372, 20160079.
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Smolka J, Baird E, el Jundi B, Reber T, Byrne MJ & Dacke M (2016) *Anim. Behav.* 111, 127–146.


No Light Pollution
Direct & Indirect Pollution
Only Indirect Light Pollution
Mean vector
Bootstrapped confidence interval
Critical vector length (p<0.05)

The spatial frequency tuning of translational optic-flow responses in hawkmoths of varying body size

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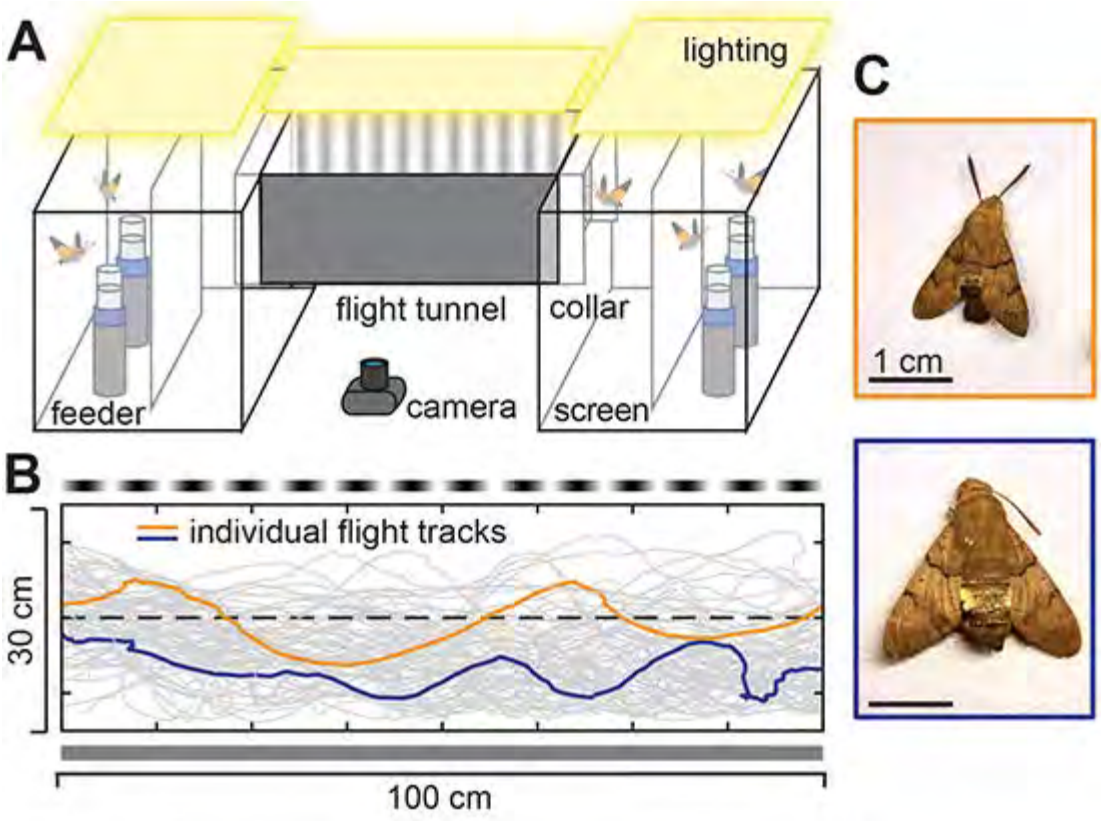
Many animals use visual information to control their flight, for example for feedback on changes of their body position and to judge their distance to nearby objects. To provide this information, insects in particular rely on the apparent image motion, or optic flow, which is generated as they move through the air. However, the perception of optic flow strongly depends on the spatial structure of the environment, and on the spatial acuity of the insect's visual system. To understand how the spatial acuity of its visual system shapes flight control in the hummingbird hawkmoth *Macroglossum stellatarum*, we tested the spatial tuning of flight control based on translational optic flow with a flight tunnel setup. Since the spatial acuity of the eye and the behavioural spatial resolution scale with body size in some insects with apposition compound eyes, we further investigated if the spatial limits of flight control in hawkmoths, which possess superposition compound eyes, showed a similar relationship to body size. While we observed distinct individual differences in flight behavior, there was no correlation between body size and spatial acuity. Interestingly, we found different cut-offs in spatial frequency with two behavioural setups in which the animals flew at different speeds, suggesting that the limits of visual resolution observed in these experiments were introduced by the temporal resolution limits of the hawkmoth's system, rather than their spatial acuity.



A combination of skylight cues is required to set the migratory direction in monarch butterflies

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Each fall millions of monarch butterflies (*Danaus plexippus*) migrate over thousands of kilometers from North America to their overwintering habitat in Central Mexico. To maintain their southerly direction over this enormous distance, these butterflies rely on celestial cues, such as the sun and polarized light, as orientation references [Mouritsen and Frost, PNAS (2002); Reppert et al., Curr Biol (2004); Stalleicken et al., JEB (2005)]. But which of these cues are essential to set and maintain their southerly direction? To investigate this, we behaviorally tested the migratory compass in wild-caught monarch butterflies while they were tethered at the center of a flight simulator and were able to freely change their bearing with respect to a visual scene. We first studied the butterflies in a flight simulator outdoors and found they kept their constant southward directions under the natural sky. When we blocked the sun from the butterflies' view and displaced it by 180° using a mirror, the animals changed their migratory direction accordingly, suggesting that the sun acts as their main migratory cue. We repeated the experiments but, this time, set a green light spot 180° to the shaded, real sun. Again, the animals turned their migratory headings by 180°, showing that they interpret the green light spot as the sun. We then performed indoor experiments and presented the green light spot, representing a mimicked sun, as the only source of orientation reference to the butterflies. Interestingly, instead of heading in the correct migratory direction, the animals exhibited constant headings in arbitrary directions which indicates that the sun is not a sufficient reference to set the migratory direction in the butterflies. When we presented the mimicked sun in combination with a simulated polarized skylight to the butterflies, the animals kept constant flight directions that accurately matched the migratory directions in nature. These findings demonstrate that the sun and additional skylight cues are essential to guide the monarch butterflies along the correct migratory direction during their long journey to Central Mexico.

The visual system of locusts as an inspiration for an anti-collision sensor for drones

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Since decades, locusts provide a model system for studying neural mechanisms enabling collision detection in a swarm. Individuals selectively respond to objects on a collision course by jumping or evasive flight maneuvers. The neuronal substrate for collision detection in locusts is based on a lateral inhibition network that results in a selective response for objects on a collision course. The sum of local excitation and lateral inhibition is forwarded to collision detector neurons (LGMDs). The image cues leading to a strong LGMD response are an increase in the velocity of edge motion and an increase in the amount of edge. Each LGMD is connected with a descending contralateral movement detector (DCMD). This neuronal network selectively responds to objects on a collision course and has inspired the development of various technical approaches of anti-collision systems. So far, however, no application of this bionic collision detection system is currently used in transport systems and drones. In the latter, efficient collision detectors with low energy demands are not yet established and the ones used often restrict flight operations to less than 25 minutes. In a novel drone project, we aim to develop a light-weight bionic sensor system for the reliable detection of impending collisions. For this purpose, we recorded movies with two cameras mounted on a drone that is operated in close proximity to various objects. We presented these movies to insect preparations from each side, while we recorded the response of both DCMD-neurons by using a pair of hook electrodes. Various collision scenarios were presented on two curved monitors covering a field of view of about 180 degrees. Based on the activity of DCMD neurons, we developed a novel bionic algorithm for collision detection that extracts the information about impending collisions from the visual scene. It also calculates a possible evasive vector based on the differential excitation of certain image regions. In a current FFG funded project, we aim to optimize this bionic algorithm and implement it into the hardware of a bionic anti-collision sensor for real time collision risk estimation. The hardware consists of two miniature cameras and an FPGA chip that is connected with the flight controller to enable avoidance responses based on the calculated collision risk.

Sun compass neurons are tuned to migratory orientation in monarch butterflies

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Every autumn, monarch butterflies *(Danaus plexippus)* migrate from North America to their overwintering sites in Central Mexico. To maintain their southward direction, these butterflies rely on celestial cues as orientation references. The position of the sun combined with additional skylight cues are integrated in the central complex, a region in the butterfly's brain that acts as an internal compass. However, the central complex does not solely guide the butterflies on their migration but helps monarchs in their non-migratory form manoeuvre on foraging trips through their habitat. By comparing the activity of input neurons of the central complex between migratory and non-migratory butterflies, we investigated how a different lifestyle affects the coding of orientation information in the brain. During recording, we presented the animals with different simulated celestial cues and found that the encoding of the sun was narrower in migratory compared to non-migratory butterflies. This feature might reflect the need of the migratory monarchs to rely on a precise sun compass to keep their direction during their journey. Taken together, our study sheds light on the neural coding of celestial cues and provides insights into how a compass is adapted in migratory animals to successfully steer them to their destination.

Activity of compass neurons in the brain of the desert locust exposed to the full natural sky

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Honeybees, desert ants, and many other insects including locusts can perceive the sky polarization pattern and are able to use it for navigation. In the laboratory, stationary flying desert locusts (*Schistocerca gregaria*) show polarotaxis when exposed to a rotating polarization filter above the animal (Mappes and Homberg 2004, J Comp Physiol A 190:61). Intracellular recordings suggest that the central complex in the locust brain serves as an internal compass based on sky polarization and solar azimuth (Zittrell et al. 2020, PNAS 117:25810). Here, we compared the responses of extracellularly recorded polarization sensitive units in the central brain of the locust under laboratory and full natural sky conditions.

The experiments were performed in a hut on the observation platform of the biology building, which provided an unobstructed view of the sky. Two hatches in the roof were closed during the laboratory part, but opened for the natural sky part of the experiments.
Animals were positioned on top of a rotational platform so that their head was directly above the center of rotation. Extracellular multi-unit recordings were made from polarization-sensitive units in the area of the central complex using custom built wire tetrodes. The laboratory experiments performed inside the dark hut consisted of two parts. First, a polarizer below a blue LED, positioned in the zenith above the stationary animal, was rotated (30°/s). Subsequently, the animal was rotated while the polarizer remained stationary. After recording responses of a unit inside the hut, the hatches were opened, the rotational platform with the animal was positioned above the roof and the animal was rotated under the full sky. Polarimetry, spectroscopy and imaging of the sky was done between rotations of the animal.

Units were recorded for one to three hours, tested with one to three repeats of the experimental sequence. All units had axial preference angles to the polarized light stimulus when tested under laboratory conditions, which has been described for identified neurons in the central complex. Axial preference angles were stable across repeated stimulation sequences. Most importantly, axial preference angles were the same whether polarizer or animal were rotated. In contrast, when rotated under the full sky, the representations of most units changed to circular preference angles about 90° relative to their axial preference angles. The results show that under the conditions of the natural daylight sky these units compute sky compass signals in ways enabling unambiguous angular coding relative to solar azimuth. Future experiments will investigate, whether direct sunlight and/or matched-filter coding of sky polarization leads to unambiguous sun-compass coding in these units.

Poster Topic

T15: Vision: Retina and Subcortical Pathways

Encoding of natural visual stimuli in the mouse superior colliculus

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The primary visual cortex (V1) and the superior colliculus (SC) are two brain regions central for visual processing in mammals. It has long been known that natural vision is encoded in a sparse manner [1] in populations of V1 neurons [2,3]. It is thought that this sparse cortical code provides a computational benefit that facilitates readout of natural scenes [2] and that inhibitory neurons play a key role in establishing this sparse representation [3,4]. Like V1, the SC circuitry contains around 20-30% of inhibitory neurons [5] and thus, potentially, natural visual stimuli could also be encoded in a sparse manner in SC. However, it is largely unknown how natural scenes are encoded on the population level in SC and therefore it is unclear whether the sparse code during natural vision is solely a cortical phenomena.
To address this open question we recorded large populations of single neurons in the mouse SC using Neuropixel probes [6]. To maximize the number of simultaneously recorded SC neurons we inserted the Neuropixel probe tangentially along the anterior-posterior axis into the brain, targeting the superficial layers of the SC. Our approach allowed us to record from many single SC neurons simultaneously covering a large area of the visual field, both needed to address the neuronal encoding of natural visual stimuli in SC on the population level. Experiments were conducted on both anesthetized and awake animals. The natural movie was taken from a set of home cage movies recorded from mice with head-mounted cameras provided by and used in Froudarakis et al. [2]. To quantify the population dynamics of SC neurons we investigated the structure of firing patterns, discriminability, reliability and variability. The neural representation of natural scenes was studied using lifetime and population sparseness measures.
Our preliminary results indicate that some SC neurons respond vigorously to a large fraction of movie frames. However, the response of the major fraction of SC neurons is sparsely activated by the natural scenes. This suggests that SC circuits might encode natural stimuli in a sparse manner.

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Pupil size indexes modulation of firing mode in the mouse dLGN

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The way in which neural sensory systems process information changes as a function of the behavioral state of the animal (Busse et al., 2017; Harris & Thiele, 2011; Lee & Dan, 2012; McGinley et al., 2015a). In sensory cortical areas, behavioral states such as arousal, which has been related to pupil size, are expressed as modulations of spontaneous and stimulus-driven activity (McGinley et al., 2015b; Reimer et al., 2014; Vinck et al., 2015). Here, we asked whether spiking activity in the dorsolateral geniculate nucleus (dLGN), an area that provides sensory input to the visual cortex, is also modulated by pupil-indexed arousal. We recorded extracellular neural activity in the dLGN of head-fixed mice while simultaneously filming the eye in order to extract pupil size. The resultant pupil size signal was made up of components oscillating over multiple time-scales. We used the Hilbert-Huang transform (Huang et al., 1998) to empirically decompose the pupil size signal and characterize its constituting components, finding that a high proportion of the power was carried by components ranging from 0.001 to 1~Hz. Next, we performed a phase tuning analysis in order to relate these component oscillations to the two types of arousal-related spiking modes in the dLGN (Sherman, 2001). We found burst and tonic spikes to preferentially occur during opposing phases of pupil size oscillations. This phase tuning was observed for multiple oscillatory components over a range of frequencies, and was stronger for bursts over this entire range. We also found that this activity modulation was not driven by changes in absolute pupil size or by transitions between periods where the animal was stationary or moving. Lastly, we found that dLGN activity was similarly modulated even when the animal was engaged in viewing naturalistic stimuli. We therefore conclude that firing patterns in the dLGN are modulated by pupil-indexed arousal over a range of time-scales, which could significantly influence the way in which sensory signals are passed on to the cortex.

Remodeling of the extracellular matrix in a novel intraocular pressure-dependent glaucoma mouse model

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Purpose

Glaucoma disease is characterized by the degeneration of optic nerve fibers and the death of retinal ganglion cells (RGCs). An increase in the intraocular pressure (IOP) is a major risk factor for glaucoma. However, the underlying molecular mechanisms of this disease are not yet fully understood. Mice heterozygous (HET) for the protein tyrosine phosphatase Meg2 (PTP-Meg2) develop progressive age-related IOP elevation and a loss of RGCs associated with reactive gliosis (Reinhard et al., 2019). Therefore, HET mice represent a valuable model for IOP-dependent glaucoma. Previous studies suggest that glaucomatous neurodegeneration is associated with alterations of the extracellular matrix (ECM) (Reinhard et al., 2017). Thus, the goal of the present study was to characterize the expression pattern of various ECM glycoproteins and proteoglycans in the retina and optic nerve of wild type (WT) and glaucomatous HET animals.

Methods

Protein levels of ECM components were analyzed in the retina and optic nerve via immunohistochemistry and Western blot (n=4-5/group). Groups were statistically compared via Students *t*-test (Statistica). Additionally, the mRNA expression was investigated by quantitative real-time PCR (RTq-PCR; n=5/group). RTq-PCR data were analyzed by a pairwise fixed reallocation and randomization test (REST software).

Results

Immunoreactivity of fibronectin ($p<0.05$) and laminin ($p<0.05$) was significantly increased in the retina and optic nerve of the HET compared to the WT group. RTq-PCR analyses of the different laminin isoforms *α4*, *β2*, *γ3* revealed an altered isoform-specific regulation in the retina ($p<0.01$) and optic nerve ($p<0.05$). Moreover, enhanced levels of tenascin-C ($p<0.001$) and its interaction partner RPTPβ/ζ/phosphacan ($p<0.05$) were observed in the HET group. However, the protein and mRNA levels of the glycoprotein tenascin-R and the proteoglycans aggrecan and brevican were comparable in both genotypes.

Conclusions

In summary, we showed a dramatic accumulation of ECM components after progressive age-related IOP elevation. The dysregulation of the blood vessel-associated glycoproteins fibronectin and laminin indicates neovascularization during glaucomatous damage. Increased levels of tenascin-C and its interaction partner phosphacan may be directly related to reactive gliosis in PTP-Meg2 HET mice. Collectively, our results suggest that remodeling of the ECM may have an influence on blood vessel formation, inflammation and glial activity during glaucomatous neurodegeneration.

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Functional diversity of visual encoding in the mouse superior colliculus

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Mouse vision is encoded already in the retina by a variety of retinal ganglion cells (RGC), estimated to form 32 different parallel streams of information (Baden et al. 2016). A recent study in the LGN reports both that 24 of these RGCs project axons in the LGN and that the LGN neuronal firing relays such diversity in a combination of 5 different inputs with 2 dominant ones (Rosón et al. 2019). To which extent the variety of these streams is also present in the superior colliculus (SC), the other main RGC's axonal recipient, is unknown. Interestingly, the mouse SC receives up to 88% of RGC's axons, while only ~30% of them project to the lateral geniculate nucleus (LGN) (Ellis et al. 2016) making the SC a better candidate to relay the 32 RGC's inputs. Using tangential Neuropixel implantation (Jun et al. 2017), we recorded several hundreds of single-units (1706) from the upper SC visual layers allowing us to characterize the functional diversity of neuronal responses. To do so, we used a combination of responses to sparse noise, moving bar and chirp, that were clustered with unsupervised Gaussian-Mixture-Model in different classes using Uniform-Manifold Approximation and Projections (UMAP) dimensionality reduction (McInnes Melville 2018). The majority of the obtained neuronal classes exhibited clear features of ON, OFF, and ON-OFF responses with both transient and sustained firing. In these classes, we observed more numerous ON-OFF-transient responses compared to the canonical 32 classes (Baden et al. 2016), together with a lower class number suggesting some level of convergence as reported in the LGN.

Poster Topic

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

T16-1 Superior colliculus and cortical circuits involved in motor preparation and navigation
Tatiana Lupashina, Ronny Bergmann, Keisuke Sehara, Sina Dominiak, Matthew Larkum, Robert Sachdev, Jens Kremkow

T16-2 Open source tool for tracking running wheel activity of group housed mice allows for individually correlated plasticity measures
Cornelia Schöne, Mihaela Guranda, Siegrid Löwel

T16-3 New perceptual channels employed for increased input variability facilitate feature discrimination in mice.
Elisabeta Balla, Christopher Wiesbrock, Jenice Linde, Björn M. Kampa

Superior colliculus and cortical circuits involved in motor preparation and navigation

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Navigation requires motor planning and the coordination between multiple sensory-motor modalities. For example, when we walk, the stepping motion is coordinated with motion of the torso, arms, head, and eyes. In our earlier work, we showed that in head-fixed mice navigating through a virtual world (a plus-maze on an Airtrack system) whisker asymmetry reflected the animal's motor plan and behavioral state (Dominiak et al, 2019). Subsequently, we have shown that during this behavior, mice also move their eyes in the direction of their future chosen turn (Bergmann et al, 2019). Here we begin to test the hypothesis that frontal-cortico-collicular circuits are causal in the expression of certain behaviors: moving forwards, moving backwards, generating whisker asymmetry, and moving the eyes. Several candidate circuits guiding this behavior, and potentially linked to each other, are in the frontal cortex (including ALM, M2, M1) and superior colliculus (SC). We targeted and recorded neuronal activity from across SC layers and in frontal cortical areas with the use of Neuropixel probes which can record simultaneously from a large population of neurons in several brain areas of an awake animal (Steinmetz et al, 2019). In the analysis of these recordings, we observed a correlation between backward and forward movement of mice in the maze and neuronal activity in both M2 and SC. We are currently assessing whether activity in the superior colliculus or cortex is associated with motor preparation or is causal in generating the entire sequence of movements associated with navigation.

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Open source tool for tracking running wheel activity of group housed mice allows for individually correlated plasticity measures

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Environmental enrichment can boost neuronal plasticity in the brain of standard-cage raised (SC) rodents (Löwel et al 2017). In the visual cortex of adult SC-mice, (>postnatal day 110) ocular dominance plasticity (ODP) induced by short-term monocular deprivation (MD) requires environmental enrichment: one week of running wheel exposure during MD is sufficient to restore ODP in adult mice (Kalogeraki et al 2014). It remains unclear which properties of running wheel enrichment promote ODP.
In order to investigate the exact relationship between running wheel activity and ODP, we induced MD in adult SC-mice, transferred them to a novel running wheel cage on the same or preceding day and tracked their running wheel activity for one week after which we performed intrinsic signal optical imaging to measure their ocular dominance.
We used a novel custom built experimental setup that allows us to track running wheel activity of individual mice in a home cage setting. Two to six female mice were housed in a standard rat cage (Dimensions: 20 cm x 25 cm) and quickly learned to enter a separate compartment containing a running wheel: they had to navigate a seesaw that would automatically block the entrance for other mice. Mice were chipped with radio frequency identification devices (RFID), which allowed them to track their interactions with the running wheel. Seesaw position, running wheel turns and RFIDs codes were registered using a raspberry pi 4 and custom written python scripts. Average number of running wheel rotations per mouse/day in this setup was consistent with previously published data (Kalogeraki et al., 2014). Using this experimental design allowed us to correlate the macro and micro architecture of running wheel activity of individual mice with their ODP. Notably, our preliminary data suggest a striking correlation between individual running wheel activity and ODP after MD that was absent in no MD mice.
Locomotor activity in mice is correlated with high gamma activity and increased arousal - brain states that have been associated with learning and plasticity and that may be the underlying substrates for promoting ODP. Future studies should look at the correlation between locomotor activity, high-gamma arousal states and ODP.

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New perceptual channels employed for increased input variability facilitate feature discrimination in mice.

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Nature challenges our visual system with a vast variability of feature distributions which simplistic visual stimuli such as square-wave gratings cannot account for. A consensus between the parametric tailoring of these well-defined laboratory stimuli and a highly variable environment, can be found in the Motion Cloud stimuli (Leon et al. 2012). Previous use thereof has asserted a negative correlation between increased spatial frequency bandwidth and perceptual speed discrimination in humans (Simoncini et al. 2012). Their proposed model places a divisive gain normalization mechanism at the center of this computation, whereby an increase in feature variability is processed by distributing the gained sensory input to newly engaged perceptual channels, thus reducing overall amplitudes. The aim of our study was to see whether this behaviorally translates to a rodent model and to investigate the neuronal aspects of increased feature variability processing. For this purpose, we first tested the influence of orientation bandwidth enrichment on spatial frequency discrimination capability and the effect of spatial frequency bandwidth broadening on orientation discrimination in a mouse touchscreen chamber-based 2AFC task. Interestingly, a significant facilitation in spatial frequency perception resulted through orientation band broadening, whereas an influence of spatial frequency bandwidth on orientation perception could not be established. Next, we addressed this question at the level of a large population of individual neurons by collecting 2-photon data from mice which were expressing the green fluorescence indicator GCaMP6f in layer 2/3 of their primary visual cortex. The animals were passively shown pseudo-random sequences of varying orientation and spatial frequency bandwidths. In accordance with our behavioral results, orientation bandwidth enrichment resulted in significant increase in both number of employed cells as well as mean fluorescence amplitudes, whereas a defined neuronal effect could not be observed with the spatial frequency bandwidth increase. Implementation of a gain normalization mechanism could not be observed here. We concluded that employment of new perceptual channels for coping with the high variability in orientation components of the stimulus was beneficial to saliency thereof, thus enabling better discrimination of the coupled feature.

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Poster Topic

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

T17-1 Evidence for BK channel-dependent electrical tuning in the Drosophila hearing organ
Xiaowei Zhang, Philip Hehlert, Thomas Effertz, Narges Bodaghabadi, Radoslaw Katana, Martin C. Göpfert

T17-2 Testing the Ca^{2+}sensor hypothesis of otoferlin
Han Chen, Qinghua Fang, Nils Brose, Tobias Moser

T17-3 Adaptation of lateral line units in the MON of Carassius auratus to constant-amplitude sine wave stimuli
Sven Lange

T17-4 How electric fish escape the curse of the dipole world
Jan Benda

T17-5 Encoding stimuli of various frequencies in an electrosensory system: Effects of noise, firing rate and population size.
Dennis Huben, Yue Yu, Jan Benda

T17-6 A low-weight power-efficient sound processor driving optical cochlear implant in freely-moving rat
Lukasz Jablonski, Tamas Harczos, Bettina Wolf, Gerhard Hoch, Alexander Dieter, Roland Hessler, Suleman Ayub, Patrick Ruther, Tobias Moser

T17-7 Fast photoswitches for control of neuronal activity in the cochlea
Aida Garrido Charles, Antoine Huet, Carlo Matera, Anupriya Thirumalai, Amadeu Llebaria, Tobias Moser, Pau Gorostiza

T17-8 Receptive field size and stimulus bandwidth are limited by axonal conduction delays.
Jan Grewe, Tim Hladnik, Jan Benda

T17-9 Developing Fast, Red-light Optogenetic Stimulation of Spiral Ganglion Neurons for Future Optical Cochlear Implant.
Antoine Huet, Tobias Dombrowski, Anupriya Thirumalai, Vladan Rankovic, Tobias Moser

T17-10 The elusive meaning of the knifefish electric signals.
Livio Oboti, Marie Ritter, Marlena Lohse, Jan Grewe, Rüdiger Krahe

T17-11 Optimality of the vestibulo-ocular reflex
Stefan Glasauer, Hans Straka

T17-12 The Johnston's organ of desert ants and its central projections
Robin Grob, Clara Tritscher, Christian Stigloher, Claudia Groh, Pauline N. Fleischmann, Wolfgang Rössler

T17-13 Effects of age and antibiotic treatment on the survival of spiral ganglion cells in the rat cochlea.
Jessica Reinhardt, Heika Hildebrandt-Schönfeld, Nicole Rosskothen-Kuhl

T17-14 Age and noise interact to determine deafness in a locust model of hearing loss.
Ben Warren, Alix Blockley, Daisy Ogle, Charlie Woodrow, Fernando Montealegre-Z, Georgina Fenton

T17-15 Hearing in Insects: Towards discovering the Mechanotransduction Ion Channel.
Thomas Austin, Ben Warren

Evidence for BK channel-dependent electrical tuning in the Drosophila hearing organ

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Ears can be endowed with mechanical and electrical mechanisms of frequency tuning. An electrical tuning is known from auditory hair cells of turtles and frogs, which display electrical resonances arising from an interplay between voltage-gated Ca^{2+}-channels and Ca^{2+}-activated potassium (BK) channels. Combining RNAseq, targeted cell ablations, and expression analyses, we found that the BK channel slowpoke (slo) is expressed in the Drosophila ear, with the slo gene being expressed in around 60-70 of the fly's 480 auditory receptor neurons. Projection analysis revealed that slo-positive receptors belong partly to class A/B and partly to class C/E, and evidence for an electrical tuning was obtained when we measured receptor responses to antennal iso-displacements. Compound receptor responses displayed an electrical resonance that cannot be accounted for by mechanical frequency filtering. Mutant analysis showed that this electrical resonance partly depends on slo, suggesting that also in Drosophila ear, slo might contribute to electrical frequency tuning. RACE PCRs identified several splice variants of slo in the fly's ear, suggesting that alternative splicing of slo might affect auditory receptor tuning, possibly explaining why not all the receptors respond to the same frequencies, though they all experience the same mechanical frequency filtering.

Testing the Ca^{2+}sensor hypothesis of otoferlin

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The ribbon synapses between inner hair cells (IHCs) and spiral ganglion neurons (SGNs) are highly specialized to achieve indefatigable afferent transmission at rates of hundreds of Hertz and with submillisecond temporal precision. IHC ribbon synapses differ fundamentally from 'conventional synapses' of the central nervous system. The protein machinery mediating exocytosis at 'conventional synapses' is composed of neuronal SNARE proteins and SNARE regulatory proteins whereby Synaptotagmins work as Ca^{2+} sensors of exocytosis. However, mature IHCs lack synaptotagmin1/2, indicating an alternative Ca^{2+} sensor of exocytosis. Otoferlin, a protein and deafness gene product with six C_2 domains specifically expressed in IHCs, is the likely candidate for Ca^{2+} sensor in IHCs and we further tested this hypothesis.

Based on clinical findings and protein structure predictions, we generated three otoferlin mouse mutants with predicted alterations of Ca^{2+} binding to the C_2E and C_2F domains and analyzed them by whole cell patch-clamp recordings of IHC presynaptic function, immunohistochemistry and auditory brainstem response (ABR) recordings. In the C_2F-DDA mutant, two potential Ca^{2+} binding aspartates of the most C-terminal C_2F domain were substituted by alanines. According to immunohistochemistry results, otoferlin remained expressed in IHCs but was abnormally localized to the apical IHC part. ABR indicated that DDA mice are deaf. While the Ca^{2+} currents of IHCs were retained, exocytosis was abolished. In C_2E-TDA mutant, three potential Ca^{2+} binding aspartates at the C_2E domain were substituted by alanines. Immunohistochemistry showed that otoferlin was successfully expressed in IHCs of DDA mice with a normal subcellular distribution. Patch-clamp revealed normal Ca^{2+} currents but greatly reduced exocytosis, suggesting a strong connection between Ca^{2+} binding and exocytosis. Finally, OTOFIT mutants (Ile1573Thr) carry a pathogenic missense mutation near a C_2E top loop leading to progressive human hearing impairment. OTOFIT mice lacked detectable ABR and exocytosis was abolished in their IHCs despite normal Ca^{2+} current. Immunohistochemistry and real-time PCR revealed that otoferlin was dramatically decreased in both protein and RNA level in OTOFIT mouse IHCs.

The newly generated mutants show that the normal expression and function of otoferlin are indispensable for IHC exocytosis. The strong synaptopathy phenotype in the OTOFIT mutant modeling the human Ile1573Thr substitution seems to contrast the human phenotype and calls for further studies of this mutant e.g. using behavioral assays of hearing. Exocytosis was completely abolished also in C_2E-TDA mutants, despite apparently normal expression and distribution of otoferlin, supporting the notion of otoferlin serving as a Ca^{2+} sensor of exocytosis.

Adaptation of lateral line units in the MON of Carassius auratus to constant-amplitude sine wave stimuli

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Fish use the lateral system to detect water movements and pressure gradients. The lateral system of fish consists of superficial and canal neuromasts. Superficial neuromasts are distributed on the surface of the fish, whereas canal neuromasts can be found in subepidermal canals. Both types of neuromasts are connected via fibres of the lateral line nerves with the central nervous system. In the past, there have been many attempts to investigate the adaptation of primary fibres to constant sine-wave stimuli. Data of these studies have shown that primary afferent nerve fibers can adapt to constant-amplitude sinewave stimuli (Mogdans et al. 2017). Here we investigated the adaptation of lateral line neurons at the first site of central integration, the brainstem Medial Octavolateral Nucelus (MON). Specifically, we wanted to know how adaptation depends on the steepness of the rising flank of the stimulus. To do so, we recorded extracellularly the responses of MON units to 50 Hz sinewave stimuli (duration 3 s) with rising flanks of 20, 50, 100, 200, 500 and 1000 ms. Responses were quantified by the degree of spike rate reduction during the steady part of the stimulus. To verify the position of our electrode, we stained the sectioned fish brain with cresyl violett.

A total of 145 responses from 13 single-units were obtained. Units responded to the presented sinewave stimuli with increased spike rates. Degrees of adaptation ranged from 1.7% to 93.3%, i.e., from no adaptation to almost complete reduction in spike rate during stimulation. Mean degree of adaptation was 45.5±9.5%. Adaptation increased with increasing steepness of the rising flank of the stimulus. The degree of adaptation increased by 53.08 % when the rising flank was reduced from 1000 ms to 20 ms.

The results demonstrate, that adaptation of MON units to constant-amplitude sinewave stimuli is not different from adaptation among primary afferents. This means that integration of the stimuli occurs at higher parts of the brain. The time course of adaptation, i.e. the time course of the response decline, was best fit by a power function. This is consistent with previous experiments on spike frequency adaptation in sensory afferents of weakly electric fish. Our results showed that the steepness of the rising flank influences the adaptation behavior.

How electric fish escape the curse of the dipole world

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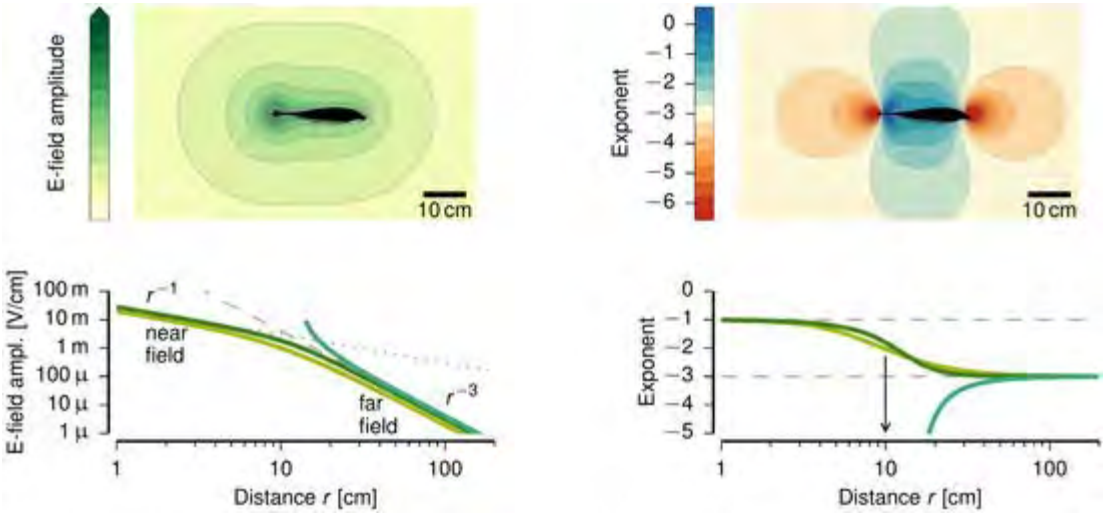
Gymnotiformes and mormyrid electric fish have evolved an electric organ whose discharges produce weak electric fields that they use for electrolocation and electrocommunication. Compared to visual and the auditory systems, electrolocation only works within about at maximum one body length distance --- apparently irrespective of the amplitude of the self-generated electric field. What limits electrolocation to one body-length distance? Let's compare the electrosensory system with the auditory one. Sound intensity emitted by a sender decays because of geometric spreading by distance squared. If a song bird wants to be heard at twice the distance it needs to raise its voice four-fold. And this is what they easily can do. Some alarm calls are loud and can be heard over much larger distances compared to other signals. This is different for electrocommunication. In the far field, the electric field generated by an electric fish is that of an ideal dipole whose electric field strength drops with distance cubed. A fish has to increase its field amplitude eight-fold to double the maximum distance for electrocommunication. For electrolocation the electric field polarizes an object, which in turn generates a dipole field that is sensed by electroreceptors in the skin of the fish. The amplitude of this electrolocation signal is the fish's electric field strength at the position of the object times the electric field strength of the induced field at the skin of the fish. In case of ideal dipoles for both the fish and the object this results in a devastating exponent of six. To double the range of object detection the electric field amplitude needs to be increased by a factor of $2^6=64$, almost two order of magnitudes! This is the curse of the dipole world. A substantial increase in signal amplitude translates into only minor gains in localization ranges. Because of fundamental physical laws there is no possibility of the fish to modify the dipole properties of the far electric field. But they can and did modify their near field. A low body resistance funnels the field along their body, resulting in collimated field lines perpendicular to the body surface. This reduces the dimensionality of the spherical symmetric field of an ideal dipole to a cylindrical symmetric field. As a result the exponent governing the power law decay of the electric field is significantly reduced, almost down to one. Consequently in the near field objects can be detected by generating appropriate electric field amplitudes. But beyond the near field at about one body length the electric field approaches that of an ideal dipole and it is therefore almost impossible to increase the detection range beyond this range. The other way out are large objects, in particular non-conducting boundaries like the water surface or big rocks. Their induced electric field can be computed by the method of image charges. That is the electric fish is mirrored on the other side of the boundary. This then resembles the same situation as for electrocommunication where the dipolar far field of a conspecific can be detected over a distance of one or two meters, since this signal only decays with distance cubed. Thus, non-conducting boundaries should be detectable up to a distance of one meter --- much more than the range of a few centimeters for electrolocation of small objects. And this range can be more easily influenced by modifying electric field amplitude. I suggest that electric fish use these signals for navigating in their limnic habitats. In summary, the physics of electrolocation is based on dipole fields which are not favorable for large distance sensing. The physics is much worse than for auditory or visual systems. Nevertheless, deviations from the dipole power laws in the near field of the fish or of large objects allow for successful electrolocation.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Distance r [cm]
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10
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Distance r [cm]

Encoding stimuli of various frequencies in an electrosensory system: Effects of noise, firing rate and population size.

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Receptor cells often need to encode a wide range of different signals. While a large number of cells helps to encode a signal, each additional cell carries a cost. There is always going to be a compromise between high encoding quality and a low number of neurons. This compromise becomes more complex considering that cells need to be able to encode a wide range of different signals.

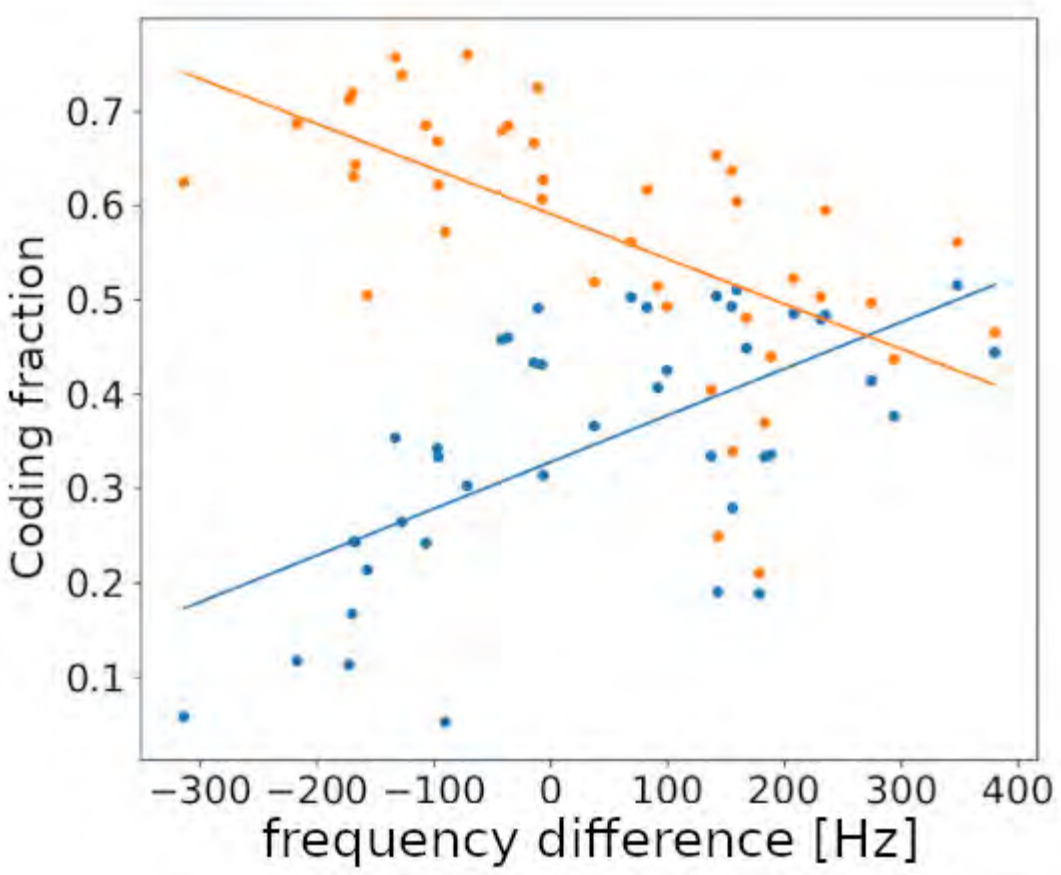
For example, electrosensory receptor cells ("p-units") in the electric fish *Apteronotus leptorhynchus* need to encode electrical signals for electrolocalization as well as electrocommunication over a range from 0 to several 100 Hz. Pyramidal cells in the brain of *A. leptorhynchus* integrate over the response of the receptor cells. The pyramidal cells are specialized, with some integrating over populations of about 10 cells and which are especially sensitive to slow electrolocation signals. Other pyramidal cells integrate over populations of about 1000 receptors and are sensitive to fast signals arising in communication contexts. We explain the relationship between population size and encoded frequency using electrophysiological measurements from the p-units of *A. leptorhynchus*. We show that large populations of receptor cells are important to encode high frequency signals, but are not necessary to encode signals of lower frequency --- matching the properties of the two different types of pyramidal cells described above.
In addition, we show that two properties of the receptor cells influence the signal frequencies they can encode well.

The first property is the relationship between the firing rate of the receptor cells and the frequencies they can reliably encode. We show that compared to the firing rate itself, the firing rate relative to the signal frequency is a better estimator for the increase in encoding quality with population size. While cells that fire fast compared to the signal frequency are efficient in small populations, they get outperformed by slower firing cells in larger populations (see attached figure).

For broadband signals that cover a relatively large frequency range (e.g. 0 to 300 Hz), cell firing rate and the effect of population size are independent. Instead the noise in the response of the receptor cell is a major determinant of the encoding quality. Cells that are not very noisy do not profit from an increase in population sizes as much as cells that are seemingly less reliable. In fact, for larger population sizes, the noisier cells actually outperform the low-noise cells. This effect is known as supra-threshold stochastic resonance and previously has not been experimentally demonstrated in electric fish. To estimate the cell's intrinsic noise level, we use the curvature of the neuron's intensity-response curve. Using experimental and simulated data, we show how this measure is superior to other commonly used measures of noisiness, like the CV of the interspike intervals, or the standard deviation of the firing rate. In particular, the measure is independent of the firing rate of the cell.

Our results contribute to the understanding of peripheral sensory neurons. We demonstrate how heterogeneous populations of these neurons are able to encode a wide range of different signals. The

results highlight the interplay between firing rate, noise, population size and signal frequency.



A low-weight power-efficient sound processor driving optical cochlear implant in freely-moving rat

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In case of deafness, cochlear implants (CIs) bypass dysfunctional or lost hair cells by direct stimulation of tonotopically organized spiral ganglion neurons (SGNs) which convey auditory inputs to the brain. The state-of-the-art implants, electrical CIs (eCIs), enables speech understanding in quiet in most of the over 700,000 users and hence are considered the most successful neuroprostheses. However, due to wide spread of SGN activation from each electrode, coding of spectral information is very limited resulting in poor speech understanding in background noise and reduced music appreciation. Thanks to the ability to confine light in space, optical CIs (oCIs) promise to overcome this shortcoming of eCIs by enabling more independent stimulation channels. This requires fast and power-efficient real-time sound analysis and control of dozens of microscale light emitters. Here, we present a low-weight (8 g), battery-powered, and wireless-controlled sound processor for driving oCIs. Behavioural experiments on rats employing presented sound processor revealed auditory percepts of the optogenetic stimulation. This proof of concept of the oCI system paves the way for the future development of medical devices for human patients.

Fast photoswitches for control of neuronal activity in the cochlea

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Light stimulation of spiral ganglion neurons (SGNs) in the ear provides a future alternative to electrical stimulation used in cochlear implants. Optogenetic manipulation of neuronal activity is based on the expression of light-sensitive proteins, which requires gene therapy. An alternative to optogenetics is offered by photopharmacology, employing light sensitive drugs (photoswitches) to control native or engineered receptors.

Among photoswitches, Targeted Covalent Photoswitches (TCPs) photocontrol endogenous ionotropic glutamate receptors. Upon ultraviolet light, neural activity can be triggered and reversible silenced with visible light (Izquierdo-Serra et al. 2016). However, their photophysical properties hamper their translational application in many fields.

By chemical design and synthesis, we obtained a fast single wavelength-TCP (TCP_{fast}). Using whole-cell patch clamp recordings of hippocampal neuron primary cultures, we show: *i*) the ability of TCP_{fast} to photomodulate neural activity through AMPA receptors without genetic manipulation; *ii*) the chemically-designed fast-relaxation in dark. To further study its utility, we tested in-vivo the potential of TCP_{fast} to photosensitize the SGNs of the cochlea. Following diffusion of the photoswitch into the gerbil cochlea, we recorded optically (λ = 473 nm) evoked compound action potentials (oCAP) in 50% of the cases. oCAPs were: *i*) evoked by light pulse as low as 1 µJ and increased in amplitude proportionally with the radiant flux; *ii*) the largest in response to 80 µs light pulse; *iii*) following repetition rate as high as 1.5 kHz.

TCP_{fast} pioneers the field of photopharmacological stimulation in the cochlea. Future studies should focus on improving stability and handling for application in the cochlea and potential hearing restoration.

Receptive field size and stimulus bandwidth are limited by axonal conduction delays.

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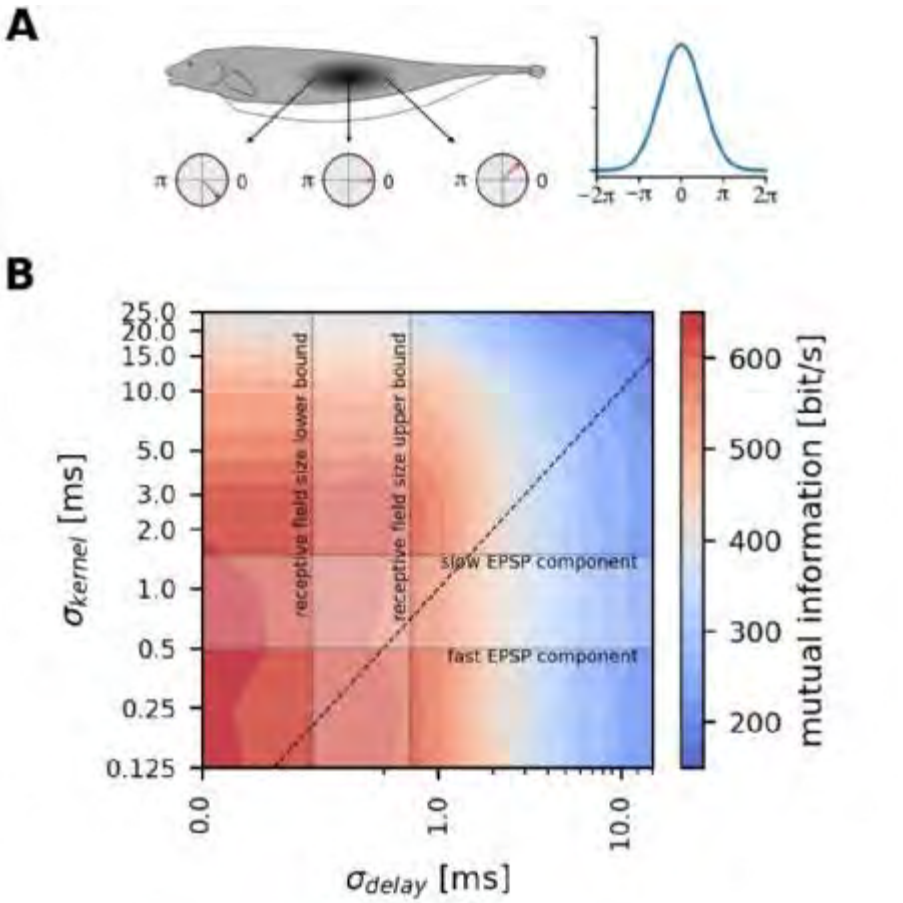
Integrating over a population of presynaptic neurons usually improves the information about a sensory stimulus. Either because presynaptic neurons encode different aspects of the stimulus space, e.g. the separate color channels in visual systems, different odors in olfaction, or the encoding of movement directions. Integrating such populations allows for distinguishing stimuli the individual neuron could not separate. Or a population of similar neurons encodes a common stimulus and the postsynaptic neuron gains from noise reduction by averaging. In the electric sense of the weakly electric fish, similar aspects of the stimulus space are encoded by a populations of a single cell type (so called P-units) and postynaptic neurons in the hindbrain integrate over sub-populations that fall within their receptive fields. P-units show a very high degree of heterogeneity in their response properties. This is expressed in a wide ranges of spontaneous firing rates and sensitivities for amplitude modulations of the fish's self-generated electric field. Such amplitude modulations occur both in navigation/prey-detection and communication contexts.
In this study we first analyze the population heterogeneity among P-units and find that these coding properties are homogeneously heterogeneous along the rostro-caudal body axis. This implies that postsynaptic neurons integrating over a subset of these neurons will always integrate over heterogeneous populations. In an information theoretical approach we then analyze the stimulus encoding with populations of P-units assembled from the pool of recorded neurons and compare the results to populations of simple leaky integrate-and-fire (LIF) model neurons. We find that (i) on average, heterogeneous populations carry more information than the average homogeneous populations of the same size; (ii) the spread of neuronal conduction delays within a receptive field strongly affects the information carried by the population response; (iii) this information filter in particular deteriorates the encoding of high-frequency information; (iv) the receptive field sizes found in the electric fish appear to be a good compromise between size, and thus the spread of conduction delays, and the coding capabilities of behavioral relevant high frequency stimuli; and (v) this is a general feature also observed in populations of LIF model neurons. The slower the axonal conduction velocity, the more severe are the constraints the conduction velocity implies on the receptive field size and the stimulus bandwidth that can be encoded. Hence, understanding receptive field sizes and stimulus encoding in populations of neurons also requires taking stimulus bandwidth and axonal conduction velocity into account.


A
π
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π
0
π
0
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−π
0
π
2π
B
25.0
20.0
15.0
10.0
5.0
3.0
2.0
1.0
0.5
0.25
0.125
receptive field size lower bound
receptive field size upper bound
slow EPSP component
fast EPSP component
σkernel [ms]
0.0
1.0
10.0
σdelay [ms]
600
500
400
300
200
mutual information [bit/s]

Developing Fast, Red-light Optogenetic Stimulation of Spiral Ganglion Neurons for Future Optical Cochlear Implant.

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[8]Equal contribution

Optogenetic stimulation of type I spiral ganglion neurons (SGNs) promises an alternative to the electrical stimulation by current cochlear implants (CIs) for improved hearing restoration by future optical CIs (oCIs). Most of the efforts in using optogenetic stimulation in the cochlea so far used early postnatal injection of viral vectors carrying blue-light activated channelrhodopsins (ChRs) into the cochlea of mice. However, preparing clinical translation of the oCI requires i) reliable and safe transduction of mature SGNs of further species and ii) use of long-wavelength light to avoid phototoxicity. Here, we employed a fast variant of the red-light activated channelrhodopsin Chrimson (f-Chrimson) and various AAV variants to implement optogenetic SGN stimulation in Mongolian gerbils. We compared AAV administration at early postnatal (p8) and adult (> 8 weeks) stages, employing different protocols for injection of AAV-PHP.B and AAV2/6 into the adult cochlea. Success of the optogenetic manipulation was analyzed by optically evoked auditory brainstem response (oABR) and immunohistochemistry of mid-modiolar cryosections of the cochlea. In order to most efficiently evaluate the immunohistochemical results an automatic procedure was developed to identify transduced cells in confocal images. Our results indicated that: i) the rate of SGN transduction is significantly lower for AAV administration into the adult cochlea compared to early postnatal injection; ii) plasma membrane trafficking of f-Chrimson is more efficient in SGNs following early postnatal AAV injection; iii) transduction rate seems independent of the capsid used; iv) slow speed AAV injection avoids the loss of SGN observed upon bolus pressure AAV injection, but is of limited transduction efficiency at the volumes applied; v) functional activation of the auditory pathway correlates positively with the extent of AAV-mediated f-Chrimson expression in the spiral ganglion. Our results highlight the need to optimize viral vectors and virus administration for efficient genetic manipulation of SGNs in the adult cochlea for successful clinical translation of the optogenetic cochlear implant and SGN-targeting gene therapy more generally. To this extend, we have recently investigated different routes of virus administration using intracochlear catether combined with slow injection.

The elusive meaning of the knifefish electric signals.

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Weakly electric fish produce a variety of electric signals which are easily detected, recorded and played back and seem therefore to represent an ideal gateway to access the function of brain circuits involved in the processing of social signals in the teleost brain.
Nonetheless, in spite of the recent advancements in the cross-species mapping of both the social electric signals and the underlying processing circuits, their behavioral meaning remains largely unclear.
Here, we focused on a widely studied gymnotiform fish, *Apteronotus leptorhynchus*. This species generates wave-type electric organ discharges (EODs) which have relatively stable frequencies and resemble electric dipoles of continuously changing polarity. On top of this carrier signal, *A. leptorhynchus* actively produces very fast frequency modulations called chirps. These are categorized in different types, depending on their amplitude and duration and have been proposed to relate to different behavioral categories (i.e. mating, aggression, etc.).
In the attempt to validate this idea, we surveyed all chirp types produced in different types of simulated social interactions. We found no evidence for a *chirp type* bias in any behavioral context analyzed. The inconsistent matching of any given chirp type to behavioral performance in social preference assays and chirp playback experiments confirmed the absence of correlation between *chirp type* and behavior. Factor analysis of a sample of more than 20.000 chirps revealed that the major component explaining chirp type variability is the frequency difference between the carrier signals of the interacting fish. Quantitative analyses of chirp diversity and analysis of chirp patterns did not reveal the existence of stereotyped temporal sequencing or any type-specific bias in any of the social interaction categories analyzed.
We conclude that the chirping behavior of *A. leptorhynchus* observed in the here examined conditions does not convey any context-related meaning. So far chirps have been seen as "social signals" conveying information between communication partners. An alternative possibility could be that chirp signal content might be salient to circuits in the sender's brain only and therefore be means to gather, rather than convey, information.

Optimality of the vestibulo-ocular reflex

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The vestibulo-ocular reflex (VOR) stabilizes retinal images by counter-rotating the eyes during head rotations. A perfect compensatory stabilization would thus require that the eyes rotate exactly opposite to the head with the same velocity and amplitude, that is, eyes versus head exhibit a unity gain factor. However, in many species, the VOR is far from compensatory with gains often considerably lower than unity. The low gain has so far been assumed to derive from the inability of a particular species to simply perform better, the actual reason for this apparent suboptimality is however still unknown.

Here we suggest that VOR gains lower than unity reflect an optimal adaptation to increased sensory and motor variability. According to this hypothesis, gaze stabilization mechanisms that aim at minimizing the overall retinal image slip have to take into account the effects of 1) sensory and motor noise and 2) dynamical constraints of peripheral and central nervous processing. We first demonstrate by computational simulation that increased sensory or motor noise leads to increases in retinal slip signals, which could be counteracted by decreasing the VOR gain. We then investigated horizontal VOR eye movements recorded in four Xenopus laevis tadpoles at four different stimulation frequencies (0.1, 0.2, 0.5, and 1 Hz) with a peak head velocity of 30°/s. Average VOR gain values varied depending on animal (0.05-0.23) and stimulus frequency (0.06-0.14), compatible with results from previous studies.

From the experimentally determined VOR gain and phase values, we estimated for each animal the underlying dynamical VOR circuit model (consisting of semicircular canals, leaky brainstem integrator, muscle activation and eye plant) and the associated overall gain factor. Using the derived dynamical model, we estimated the motor and sensory noise level for which the experimentally occurring VOR gain at 0.5 Hz would be optimal. Finally, we simulated the experimental data using the model. Importantly, the dynamical model, which is based only on the optimality assumption that determines the model noise and the experimentally observed gain and phase values, allows predicting eye movement variability in the data. Therefore, as a test of our hypothesis, we compared experimentally observed and simulated eye movement variability. Variability of eye position was determined by first calculating the standard deviation of eye position for each time point of the stimulation cycle and then averaging over time points. A repeated measures ANOVA (4 animals, 4 frequencies) showed that the average variability varied across animals ($p<0.001$; 0.18° to 1.34°) but not significantly across frequency ($p=0.3$). Correlation between variability predicted by the model and experimental variability was highly significant ($n=16$, $r=0.89$, $p<0.001$).

Thus, our analysis supports the hypothesis that the magnitude of the VOR gain is optimized to achieve minimal retinal image slip given sensory and motor noise as well as dynamical constraints by central nervous and peripheral processing. We suggest that the results found here for Xenopus tadpoles generalize to other species and might also explain why VOR gains decrease after peripheral lesions or with age. Under both circumstances, increased levels of sensory and motor noise might favour a lower VOR gain to optimally stabilize gaze.

Acknowledgements: Funded by DFG GL 342/2-1.

The Johnston's organ of desert ants and its central projections

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The Johnston's organ (JO) in the insect antenna is a multisensory organ involved in several navigational tasks including wind-compass orientation, flight control, graviception, and, possibly, magnetoreception. Here we present the three dimensional anatomy of the JO and its neuronal projections into the brain of the desert ant *Cataglyphis*, a marvelous long-distance navigator. The JO of *Cataglyphis nodus* workers consists of 40 scolopidia comprising three sensory neurons each. The numbers of scolopidia slightly vary between different sexes (female & male) and castes (worker & queen) with the highest number found in males. Individual scolopidia attach to the intersegmental membrane between pedicel and flagellum of the antenna and line up in a ring-like organization. Three JO nerves project along the two antennal nerve branches into the brain. Anterograde double staining of the antennal afferents revealed that JO receptor neurons project to several distinct neuropils in the central brain. The T5 tract projects into the antennal mechanosensory and motor center (AMMC), while the T6 tract bypasses the AMMC via the saddle and forms collaterals terminating in the posterior slope (PS) (T6I), the ventral complex (T6II), and the ventrolateral protocerebrum (T6III). Double labeling of JO and ocellar afferents revealed that input from the JO and visual information from the ocelli converge in tight apposition in the PS. The general JO anatomy and its central projection patterns resemble situations in honeybees and Drosophila. The multisensory nature of the JO together with its projections to multisensory neuropils in the ant brain likely serves synchronization and calibration of different sensory modalities during the ontogeny of navigation in *Cataglyphis*.

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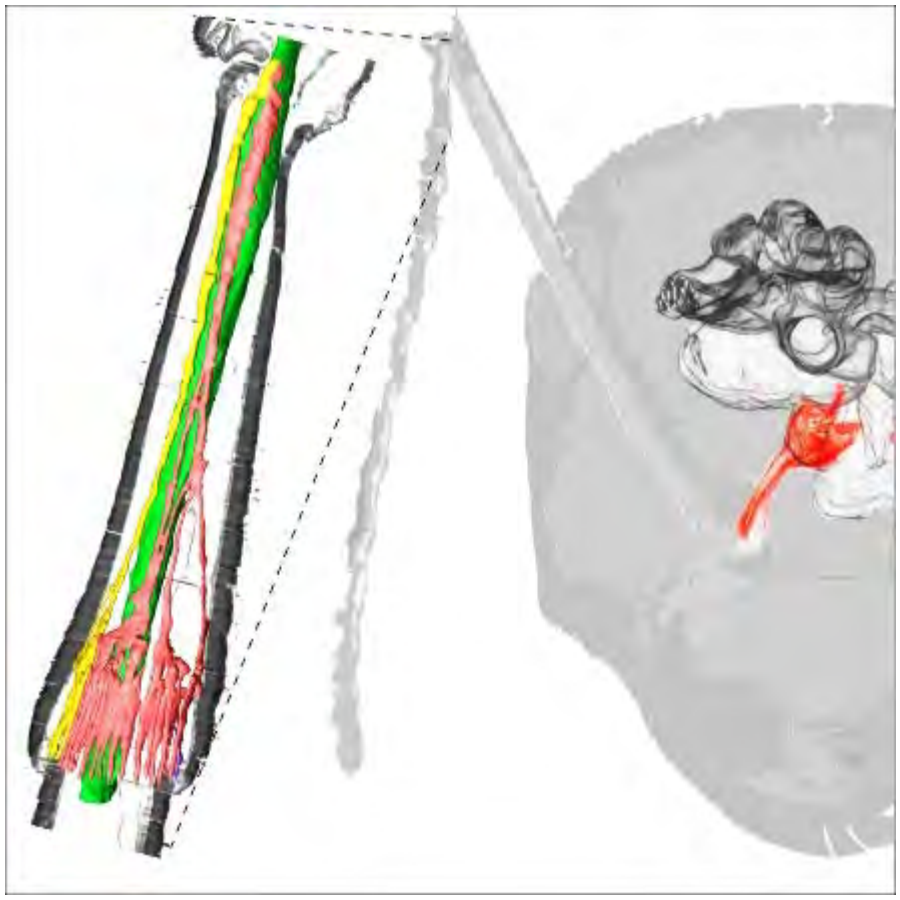

Effects of age and antibiotic treatment on the survival of spiral ganglion cells in the rat cochlea.

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Spiral ganglion cells (SGCs) build the bridge between the peripheral and the central auditory system by transferring input from the sensory hair cells (HCs) to the cochlear nucleus. Among others, aging, pharmacological treatment (e.g. by antibiotics), and noise trauma can induce HC degeneration followed by SGC loss. Previous studies have shown SGC degeneration only in high frequency regions of the cochlea for different animal models. We here investigate how age and/or pharmacological/antibiotic treatment affect the degeneration of SGCs not only in the basal, high-frequency area of the cochlea but also in all four turns of the rat cochlea.

The time course of SGC degeneration was examined over all cochlea turns of normal hearing (NH) and neonatally deafened (ND) Wistar rats. Profound hearing loss was induced by systemic kanamycin injections from postnatal day (P) 10 to P20, resulting in a rapid and permanent rise of hearing threshold (>90 dB) after complete loss of HCs. SGC density was quantified by immunofluorescence staining using the neuronal marker HuC/HuD. In addition, we investigated alterations in the composition of SGC subpopulations by using fluorescence staining of the calcium binding proteins (CBPs) calbindin and calretinin, which may have cell protective effects. SGC degeneration was studied at four to five different points in time, P18, P30, P77, P252 (and P365).

Following pharmacologically induced destruction of HCs, SGCs degenerate significantly over all cochlea turns (basal, lower middle, upper middle, apical turn) within two months. In detail, SGC degeneration was significantly stronger in the higher frequency (basal/lower middle) areas compared to the lower frequency (apical/upper middle) areas of the rat cochlea. In the apical turn, about 50% of the SGCs were still present after two months of deafness. Simultaneously, the percentage of CBP-positive cells of all surviving SGCs increased significantly in ND animals wit the duration of deafness. In comparison, NH animals did not show any significant SGC degeneration or change in the percentage of CBP-positive cells of all SGCs.

Overall, our data suggest that although SGCs degenerate rapidly throughout the cochleae in ND animals, neurons in the apical, low-frequency region, are more resistant to input loss from HCs, resulting in significantly more surviving SGCs. An increasing percentage of all surviving SGCs expressing CBPs could indicate a protective effect of these proteins also for the survival of SGCs after deafness.

Age and noise interact to determine deafness in a locust model of hearing loss.

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We experience an inevitable decline in our hearing ability as we age. Exposure to loud noise – such as those in attendance at the infamous biennial Göttingen NWG NeuroDisco – leads to a temporary hearing loss which recovers. But repeated and frequent exposure to noise, through either work or recreation, adds to our age-related hearing loss in an additive manner. We currently use this 'additive model' to quantify and protect against hearing loss (International standard ISO 13.140). However, this simple additive model fails to accurately capture the extent of hearing loss (Corso, 1992; Miller et al., 1998) especially in most social environments with background noise. This leaves us in the dark as to what to classify as 'safe' noise exposure and in our understanding of the long-term physiology of hearing loss that will affect us all.

In this preliminary high-powered (Power>0.95) study, we used the tympanal ear of the desert locust to quantify the interaction of noise and age to determine hearing loss over a life course. Ben Warren rigorously quantified the electrophysiological properties of auditory neurons, including their sound-evoked transduction currents in noise-exposed and age-matched controls. Alix Blockley recorded the sound-elicited compound spikes from the auditory nerve and Charlie Woodrow measured nanometer displacements of the tympanum in response to sound. Daisy Ogle quantified the loss of cell types in the locust's hearing organ, including morphological features of the auditory neurons.

We report that age and noise interact to determine the extent of deafness following similar patterns over a life course as reported in other mammalian models including humans. We show that temporary hearing loss recovers within 24 hours but repeated exposures lead to permanent deafness. The deficits in hearing stem from a reduction in the transduction current, despite a decrease in the resting potential of noise-exposed auditory neurons. Mechanical deficits are detected at the tympanum with a paradoxical increase in its sound-induced movements after noise-exposure. Morphological age-related effects include a loss of cells in the auditory organ but not in the number of auditory neurons.

Age-related and noise-induced hearing loss occur in a wide variety of animal hearing models. These parallels in hearing loss across animals presumably reflect the multifaceted assault on different vulnerable components in the ear of all animals. Our short-term five-year goal is to integrate our flow of biological data into a predictive multivariate model that can accurately quantify hearing loss in multiple biological hearing models. Our long-term ten-year goal is to bring this approach to accurately predict human hearing loss.

Hearing in Insects: Towards discovering the Mechanotransduction Ion Channel.

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Hearing in all animals relies upon mechanotransduction ion channels that convert sound-induced mechanical vibrations into electrical potentials. To fully understand this fundamental principle of hearing, insects offer a powerful resource. In insects, the elusive mechanotransduction ion channel is still being uncovered, but there are two promising candidates; two Transient Receptor Potential channels localised in the cilium of auditory neurons, where transduction takes place. It remains controversial as to which of these hopeful channels is the mechanotransducer: NompC, located at the tip of the cilia of auditory neurons; or Nanchung-Inactive, localised at the proximal cilium. NompC is the only channel that has been shown to act as a mechanotransduction channel when expressed in heterologous systems, making it the front runner in the race to reveal the mechanotransduction channel [1]. However, Nanchung-Inactive has come back into contention recently as intracellular recordings of the transduction current of the auditory neurons suggest that Nanchung-Inactive could be the mechanotransduction channel [2]. Currently, there have been no transduction current recordings coupled with genetic manipulations of the channels themselves.
To quantitatively record transduction currents directly from individual auditory neurons, the neuron must be accessible and be large enough to be patch clamped. To this end, the desert locust (*Schistocerca gregaria*) provides an ideal model organism, because its auditory neurons are accessible, compared with other more widely studied insect models such as fruit flies (*Drosophila melanogaster*). Here, we employed RNAi to knock down the expression of the genes encoding NompC, Nanchung, and Inactive in *S.gregaria*, and measure the corresponding transduction current. Knockdowns were quantified using qPCR, and transcriptome analysis of knockdown animals reveals effects upon the expression of other genes within the locust's ear. This provides us with a novel insight into the role of *nompC, nanchung* and *inactive*, and provides crucial evidence towards finally unlocking the secret behind auditory transduction in insects.

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2: Warren, B., & Matheson, T. (2018). The Role of the Mechanotransduction Ion Channel Candidate Nanchung-Inactive in Auditory Transduction in an Insect Ear. The Journal of neuroscience : the official journal of the Society for Neuroscience, 38(15), 3741–3752.

Poster Topic

T18: Auditory System: Subcortical and Cortical Processing

T18-1 Predictive coding underlies adaptation in the human subcortical auditory pathway
Alejandro Tabas, Glad Mihai, Stefan Kiebel, Robert Trampel, Katharina von Kriegstein

T18-2 Kv3.3 (kcnc3) subunits mediate action potential repolarization at the calyx of Held presynaptic terminal
Ian D. Forsythe, Amy Richardson, Mihai Stancu, Victoria Ciampani, Conny Kopp-Scheinpflug

T18-3 Auditory processing by descending neurons: a missing link in song recognition?
Ali Cillov, Andreas Stumpner

T18-4 Central compensation of cochlear synaptopathy is not dependent on age, but on LTP/BDNF recruitment
Daria Savitska, Philine Marchetta, Angelika Kübler, Giulia Asola, Marie Manthey, Dorit Möhrle, Thomas Schimmang, Lukas Rüttiger, Marlies Knipper, Wibke Singer

T18-5 Auditory cortex responsiveness during silent reading
Julia Gelenar Marae, Valentina Niccolai, Alfons Schnitzler, Katja Biermann-Ruben

T18-6 Synaptic integration in the lateral superior olive analyzed via dynamic-clamp
Jonas Martin Fisch, Nicolas Freche, Matthew Xu-Friedman, Eckhard Friauf

T18-7 Neuronal mechanism underlying detection of amplitude modulated sound in mice
Aaron Benson Wong, Maurits M van den Berg, Esmée Busscher, Jean P H Slenter, Marcel van der Heijden, Peter Bremen, J Gerard G Borst

T18-8 Simulated dendritic inputs to realistic gerbil bushy cell dendrites
Thomas Künzel

T18-9 Use of varying metabolic pathways in neurons exhibiting a broad dynamic range of activity
Nicola Palandt, Patricia Unterlechner, Valentin Winhart, Otto Albrecht, Lars Kunz

T18-10 Role of Brain Oscillations for Information Processing in the Auditory Space Map of Awake Barn Owls
Roland Ferger, Andrea Bae, Keanu Shadron, Jose Pena

T18-11 Investigating frequency discrimination using the acoustic startle response in rats

Lisa Koch, Markus M. Middeke, Eva Dunkel, Bernhard H. Gaese

T18-12 Hyperacusis masks tinnitus response pattern of sound-evoked and resting state BOLD fMRI
Jakob Wertz, Jörg Saemisch, Benedikt Hofmeier, Fatma Refat, Pauline Hinrichs, Wibke Singer, Lukas Rüttiger, Uwe Klose, Marlies Knipper, Stephan Wolpert

T18-13 Neuromodulation in auditory thalamus upon associative fear learning
Joana Amorim Freire, James Alexander Taylor, Masashi Hasegawa, Marvin Weiss, Marine Theodore, Sabrina Innocenti, Chloe Benoit, Dan Ganea, Benay Baskurt, Ziyan Huang, Jan Gründemann

T18-14 Estimating electrical hearing thresholds using eCAPs as an objective measure in cochlear implanted common marmosets
Amirouche Sadoun, Antoine Tarquin Huet, Stefan B. Strahl, Gerhard Hoch, Lukasz Jablonski, Tobias Moser, Marcus Jeschke

T18-15 Locomotion modulates spontaneous and sensory-evoked neuronal activity in the mouse dorsal auditory field

Julia Henschke, Alyssa M. Price, Janelle M. P. Pakan

T18-16 Anatomy of neurotransmitter-specific connections between the auditory cortex and mid- and hindbrain in Mongolian gerbils
Alina C Zacher, Julia U Henschke, Max FK Happel, Eike Budinger

T18-17 Linking endogenous oscillations to sound-driven responses in the auditory cortex
Luciana Francisca López Jury, Julio Hechavarria

T18-18 Cortical and subcortical differential processing in forward suppression with natural sounds
Eugenia González Palomares, Francisco García Rosales, Julio C. Hechavarría

T18-19 Individual and group-level flexibility in social vocalization timing in bats

Ava Kiai, Julio Hechavarría

T18-20 Female zebra finch presence shapes juvenile song performance and premotor circuitry
Linda Bistere, Philipp Norton, Martin Rötzschke, Constance Scharff, Daniela Vallentin

T18-21 A continuum of biophysical time scales of neurons in the intermediate nucleus of the lateral lemniscus
Kathrin Wicke, Felix Felmy

T18-22 Dendritic morphometry of principal neurons in the medial nucleus of the trapezoid body
Christina Pätz, Felix Felmy

T18-23 The extracellular matrix regulates cortical layer dynamics and cross-columnar frequency

integration in the auditory cortex
Ebru Acun, M El-Tabbal, H Niekisch, JU Henschke, E Budinger, R Frischknecht, M Deliano, MFK Happel

T18-24 Object specific Adaptation in the Auditory Cortex of Bats
Jan David Pastyrik, Uwe Firzlaff

T18-25 Characterization of the auditory spatial receptive fields in the optic tectum of the domestic chicken (*Gallus gallus domesticus*)
Gianmarco Maldarelli, Uwe Firzlaff, Harald Luksch

T18-26 Neuronal representation of spectral and temporal song features in juvenile zebra finches
Stefan Wilczek, Avani Koparkar, Daniela Vallentin

T18-27 Optogenetically controlled aggregation of calcium channels in the auditory cortex causes deterministic population dynamics and suppressed impulse responses
Katrina E Deane, Jennifer Heck, Melanie Mark, Stephan Herlitze, Max FK Happel

T18-28 Central stress responses account for auditory nerve sensitivity
Philine Marchetta, Philipp Eckert, Wibke Singer, Lukas Rüttiger, Marlies Knipper

T18-29 Can tinnitus and tinnitus with co-occuring hyperacusis be reflected in high-frequency brain oscillations?
Jörg Saemisch, Jakob Wertz, Stephan Wolpert, Uwe Klose, Ann-Christine Ehlis, Lukas Rüttiger, Marlies Knipper, Matthias H. J. Munk

T18-30 Studying the hearing of bats in a minimally invasive way: ABRs and FFRs to simple and complex tones in the bat species *Carollia perspicillata*
Johannes Wetekam, Manfred Kössl

Predictive coding underlies adaptation in the human subcortical auditory pathway

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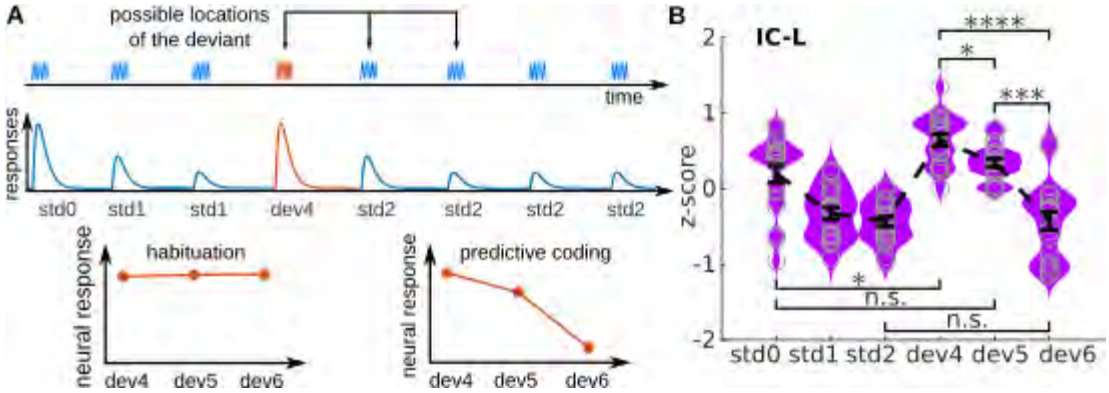
The subcortical sensory pathways are the fundamental channels for mapping the outside world to our minds. Sensory pathways efficiently transmit information by adapting neural responses to the local statistics of the sensory input. The longstanding mechanistic explanation for this adaptive behaviour is that neural activity decreases with increasing regularities in the local statistics of the stimuli due to habituation. The predictive coding theoretical framework offers an alternative account: that neurons showing adaptation encode prediction error with respect to an internal generative model of the sensory input.

Previous studies have shown that single neurons and neuronal ensembles of subcortical sensory pathway nuclei exhibit stimulus specific adaptation (SSA), where they adapt to so-called standards (frequently occurring stimuli) yet show restored responses to so-called deviants (rarely occurring stimuli). In the auditory modality, SSA is typically elicited using sequences consisting of repetitions of a standard tone (typically a pure tone of a given frequency) incorporating a single, randomly located, deviant (e.g., a pure tone with a different frequency). These studies manipulated predictability by changing the ratio between deviant and standards, rendering their results ambiguous about the neural mechanisms underlying SSA.

Here we introduce a variation of the classical SSA paradigm in humans where we manipulate the predictability of the stimuli without perturbing the local stimulus statistics. A trial consisted on a train of 8 sounds: 7 standards and only one deviant (Figure A). Participants were instructed to monitor the sequences and report the position of the deviant within the sequence pressing a button. Expectations for each of the deviant positions were manipulated by restricting the deviant location to positions 4, 5, or 6. Although the three deviant positions were equally likely, the probability of finding a deviant in position 4 after hearing 3 standards is 1/3, the probability of finding a deviant in position 5 after hearing 4 standards is 1/2, and the probability of finding a deviant in position 6 after hearing 5 standards is 1. This paradigm yields opposing predictions for the habituation and predictive coding scenarios. According to habituation, responses to the deviant only depend on the ratio between deviants and standards and should be roughly independent of its position. According to predictive coding, responses should scale inversely with the expectations that the incoming stimuli is a deviant: responses to deviants in location 4 should be stronger than responses to deviants in location 5 which should be stronger than responses to deviants in location 6.

We used 7 Tesla functional-MRI to measure BOLD responses in IC and MGB while participants completed the task. BOLD responses to deviants with low likelihoods were much stronger than to deviants with high likelihoods, in full agreement with predictive coding. Specifically, responses to deviants in location 4 (those with a likelihood of 1/3) were higher than the responses to the first sound of the trial, while responses to deviants in location 6 (those with a likelihood of 1) were comparable to the responses to the repeated standards (Figure B).

These results provide first unambiguous evidence of abstract processing in a subcortical sensory pathway, indicating that the neural representation of the outside world is altered by our prior beliefs even at initial points of the processing hierarchy.



Kv3.3 (kcnc3) subunits mediate action potential repolarization at the calyx of Held presynaptic terminal

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The voltage-gated potassium channel family 3 has four members (Kv3.1-Kv3.4) which are broadly expressed in the nervous system; they are high voltage-activated and therefore have a role in action potential (AP) repolarization. Functional Kv3 channels in the plasma membrane are composed of four alpha subunits, but the specific configuration of native channels *in vivo* has been difficult to determine unequivocally. In the auditory brainstem, we have previously demonstrated that only Kv3.1 and Kv3.3 subunits are expressed significantly. All Kv3 channels are effectively blocked by low concentrations of tetraethylammonium (TEA, 1mM) and we can constrain Kv3 channel composition within nuclei of the auditory brainstem by employing transgenic knockout mice for Kv3.1 or Kv3.3. In MNTB principal neurons, Kv3 channels possessing either Kv3.1 and/or Kv3.3 subunits can support AP repolarization. In contrast, Kv3 channels in LSO principal neurons must include at least one Kv3.3 subunit (Choudhury *et al.*, 2020) and need not include any other Kv3 subunit (i.e. they may be Kv3.3 homomers).

While longer APs generated by postsynaptic neurons influence the rate and pattern of neural firing, changes in presynaptic AP duration could independently and additionally affect transmitter release. Therefore, we have asked if presynaptic Kv3 channels at the giant synaptic terminal of the calyx of Held have a particular requirement for Kv3.1 or Kv3.3 subunits, and then determined the impact on transmitter release at this glutamatergic synapse.

Whole-cell patch-clamp recordings were made from the calyx of Held and MNTB neurons during stimulation of the presynaptic axons over a wide range of frequencies from 100 to 600Hz. We compared EPSCs and evoked APs from each of three different genotypes: CBA/Ca (WT), Kv3.3KO and Kv3.1KO. These *in vitro* recordings demonstrated that presynaptic Kv3 channels required the presence of Kv3.3 subunits to form functional channels at the calyx of Held. In the absence of Kv3.3 subunits, presynaptic APs were of longer duration, giving rise to larger initial EPSCs, but which exhibited a greater level of short-term depression compared to WT animals.

In vivo recording of evoked and spontaneous potentials from the MNTB consist of a compound presynaptic and postsynaptic AP (Kopp-Scheinpflug *et al*., 2003). These extracellular recordings permitted the function of Kv3.3 containing channels to be determined in auditory processing. Comparing MNTB recordings between WT and Kv3.3KO mice revealed significantly increased durations for both pre- and postsynaptic APs, as well as longer synaptic delays. In response to sound, Kv3.3KO mice showed longer latencies, increased latency jitter and altered firing rates compared to WT controls.

We conclude that Kv3.3 subunits are essential for presynaptic Kv3 channels at the calyx of Held; they accelerate AP repolarization, thereby maintaining short duration APs to enhance temporal precision during synaptic transmission to the MNTB neuron. Loss of temporal precision and changes in AP firing rates at such a crucial junction in the auditory pathway has implications for multiple temporal processing tasks, including sound localization and acoustic communication.

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Auditory processing by descending neurons: a missing link in song recognition?

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Auditory processing in insects is often seen as a simple process. Yet, orthopteran insects display surprisingly complex and diverse auditory behaviour patterns – sometimes apparently with a relatively small number of neurons. *Ancistrura nigrovittata* is a duetting bush cricket with males singing with a distinct temporal pattern at about 16 kHz and females responding with a short click at about 28 kHz and a delay of sometimes less than 30 ms. As for auditory processing, *A. nigrovittata* is one of the most thoroughly investigated species. However, the recent discovery of a large cluster of local auditory interneurons and the unknown mechanism behind the species' duetting behaviour highlight the limited knowledge even in this system.

One little-studied, but intriguing group of neurons in bush crickets is the auditory descending neurons (DNs). Previous data on DNs are limited and lack distinct hypotheses of their function – additionally being fragmented over various species. As an exception, DNs of *Mecopoda* have been shown to present correlates to behavioural performances [1]. Our preliminary results from intracellular recordings and stains in *A. nigrovittata* demonstrate the existence of a group of ten or more auditory DNs in the prothoracic network with diverse response patterns. The complete spectrum of the population is still unknown. However, some of these DNs are tuned to the male calling song frequency and are therefore potentially involved in processing of communication signals. We hypothesize that one or multiple DNs with such frequency tuning may be responsible for triggering the female reply to the male calling song after being primed by previous processing in the brain. This response occurs too fast to be directly generated by brain interneurons, which have latencies of 30 ms and more [2]. Thus, DNs could constitute the missing link between auditory processing and behaviour.

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Central compensation of cochlear synaptopathy is not dependent on age, but on LTP/BDNF recruitment

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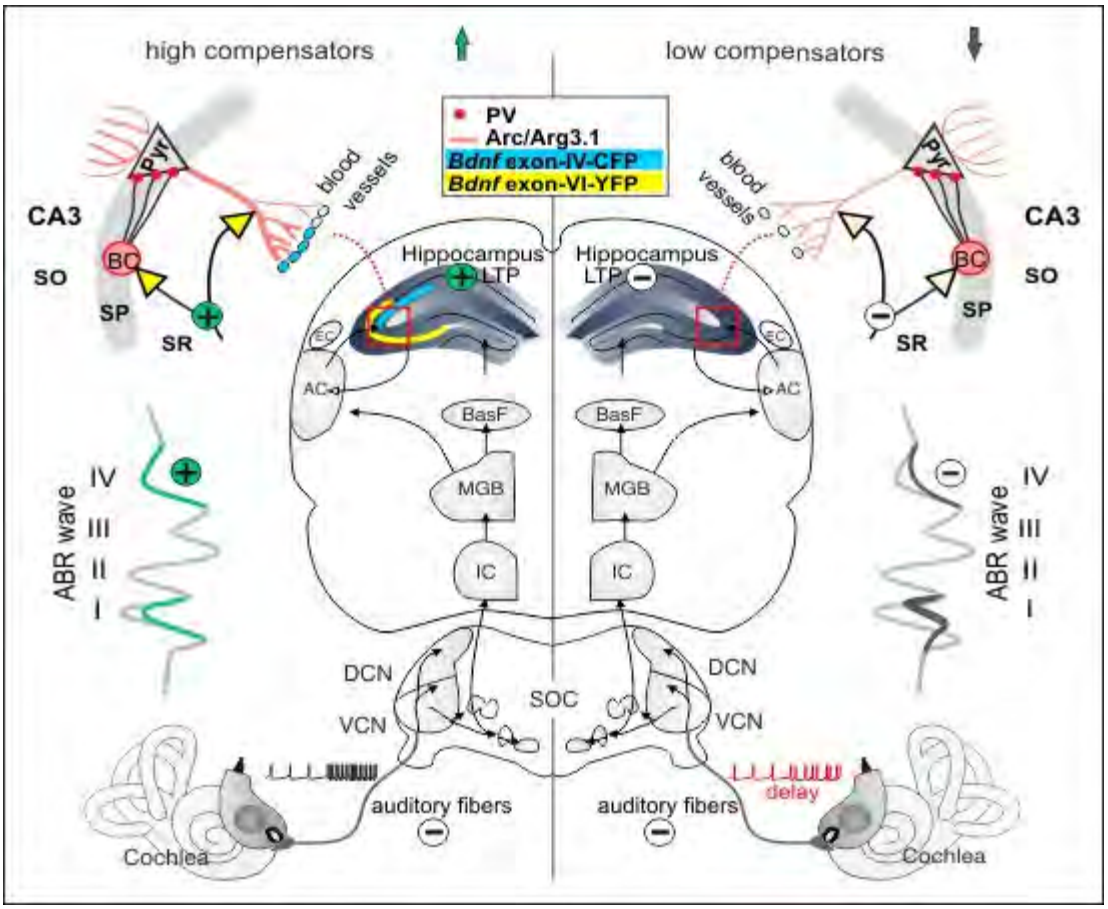
Age-related loss of synaptic contacts between inner hair cells and auditory-nerve fibers (cochlear synaptopathy) has been linked to temporal processing deficits and impaired speech-in-noise recognition. In individual cases age-dependent temporal discrimination loss may be attenuated due to central compensation mechanism (neural gain). We hypothesize that cochlear synaptopathy, central neural gain and the ability for fast auditory temporal processing are connected to changes in hippocampal long-term potentiation (LTP) and brain-derived neurotrophic factor (BDNF) expression independent of the age. Here, we investigated middle-aged and old BDNF-live-exon-visualization (BLEV) reporter mice and analyzed auditory brainstem responses, auditory steady state responses and hippocampal field excitatory postsynaptic potentials. In both, middle-aged and old groups, animals with lower or higher ability to centrally compensate reduced auditory nerve activity were found. The low compensators exhibited attenuated responses to amplitude-modulated tones and a reduction of hippocampal LTP and *Bdnf* transcript levels in comparison to high compensators. These results suggest that not the age itself but rather the diminished capacity for central compensation and LTP/BDNF recruitment play a key role in age-related loss of central auditory function caused by cochlear synaptopathy. This work was supported by grants from the Deutsche Forschungsgemeinschaft FOR 2060 project RU 713/3-2, Projektnummer 335549539/GRK 2381, SPP 1608 RU 316/12-1, and KN 316/12-1, Siegmund Kiener Stiftung (D.S.) and BFU2016-76580-P.


high compensators
low compensators
PV
Arc/Arg3.1
Bdnf exon-IV-CFP
Bdnf exon-VI-YFP
CA3
SO
SP
SR
BC
PYr
blood vessels
Hippocampus LTP
EC
AC
BasF
MGB
IC
ABR wave
IV
III
II
I
DCN
VCN
SOC
auditory fibers
delay
Cochlea

Auditory cortex responsiveness during silent reading

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The theory of embodied cognition postulates that language and concepts are processed not only by semantic brain circuits, but also by neural sensory and motor systems. As for the auditory system, there is some evidence for its engagement during the visual processing of words referring to sounds or acoustic features especially in the posterior temporal gyrus (pSTG) and middle temporal gyrus (MTG). The present study further examines whether auditory cortex responsiveness to a same acoustic stimulus is differentially modulated by earlier processing of “loud” (e.g., to shout) as compared with “quiet” (e.g., to whisper) action verbs (1) and whether processing of visually presented words characterized by different levels of associated loudness differentially activates the auditory cortex itself (2). Loud tones cause a greater activation of the auditory cortex compared to quiet tones, and repetitive auditory stimulation results in habituation. According to this sensory suppression, we expect (1) a lower activation of the auditory cortex in response to a tone stimulus following a “loud” compared to a “quiet” action verb. Further, we expect (2) to find stronger activation of the auditory cortex for “loud” than “quiet” verbs.

Twenty healthy participants were measured with magnetoencephalography (MEG) while listening to loud and quiet 440-Hz tones serving as a functional localizer task for the auditory cortex. The word paradigm required participants to semantically process visually presented German verbs describing human actions typically producing loud or quiet sounds. Verbs were matched according to word length, frequency, bi-/trigram frequency and number of facial versus limb/whole body actions in both conditions (loud vs. quiet) and were inflected in either the first or the third singular person, present tense. Verbs were followed by a short 440 Hz tone of stable sound pressure level. After the tone a visual prompt indicated if the subject either had to select the perspective (1st or 3rdperson) or the body part involved in this action by manual button press. We performed dipole source modeling of the N100 event-related component in the localizer task, which served as a proxy for auditory activation in the other conditions under consideration: (1) tones following word presentation and (2) putative auditory activation during silent reading of inflected verbs associated with different levels of loudness.

In line with previous study results the localizer study showed a statistically significant larger peak amplitude of the auditory N100-sources induced by loud tones as compared to quiet tones bilaterally ($p=0.025$ left, $p=0.048$ right; Wilcoxon, corrected). In the word paradigm, the lefthemispheric N100-peak evoked by an identical tone (1) tended to be lower following “loud” actions versus following “quiet” actions ($p=0.085$; Wilcoxon, corrected); no difference emerged in the right hemisphere ($p=0.717$). During visual word processing (2) the area-under-the-curve related to the N100-source in the time window of 100-215 ms after visual word onset showed a trend towards stronger auditory involvement while reading “loud” compared to “quiet” verbs in the left ($p=0.057$; Wilcoxon, corrected) but not in the right hemisphere ($p=0.550$). The current results suggest specific and left-lateralized contribution of the auditory cortex to the processing of sound-related action words, thus supporting the theory of embodied cognition.

Synaptic integration in the lateral superior olive analyzed via dynamic-clamp

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Excitatory and inhibitory synapses are the major contributors to neuronal information processing. The robust interplay of both types is of utterly importance in the lateral superior olive (LSO), a prominent hub in the mammalian auditory brainstem. LSO neurons are involved in sound localization by analyzing inter-aural level differences in a subtraction-like computation. They do so by integrating excitatory and inhibitory inputs coming from the ipsilateral and contralateral ear, respectively. LSO neurons are tonically activated at high frequency (>100 Hz) upon acoustic stimulation. Moreover, information processing is ultrafast and temporally precise. Together, the LSO and its input nuclei (cochlear nucleus and medial nucleus of the trapezoid body) form a perfect neural circuit for studying integration mechanisms of excitation and inhibition over periods of milliseconds to minutes. To investigate the importance of timing and strength on integration, several technical difficulties must be overcome. Here, we use the dynamic-clamp method (DC, aka conductance-clamp) to simulate synaptic activity. The DC method allows a precise and independent control of excitatory and inhibitory inputs regarding timing, strength, and reproducibility. This poster will focus on our establishing procedure in the LSO. In whole-cell voltage-clamp experiments from acute slices of prehearing mice, we first measured and reanalyzed unitary evoked excitatory and inhibitory postsynaptic currents (eEPSCs and eIPSCs). Current templates displaying onset and decay kinetics characteristic for a mean eEPSC and a mean eIPSC were generated, and resulting waveforms were converted to conductance templates. Next, we modeled synaptic depression upon 100-Hz stimulation in order to analyze its effect on postsynaptic membrane potential changes. Preliminary results suggest a major contribution of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels on the summation of inhibitory postsynaptic potentials and rebound depolarizations by inhibitory postsynaptic conductance (IPSG) stimulations. These findings are in line with post-inhibitory facilitation described by Beiderbeck et al (2018, Nat Commun, PMID: 29720589) and Leao et al (2006, J Physiol, PMID: 16916913). We currently analyze the effects of different strength and timing of inhibitory input conductance (trains of at least 10 IPSGs) on action potential firing probability in LSO neurons. Results from these ongoing experiments will also be presented and discussed on the poster.

Neuronal mechanism underlying detection of amplitude modulated sound in mice

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Amplitude modulation (AM) is a common feature of natural sounds, including speech and animal vocalizations. Here, we used operant conditioning and in vivo electrophysiology to determine the AM detection threshold of mice as well as its underlying neuronal encoding. B6CBAF1/JRj mice were trained in an operant conditioning task to detect the transition from unmodulated, broadband, pink noise to noise that was sinusoidally modulated at frequencies ranging from 4 to 1024 Hz. Noise stimuli were designed to compensate for the inevitable addition of spectral side-bands in the modulated sounds. Behavioural response rates at different modulation depths were converted to the bias-correcting metric *d'*, and detection thresholds were set at *d'* = 1. Our results indicated that mice, in comparison to other species, detect high modulation frequencies up to 512 Hz exceptionally well, but show poor performance at low frequencies. In addition, we performed in vivo multi-electrode recordings in the inferior colliculus of anesthetized mice to measure single unit responses to AM sounds. Remarkably, we found units that showed statistically significant phase-locking to modulation frequencies up to 512 Hz. However, using a PSTH-based classification method (Hoglen et al, 2018), we found that classification generally improved with larger time bins, suggesting that already at the level of the inferior colliculus, mice predominantly use a rate code to encode amplitude modulations in sound.

Simulated dendritic inputs to realistic gerbil bushy cell dendrites

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Processing of auditory information in bushy cells of the ventral cochlear nucleus is dominated by a low number of axosomatic inputs per neuron, the endbulbs of Held. At least in low-frequency tonotopic areas already a single endbulb input can often be suprathreshold. Despite this fact, bushy cells receive additional regular axodendritic inputs from the auditory nerve. The role of these small dendritic inputs in hearing, and the way they interact with the powerful main input, is not well understood.

In a recent study (Koert and Kuenzel, bioRxiv 2020.06.03.131516) we explored these questions in silico, using NEURON compartment models of 3D-reconstructed bushy dendrites from neurons recorded in acute gerbil brain slice preparations. There we found that phase-locked dendritic inputs from the same cochlear origin were subthreshold for plausible physiological parameters. However, in interaction with the main endbulb input, they enhanced action potential generation, entrainment, and precision of phase-locking. This was strongly dependent on input frequency and tuning. To better understand this we estimated the complex dendrite-to-soma transfer impendance. Bushy dendrites showed high-frequency resonant behavior (f_{res}>100Hz). Not unexpectedly, both the amount of resonance and the resonance frequency changed with dendrite-to-soma path length for a given input. The exponential decay constant (or in the case of the change of resonance frequency: the linear slope) of this reduction differed from the steady state length constant of average bushy dendritic paths (λ=75µm). However, for very common intermediate pathlengths (50-100µm) the frequency dependence of dendritic input efficacy varied most strongly between the different dendritic models.

This lead me to explore the interplay between input frequency and dendritic morphology in greater detail. For this I analyzed N = 18 reconstructed gerbil bushy dendrites. These dendrites on average did not reach very widely (maximal distance to soma 83 ± 19 µm), but showed remarkable complexity relatively close to the soma (36 ± 16 branchpoints, total length 1463 ± 667 µm, Sholl critical value 18 ± 3 intersects at a critical range of 38 ± 12 µm). I wanted to establish whether dendritic morphological parameters correlated with the most effective input frequency for dendritic enhancement in bushy cells. Furthermore, I explore here how the properties of the dendritic input axons (characteristic frequency, ratio of high/medium/low spontaneous rate fibers) influenced the dendritic enhancement.

Overall it became clear that small phase-locked dendritic inputs significantly influenced processing of auditory information in bushy cells. Did they, however, really need to be on the dendrite? Indeed, the same amount of extra excitatory inputs placed on the model soma was strongly detrimental for output generation. On the other hand, long dendritic paths quickly attenuated all benefits of dendritic inputs. Thus, the peculiar shape of the bushy dendrite might be a biological compromise between these opposing contraints.

Use of varying metabolic pathways in neurons exhibiting a broad dynamic range of activity

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Generation of action potentials is costly in terms of energy, and, thus, a neuron's firing rate determines its total energy consumption. Neurons – like any other cell type – use a dynamic system of various metabolic pathways for ATP production. We assume that a neuron can adapt the fractional contribution of these different metabolic pathways to the actual firing rate.
To study this topic, we monitored metabolic activity (changes in levels of NADH, FAD and O_2) in the medial nucleus of the trapezoid body (MNTB) in acute brainstem slices of the Mongolian gerbil (Meriones unguiculatus) following electrical stimulation of MNTB fibre inputs. As MNTB neurons can generate action potentials with a frequency up to 1000 Hz, this nucleus represents an apt model for studying which metabolic pathways neurons use at different physiologically relevant firing frequencies.

In the range between 10 Hz and 1000 Hz our results show a lower O_2 consumption for higher frequencies as well as a decreasing NADH and $FADH_2$ consumption for higher and very low frequencies (below 100Hz). This different behaviour for lower frequencies compared to higher ones is an indication for a metabolic switch to different pathways for higher frequencies. Especially the changing O_2 consumption might be explained with a varying contribution of the oxidative phosphorylation (OXPHOS).
For further investigation, we blocked the OXPHOS by a mixture of rotenone, antimycin A and cyanide and found a strong and partially reversible reduction in $FADH_2$ consumption during ATP regeneration. We observed a similar reversible reduction in NADH and O_2 consumption.
For a better interpretation of our findings we are working on a linear kinetics-dynamic flux balance analysis (LK-DFBA). This model allows us to calculate how much each metabolic pathway contributes to the generation of action potentials at different firing frequencies.

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Role of Brain Oscillations for Information Processing in the Auditory Space Map of Awake Barn Owls

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Brain oscillations evoked by sensory stimuli are fluctuations in field potentials reflecting the combined activity of neural populations. Specifically, gamma oscillations (25-140 Hz) have been linked to inhibitory input which shapes population activity in barn owls' midbrain regions involved in sound localization. Earlier in vivo recordings in the owl's optic tectum (OT) have shown that gamma oscillations are spatially tuned to both visual and auditory information. Whether these oscillations contribute to information processing and/or coding in the space map or merely reflect spiking activity is yet to be determined. The OT of barn owls, a well established model for sound localization, provides a unique opportunity to evaluate the role of brain oscillations.

Gamma oscillations are sensitive to anesthesia and have been implicated in awake processes, like attention and stimulus selection. However, previous studies characterizing gamma oscillations in the barn owl have relied on light tranquilization with nitrous oxide or anesthesia with ketamine – which may affect gamma oscillations to different extents. Therefore, recordings in awake owls are imperative to understanding the role of gamma oscillations in the owl's sound localization and stimulus selection network.

In a novel approach, we chronically implanted drives loaded with tetrodes in OT and recorded spikes and local field potentials. We investigated spontaneous and sound evoked neural activity in the midbrain of awake barn owls and compared this to recordings from anesthetized animals. Additionally, we compared spontaneous recordings from anesthetized animals before and after injections of ketamine (usually given every 1-2 hours to maintain anesthesia) to elucidate the generalizability of anesthetized recordings to awake processes.

Comparing activity pre- and post-injection of ketamine, we show that anesthesia diminishes spontaneous spiking activity significantly. Power spectra from local field potential recordings in these same recordings also show increases in power below 10 Hz post-injection, suggesting that low frequency signals are especially sensitive to anesthesia. The pandemic has delayed our experiments and awake recordings. The first animals are recovering from implantation surgery as we submit this abstract. We're confident to record data from multiple owls before the end of the year.

In this work, we first demonstrate the technical feasibility of chronically implanted electrodes to record from the avian midbrain. This will bridge our understanding of auditory processes between awake and anesthetized states and further our knowledge about the role of brain oscillations in midbrain computations. Comparisons between recordings from awake and anesthetized animals will allow to assess generalizability between the two in an already well-known network. In the future, we plan to record from forebrain regions, possibly simultaneously with OT, to shine light on the interactions across the brain, which have been historically difficult to study in anesthetized animals.

Investigating frequency discrimination using the acoustic startle response in rats

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Investigating frequency discrimination in rodents mostly requires time-consuming training of animals. Here we explored the possibilities of applying a reflex-based behavioral method for a time-efficient measurement of frequency discrimination. The acoustic startle response is elicited in response to sudden loud acoustic stimuli with reflexive contraction of skeletal muscles. A preceding weaker acoustic stimulus can reduce the startle response amplitude, a phenomenon called prepulse inhibition. This procedure does not require training of animals. Based on the prepulse inhibition of the acoustic startle reflex, animals were tested with a modified behavioral paradigm combining a continuous pure tone background stimulus with a shift in pure tone frequency acting as a startle-modifying prepulse. Three parameters were systematically changed for a thorough characterization of this procedure: i) frequency of background stimulation, ii) step size of frequency shifts and iii) timing of shift occurrence.

Prepulse inhibition increased with increasing step sizes of frequency shifts, reaching a plateau at a step size of Δ10% and above. Maximal inhibition depended also on background frequency, with strongest inhibition at the lowest tested frequency, 8 kHz (around 80%) and lowest at highest tested frequency, 18 kHz (around 30%). Timing of the prepulse in the range below 130 ms before the startle stimulus did not have a significant effect on the maximal inhibition.

Interestingly, a strong increase in response amplitude at -2% frequency shift was detected at all tested background frequencies. This effect was reduced when the prepulse (i.e. frequency-shifted pure tone) ended clearly (50 ms) before the startle stimulus. Thus, a stronger increase of startle response amplitudes was found for paradigms where the shift ended with the beginning of the startle stimulus.

Possible underlying mechanisms can be investigated by combining behavioral with electrophysiological measurements. Those experiments are urgently needed and planned.

In summary, the modified acoustic startle response is a reliable measure for frequency discrimination in rodents. Fascinating effects were found that could not have been detected by operant conditioning and that need further electrophysiological investigation.

Hyperacusis masks tinnitus response pattern of sound-evoked and resting state BOLD fMRI

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Hearing loss is often accompanied by symptoms such as tinnitus, a constant ringing sound. Due to the high prevalence of 10 to 20% in the population, the development of successful approaches for causal tinnitus therapies is required.
Progress of curative therapy is mostly impeded by controversial views on the neural correlate of tinnitus. We hypothesize that the neural correlate and the success of tinnitus therapy varies greatly with the co-occurrence of hyperacusis, a perception of moderate sounds as too loud or even painful.
Forty-three controls and fifty tinnitus patients with and without co-occurrence of hyperacusis were compared using a questionnaire, tympanometry, acoustic reflex measurements, auditory brainstem responses, pure tone audiometry, evoked-, and resting-state fMRI in anatomically predefined brain regions.
Patient groups with tinnitus and patient groups with a comorbidity of hyperacusis were significantly different for tinnitus questionnaire (Goebel-Hiller) score, ABR waves, sound-evoked and resting-state fMRI BOLD activity.
Our results indicate that a so far undiagnosed coincidence of hyperacusis in tinnitus patients is an important confounder for studies of the neural correlate of tinnitus. These findings should guide medical tinnitus practice toward personalized therapies based on objective diagnostic markers identified for tinnitus and hyperacusis.

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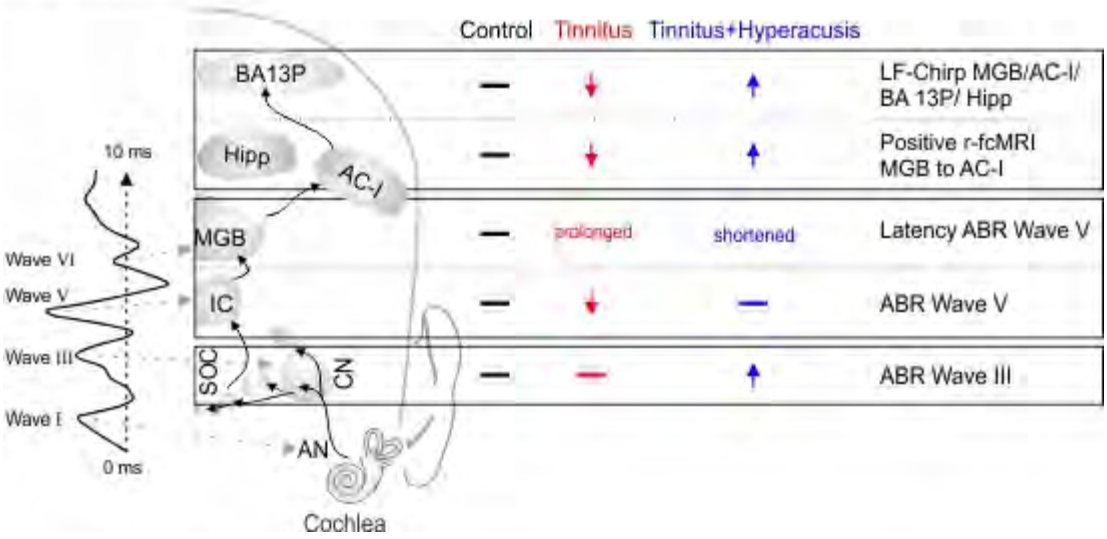
Graphical abstract



Neuromodulation in auditory thalamus upon associative fear learning

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Associative learning links sensory, predictive stimuli from the environment with their outcomes and depends on reliable integration of sensory inputs to shape behavioral adaptations and ensure an animal's survival. Several cortical and limbic brain areas have been identified as sites for associative learning. However, the role of thalamic structures that process, relay and store associative learning-related sensory information remains largely unknown. The medial geniculate body (MGB), or auditory thalamus, is a site of convergence for auditory as well as somatosensory information. It receives feedforward sensory as well as neuromodulatory input (e.g. acetylcholine). One prominent cholinergic input to MGB originates in the pedunculopontine tegmental nucleus (PPT). Acetylcholine is described as a key component to promote learning. However, the role of brainstem cholinergic inputs during associative learning remains unknown. Here we use a combination of deep brain calcium imaging as well as optogenetics to unravel the functional role of cholinergic projections in MGB during associative fear learning. We find that tonic brainstem cholinergic projections modulate sensory responses of MGB neurons during fear conditioning. Furthermore, optogenetic manipulation of cholinergic PPT inputs in MGB during fear acquisition affects associative learning. This study broadens our view on how neuromodulators contribute to aversive associative learning in thalamic areas.

Estimating electrical hearing thresholds using eCAPs as an objective measure in cochlear implanted common marmosets

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The electrical cochlear implant (eCI) is the most successful neuroprosthesis and allows for open speech comprehension in majority of more than 700,000 users. Typically, in adult humans, eCIs are fitted to the individual patient via extensive patient feedback sessions in a multistage process. In contrast, newborns' eCIs are fitted using a set of objective measures. Similar objective approaches should be automated in animal models. The common marmoset (Callithrix jacchus) has become a prime model for auditory neuroscience in general and evaluation of the eCI in particular. In this study, marmosets were unilaterally (left ear) deafened and implanted with an eCI. We evaluated two electrically evoked far-field potentials (electrically evoked auditory brainstem response, eABR; electrically evoked compound action potential, eCAP) for use in marmoset eCI fitting and compared them with behavioral data from eCI stimulation. The eABR and eCAP correspond to the synchronous activation of the auditory pathway and auditory nerve fibers, respectively. Both approaches are widely used by clinicians to evaluate changes in the peripheral auditory system without requiring patient cooperation such as in children.
In marmosets implanted with eCI, eCAPs were recorded using biphasic electrical pulses and artifact reduction methods. Employing a Go-No Go task, behavioral thresholds for electrical stimulation intensity were estimated in four marmosets. To estimate eCAP thresholds, in addition to a manual evaluation by an independent expert, we used an automated method which performs a projection to the baseline (linear extrapolation) of the linear fitting of the amplitude growth function's (AGF) ascendant part using a robust statistical method. Likewise, we have taken into consideration AGF slopes, obtained using the linear fitting.
Preliminary results showed, for the first time in this species, to our knowledge, reliable, stable, and reproducible eCAPs in four out of 5 tested animals for most channels. Moreover, eCAP thresholds obtained via the two methods (automated vs. manual) were correlated. Likewise, AGF slopes were more correlated to behavioral thresholds for electrical intensity compared to eCAP thresholds.
In conclusion, our work paves the way for future research on eCAP-based automatic fitting of eCI.

Locomotion modulates spontaneous and sensory-evoked neuronal activity in the mouse dorsal auditory field

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As we move through the environment, our sensory input is constantly updating and our spatial perspectives are changing; to survive we must perceive this dynamic environment and merge our sensory experience with motor behaviours. It has been shown that an active process like locomotion increases the sensory evoked neuronal activity in primary visual and somatosensory cortices. In contrast, there is some evidence that locomotion largely suppresses neuronal responses in the primary auditory cortex (A1), supporting a theory that there is suppression of predictable movement-generated sounds, leading to an increased sensitivity to relevant environmental sounds. However, it is unknown whether neurons in higher-order auditory fields are modulated by locomotion in the same way as in A1. In mice, neurons in association auditory fields, such as the dorsal auditory cortex (AuD), have been shown to have different physiological response properties (e.g. broader frequency tuning and a non-tonotopic organization), protein expression patterns (e.g. calcium-binding proteins, such as parvalbumin [PV], and non-phosphorylated neurofilaments) as well as thalamic and cortical connections, in comparison to A1. Further, we have previously shown that AuD is linked with the cerebellum via a direct cortico-pontine-cerebellar pathway, whereas cortico-cerebellar pathways of A1 are more polysynaptic, involving collicular and vestibular nuclei. Based on this, we hypothesize that AuD may show an increased gain in neuronal responses during locomotion in comparison to A1.

Using two-photon Ca2+ imaging in head-fixed awake mice, freely running on a treadmill, we investigated the movement-evoked neuronal activity in AuD while presenting ambient noise vs. frequency modulated sweeps as auditory stimuli. We imaged two subpopulations of layer 2/3 neurons in order to disentangle neuronal activation and/or suppression mechanisms due to locomotion, namely, excitatory (Thy-GCamp6f mice) and inhibitory populations (cre-dependent PV expression of GCaMP6). Further, we identified specific projection patterns from cortical motor regions (primary and secondary motor cortices) to AuD and A1 using a mono-trans-synaptic anterograde viral tracer. Through additional immunohistochemistry against PV, we were also able to quantify the PV positive target neurons of these projections in the auditory cortex.

We defined the proportion of auditory cortex neurons that are responsive to locomotion and classified the depth of their modulation by means of a locomotion modulation index. We found that both spontaneous (ambient noise) and sound-evoked (sweeps) activity in AuD is modulated by locomotion and that the proportion of responsive neurons and the depth of modulation is different than that observed in A1. This finding is supported by our anatomical results, since we found more projections from cortical motor areas to AuD than to A1. In conclusion, secondary auditory regions such as AuD may integrate movement-related information in a completely different way than primary areas, which may be important for down-stream auditory processing pathways (in particular to the cerebellum) during movement and acoustically guided behavior.

Anatomy of neurotransmitter-specific connections between the auditory cortex and mid- and hindbrain in Mongolian gerbils

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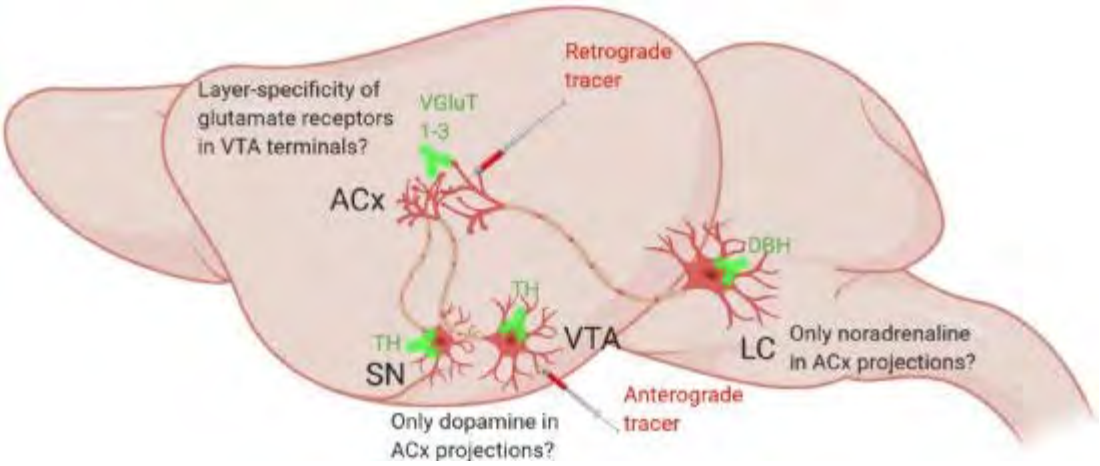
In the brain, neuronal circuits undergo permanent structural and functional changes of their connections, which is fundamental for learning and memory. Synaptic rearrangements need a reinforcing factor to promote associative learning. One key player known for learning is the neurotransmitter dopamine, which is mainly released by neurons of the substantia nigra (SN) and ventral tegmental area (VTA). Particularly the neurons in the VTA are involved in the generation of the reward prediction error, which is a main component of learning. Within this process, the dopaminergic neurons strengthen rewarding situations and weaken aversive situations to predict the outcome of a following alike situation. Likewise, noradrenaline and adrenaline from the locus coeruleus (LC) modulate distinct forms of learning such as novelty learning. Dopamine as well as glutamate from the VTA and SN, and (nor-) adrenaline from the LC are released in the cerebral cortex during sensory based learning, as has been demonstrated extensively for the auditory cortex (ACx) of the Mongolian gerbil.

Here, we are interested in the still elusive exact anatomical connections between the ACx and the VTA, SN, and LC, respectively, of the Mongolian gerbil (*Meriones unguiculatus*), a valuable animal model in neuroscientific research. Methodologically, anterograde and retrograde neuronal tracing combined with immunohistochemistry of labelled cell bodies and presynapses were used (Fig. 1). In the VTA and SN, we found 60 – 70% co-localisation between tyrosine hydroxylase (i.e. dopamine) and retrogradely labelled cell bodies, which project to the ACx. In the LC, the co-localisation of dopamine beta-hydroxylase (i.e. noradrenaline and adrenaline) and the retrogradely traced cell bodies from the ACx was around 90%. In the ACx, we found no significant co-localisation of vesicular glutamate transporters (VGluT 1-3) and anterogradely tracer-labelled VTA terminals. Our results indicate that the interplay between various neurotransmitters arising from the mid- and hindbrain to the ACx are more complex than expected and require further investigations.


Retrograde
tracer
Layer-specificity of
glutamate receptors
in VTA terminals?
VGluT
1-3
ACx
DBH
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TH
VTA
LC
Only noradrenaline
in ACx projections?
SN
Anterograde
tracer
Only dopamine in
ACx projections?

Linking endogenous oscillations to sound-driven responses in the auditory cortex

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Neural oscillations have been associated to neural selection processes as they can amplify or attenuate responses triggered by sensory inputs. In this study, we assess the contribution of endogenous oscillations to the processing of auditory stimuli occurring at unpredictable (random, nonrhythmic) time points. Our working hypothesis was that the strength of auditory responses can be predicted by the properties (phase, power, etc…) of endogenous ongoing oscillations in the auditory cortex.
Our approach allows to study how endogenous local field potential oscillations leading to stimuli presentation determine sound-driven spiking in cortical neurons.

Cortical and subcortical differential processing in forward suppression with natural sounds

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Forward suppression – a reduction in the response to the second sound in a pair of identical sounds-is considered to be important for cortical processing and/or stimulus perception. Forward suppression has been extensively studied in the context of artificial sound pairs often in anesthetized animals. In this context, responses to the second sound (probe), are reduced in comparison to responses to the first sound (masker), with recovery times ~100 ms for cortical neurons. How forward suppression operates in naturalistic contexts in awake animals that possess rich vocal repertoires remains unknown. Here, we used awake bats (*Carollia perspicillata*) and recorded extracellular activity using laminar probes (16 channels) in the inferior colliculus and auditory cortex. As stimuli, we used 8 natural distress sequences recorded from the same bat species, which consisted of seven syllables and which differed in the silent time between the second and third syllable of the natural sequence. We found that cortical neurons had a greater decrease in response due to recent stimulation history, i.e. had a longer lasting suppression, and that the inferior colliculus neurons barely suffered the effects of the masker syllables. Our results support the notion that cortical suppression stems mostly from thalamic input and intracortical circuits. Although they were described using artificial sounds, our results indicate that forward suppression circuits do influence the way natural sounds are processed in the brain.

Individual and group-level flexibility in social vocalization timing in bats

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Bats are highly gregarious and vocal animals that live in groups of up to several hundred individuals. In addition to sonar vocalizations, used for navigation, bats exchange social calls in highly enriched, acoustically challenging environments. How individuals are able to overcome this challenge to communicate effectively is little understood. Previous work has demonstrated that adult bats are able to modify distinct parameters of their vocalizations, notably amplitude and frequency parameters, in response to acoustic playback of sonar calls and broadband noise, respectively. This vocal plasticity theoretically aids in reducing acoustic interference between bats' own calls and environmental noise, thus improving the transmission quality of vocalizations. Our study examines the degree to which individuals and groups of individuals are able to apply similar signal-optimizing strategies in the temporal domain, i.e. whether they are able to adapt the temporal rate of social calling in order to exploit the temporal regularity of noise in their environment. Our findings suggest that individuals tend to vocalize in silent periods between bouts of playback distress sequences, as well as in the brief silences between distress syllables, revealing sensitivity to external rhythms and temporal flexibility in social vocal production. We predict these findings are the result of neural entrainment to external temporal patterns, as well as synchronization of oscillatory activity between key structures in the vocal-motor network.

Female zebra finch presence shapes juvenile song performance and premotor circuitry

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Learned motor behaviors are often shaped by social influences. Human infants learn their first words by imitating their parents and observing their feedback. Similar to human vocal learning, young zebra finches learn their song from their fathers. To some extend young birds are also able to learn to imitate from playbacks of tutor song. However, the contribution of female zebra finches to song learning is less clear. We 1) investigate whether the presence of a female supports song learning, 2) characterize the vocal feedback of females in response to song performance and 3) measure the impact of female feedback onto the premotor circuitry. We find, that female zebra finches have an impact on the learned song tempo i.e. zebra finches, that were raised with a female copied song tempo more accurately compared to birds, that grew up socially isolated. Female birds give feedback by calling before, during or shortly after a song practiced by a juvenile. We performed intracellular recordings in awake and listening juvenile zebra finches while presenting playbacks of songs that were accompanied with female calls. We found that a female call had the potential to elicit changes in spike firing rate and subthreshold activity in premotor neurons. We hypothesize that female feedback contributes to neural plasticity within a premotor circuit which leads to improved learning of the song tempo in juvenile zebra finches.

A continuum of biophysical time scales of neurons in the intermediate nucleus of the lateral lemniscus

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The intermedial nucleus of the lateral lemniscus (INLL) is an auditory brainstem structure within the fibre bundle of the lateral lemniscus. The INLL receives inputs from the cochlear nuclei and all major nuclei of the superior olivary complex. This connectivity suggests that INLL neurons process complex auditory information, which is fed forward to the inferior colliculus. Little is known about the functional role of the INLL and the basic biophysical characteristics of these neurons have not been studied yet.

Here we describe the biophysical input-output functions and synaptic properties of INLL neurons of Mongolian gerbils of 25 – 40 days of age. Using patch-clamp recordings from visually identified neurons in acute slices we observed a wide range of firing patterns as well as heterogeneous biophysical membrane properties, contrasting the typically homogeneous characteristics of other auditory brainstem nuclei. The firing behaviour included onset, adaptive to sustained firing patterns. The type of firing pattern appeared to be correlated with the membrane time constants, which stretched over three magnitudes (0.4 to 150 ms) as a continuum.

Since the membrane time constant (τ_{mem}) is a critical factor of the neuron's integrational properties, we probed the biophysical and synaptic parameters for correlations with τ_{mem}. The timing of generated action potential of neurons with different firing patterns correlated with τ_{mem} and matched differences in the subthreshold voltage excursions and current thresholds. Furthermore, the properties of synaptic inputs were assessed by recording inhibitory and excitatory evoked postsynaptic currents as well as their spontaneous events. The kinetics of these currents appear to correlate with the respective τ_{mem} of the neuron. Other cellular parameters, such as action potential induced calcium influx or dendritic morphometry showed no significant correlation with τ_{mem}.

The heterogeneous biophysical properties as well as the correlating kinetics of the postsynaptic currents indicate that the different neurons operate with different integration time windows and might utilize different computation modes. However, no spatial arrangement in a dorsoventral or mediolateral axis could be observed and therefore the heterogeneity seems to be independent of the INLL's proposed tonotopy. Thus, the INLL neurons might be able to process varying temporal features of the auditory information on a broad range of timescales, possibly serving as integrators of different sound frequencies or patterns.

Dendritic morphometry of principal neurons in the medial nucleus of the trapezoid body

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The medial nucleus of the trapezoid body (MNTB) is one of the major nuclei in the superior olivary complex of mammals. It is functionally viewed as a relay station between the cochlear nucleus and the medial and lateral superior olives, playing a pivotal role in binaural processing. At least in rodents, the MNTB is tonotopically organized along its medial-lateral axis. MNTB principle neurons show functional and morphological similarities across mammalian species. Their input-output function is dominated by the large somatic calyx of Held synapse originating from neurons in the cochlear nucleus. Moreover, principle cells possess one to three thin primary dendrites, whose function and input pattern is largely unknown. So far, there is no quantitative morphometry of the dendritic structure of MNTB neurons in any species.
We, therefore, perform a quantitative analysis of MNTB dendrites using 3D reconstructions of single electroporated neurons combined with immunofluorescent labeling. We extend this experimental paradigm to a comparative approach, where we seek species differences between low and high frequency hearing mammals including the Northern treeshrew (*Tupaia belangeri*), gerbil (*Meriones unguiculatus*), mouse (*CBA/J*), Etruscan shrew (*Suncus etruscus*) and bat (*Carollia perspicillata*).
In all species, immunoreactivity for vesicular glutamate transporter 1 and 2 identifies calyceal terminals surrounding somata of MNTB principal neurons. With this labeling, species-dependent shapes and proportions of this nucleus were extracted. Principle cells in all species have a directionality of their partially wide spread dendritic fields often extending far beyond the borders of the MNTB. Dendrites in mice and Etruscan shrews tend to have comparatively shorter lengths. Principle neurons in tupaia, gerbil and mice have an average number of two primary dendrites, while those in Etruscan shrews and bats are biased to one. The number of dendritic nods and endings appears to be similar between the tested species except for Etruscan shrews, manifesting in considerably less branching. Together, these findings imply that there are species-specific differences in the structure of MNTB dendrites. We further correlated dendritic structure with soma size and the location within medial-lateral axis of the MNTB. These data indicate that dendritic parameters in gerbil are associated with soma size and tonotopic location, while in bat no correlation with tonotopy is apparent. We therefore may speculate about a species-specific cue for dendritic pattering by hypothesizing, that sound frequency during development might only be relevant in some species. Moreover, immunofluorescent labeling showed that MNTB dendrites receive glutamatergic and glycinergic input and, at least in gerbils, express voltage-gated $K_V1.1$ potassium channels. Thus, MNTB dendrites are active cellular compartments implying functional significance.

The extracellular matrix regulates cortical layer dynamics and cross-columnar frequency integration in the auditory cortex

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In the adult vertebrate brain, enzymatic removal of the extracellular matrix (ECM) is increasingly recognized to promote learning, memory recall, and restorative plasticity. The impact of the ECM on translaminar dynamics during cortical circuit processing is still not understood. Here, we removed the ECM in the primary auditory cortex of adult Mongolian gerbils using local injections of hyaluronidase (HYase). Using laminar current-source density (CSD) analysis, we found layer-specific changes of the spatiotemporal synaptic activity patterns with increased corticocortical integration in supragranular layers with simultaneous weakening of early local sensory input processing within infragranlar layers Vb. The spatiotemporal synaptic changes were associated with an oscillatory fingerprint in the beta-band (25-36 Hz) selectively within infragranular layers Vb. To understand the laminar interaction dynamics after ECM digestion, we used time-domain conditional Granger causality (GC) measures, revealing a stronger drive from supragranular layers I/II into late infragranular layer VI, which also showed increased drive towards early infragranular layer Vb. These results show that ECM degradation caused an altered translaminar cortical network dynamic with stronger supragranular lead of the columnar response profile and increased cross-columnar frequency integration (El-Tabbal et al., 2020; bioRxiv).
Recently, we further described that behavioral auditory training leads to transient downregulation of the ECM protein brevican in auditory cortex (Niekisch et al., 2019; J Neurosci.). Brevican degradation has been shown to depend on dopaminergic neuromodulation via activation of the protease ADAMTS-4/5 (Mitlöhner et al., 2020; Cells).
Our hypothesis is that during acquisition learning, dopamine release triggers an initial downregulation of the ECM to optimize the dynamics of corticocortical networks for synaptic plasticity. We speculate that in the adult brain, such learning-dependent regulation of the ECM needs concomitant action of reinforcing dopaminergic neuromodulation coding the behavioral relevance of events.

Object specific Adaptation in the Auditory Cortex of Bats

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To quickly differentiate stimuli is an important function of the auditory system. Especially echolocating bats have to negotiate incoming auditory stimuli in the context of navigation, predator perception, foraging and background noise. Given that bats have to filter auditory stimuli quickly for relevancy during flight, we assume that there is a neuronal filtering mechanism at play, such as Stimulus Specific Adaptation (SSA). SSA is a process whereby the excitability of a given neuron is reduced by prolonged exposure to the same stimulus, but not to others (Khouri and Nelken 2015). Such a mechanism could account for quick differentiation between behaviourally important information from background clutter.
To test this, we played sequences of echoes of virtual objects to anaesthetised bats of the species *Phylostomus discolor*, and recorded extracellular responses in the auditory cortex (AC) The stimuli were generated by convolving a *P. discolor* echolocation call with artificially generated impulse responses, creating virtual echoes differing in spectral and temporal structure, but having the same overall duration (6 ms) and RMS amplitude. The virtual echoes were then arranged in sequences, whereby one virtual object echo was repeated ten times (standard echo) and a second different virtual object echo was played in the end once (deviant echo). Sequences were generated with different inter pulse intervals (IPI:10 to 100 ms) and presented with 20 repetitions.
We recorded from 44 cortical units. In 48 % (21/44) of these units responses adapted to the standard echo, but showed a strong response to the finally presented deviant echo. This effect was strongly IPI dependent. These results suggest that neurons in the AC of bats show specific adaptation to echoes from virtual object with different spectro-temporal properties. In a behavioural context, these neuronal properties might facilitate the detection of rare object while bats forage on the wing in complex environments.

Characterization of the auditory spatial receptive fields in the optic tectum of the domestic chicken (*Gallus gallus domesticus*)

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The ability to locate a sound source is crucial for the survival of birds. During the last decades, the research in avian auditory neuroscience has mainly focused on the barn owl, which has unique peripheral specializations and hypertrophied brain structures which enable outstanding localization performances. However, little is known about the neural representation of the auditory space in generalist birds - i.e., the birds with symmetrical ears – which do not show comparable auditory capabilities. Therefore, we investigated auditory processing in a presumably multimodal midbrain area involved in the avian auditory pathway, namely the deep layers of the optic tectum in the chicken.

We conducted *in vivo* extracellular recordings of single units in the optic tectum of anaesthetised chickens, while presenting binaurally broadband noise filtered by the head-related transfer function (HRTF), in order to present sounds coming from specific locations within the virtual auditory space (VAS). Thus, it was possible to characterize the auditory spatial receptive fields (aSRFs) of these neurons. Moreover, we presented binaural broadband noise manipulating either the interaural time difference (ITD) or the interaural level difference (ILD), in order to investigate the contribution of these acoustic cues in the formation of the aSRFs.

We found two main types of aSRFs: round-shaped and ring-shaped (a form we called ‘annulus’). Most of the units showed receptive fields located on the contralateral hemifield and ITD and ILD tuning to contralateral values. However, the annulus aSRFs showed significantly shorter best ITDs compared to the round aSRFs, suggesting that the ITD tuning is crucial to the formation of the aSRF shape. Moreover, both aSRF types are centred to the interaural axis, i.e., at 90° in azimuth and 0° in elevation. These results suggest a concentric arrangement of the receptive fields around the auditory and visual axis which, as far as we know, has never been described before. Altogether, these results lead to some speculations about the way a sound source is perceived and localized by the generalist birds.

Neuronal representation of spectral and temporal song features in juvenile zebra finches

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Similar to human language acquisition, juvenile male zebra finches learn their song by copying an adult tutor bird during early development. This complex vocal learning task requires a combination of memorizing an auditory template, vocal practice, feedback integration and error detection. During this learning process, juvenile song renditions fluctuate broadly in both, the spectral and temporal domain. Once birds reach adulthood, their song becomes crystalized and remains unchanged for the rest of their life. Adult song motifs consist of individual syllables with stereotyped spectral features and syllable sequences. How these characteristics are represented in the brain of a juvenile bird while it is aiming to master the complex sensory-motor task remains unclear. The premotor nucleus HVC (proper name) is known to be involved in both song learning and production and assumed to be homologous to parts of mammalian premotor cortex. HVC receives inputs from auditory areas and relays information to Area X in the anterior forebrain pathway, which is involved in early song learning and to the motor output nucleus RA (Robust nucleus of the arcopallium), responsible for output towards vocal muscles. Both of these projection neuron types are known to develop a precise and stereotyped firing pattern during song production, and perception in juveniles. However, these cells remain silent during tutor song stimuli in adult males. Local inhibitory interneurons are known to protect learned parts of the song by gating off auditory input.

We hypothesized that the sparse song encoding of premotor neurons in HVC is achieved through the refinement of local inhibitory network activity and that individual song features, especially song timing, are encoded locally in HVC. To test this hypothesis, we used a set of spectral and temporal playback modifications based on the birds own song. We performed intracellular and extracellular recordings while presenting these stimuli to head fixed, awake birds, to shed light on the differences of neuronal representations of song syllables and features in HVC across developmental stages.

We were able to demonstrate that unlike adult projection neurons, juvenile projection neurons do not only respond to birds own song, but encode syllables' identity, independent of their position within a motif. These responses are represented by substantial changes in firing rates as well as spiking precision during a syllable. We further show, that inhibitory interneurons display similar activation patterns in both juvenile and adult birds. Bursts in neuronal activity are time-locked to individual syllables and follow their respective syllable.
To further support our findings, we pharmacologically disrupted the inhibitory network in adult birds using a GABAA-Inhibitor to render projection neurons vulnerable to auditory input again. We were able to show that in combination with our playback paradigm, birds rapidly started to diverge from their learned song structure, incorporating new syllables and producing altered syllable sequences.

Together, these findings indicate that the HVC local network is sensitive to auditory input and responsible for

protection and execution of the temporal dynamics underlying song production and learning. While in adult birds, auditory feedback is generally tuned out by the refined local inhibitory system, juvenile birds still require this feedback to adjust and improve parts of their vocal output pattern.

Optogenetically controlled aggregation of calcium channels in the auditory cortex causes deterministic population dynamics and suppressed impulse responses

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Neuronal communication is critically mediated by the release of chemical transmitters from presynaptic vesicles. This neurotransmitter release is controlled by voltage gated calcium channels (VGCCs) which trigger the influx of calcium ions to the presynaptic bouton upon action-potential-induced membrane depolarization. However, intracellular calcium is strictly controlled and immediately buffered from the intracellular space due to its substantial function as a second messenger molecule. The transient and local action of calcium requires a close proximity between vesicular calcium sensors and the VGCC pore, known as nanodomains, to efficiently initiate vesicle fusion and transmitter release. The probability that enough channels are perfectly situated in nanometer distances in the moment when an action potential arrives in the presynapse is not 100%. This lack of determinable neurotransmitter release by action potential is part of what gives us a probabilistic firing rate. Network oscillations emerge naturally out of a system like this. When neuronal VGCCs were acutely aggregated using the optogenetic clustering of an cryptochrome mutant , CRY2olig[1], under blue light, this probabilistic firing mode was shifted to a deterministic one[2]. Upon VGCC clustering, cultured primary hippocampal neurons generated a strong and reliable paired-pulse depression of consecutive responses while control neurons showed almost no reduction in response amplitudes during the train of stimulation. While this method was used in vitro to render a single cell from a more random to a more predictable firing mode, the question we sought to answer was how this would affect a neuronal network. For this purpose, we have combined the usage of CRY2olig with a transgenic mouse model that allows for the optogenetic clustering of VGCCs in vivo. In particular, this knock-in line expresses a Citrine tag, a YFP/GFP derivate, at the N-terminus of the neuronal VGCC type $Ca_V2.1$[3]. This Citrine tag, and in turn the $Ca_V2.1$ channel, were coupled to Cry2olig via a feed-back-controlled anti GFP-intrabody which was transduced to the primary auditory cortex (A1) in a lentivirus. We recorded local field potentials down the depth of the A1 using a 32-channel shaft electrode and transformed the output into current source density (CSD) profiles over consecutive measurements under ketamine-xylazine anesthesia. Cortical response to auditory stimuli in the form of click trains and amplitude modulated tones was recorded, as well as spontaneous brain activity. We demonstrated through cortical layer activity and average rectified CSD profiles that there is a significant suppression of the impulse response to click stimuli. Interestingly, response

to amplitude modulated tones and spontaneous activity did not show this same level of suppression. With applying the more elaborate spectral analysis of power and phase coherence of oscillations in layer-specific neuronal activity, we aim to disentangle the influences of VGCC dynamics on the dominant impulse response, provided by click stimulation, and on the more temporally dispersed coordinated response characteristics, represented in response to amplitude modulation.

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Central stress responses account for auditory nerve sensitivity

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Stress comprises two contrasting effects on the auditory function: On the one hand, stress related glucocorticoids (GCs) are protective for the hearing organ during damaging situations. On the other hand, chronic stressors are also risk factors for hearing disorders, as they negatively influence tinnitus symptoms, among others. The link between both contrasting stress actions has remained elusive. We hypothesize that the two different stress receptors mineralo- (MR) and glucocorticoid receptors (GR), which translate the different physiologic stress responses depending on the level of the stress, play a guiding role via top down, central auditory to peripheral cochlear, signaling.

We performed a timed conditional deletion of stress receptors in adult tamoxifen inducible MR- and/or $GR^{CaMKII\alpha Cre}$ KO mice, which leads to selective deletion of the stress receptors in the frontal brain regions (e.g. hippocampus) within 4 weeks, and we analyzed the functional consequences in the hippocampus and the auditory pathway using LTP, DPOAE, ABR thresholds and supra-threshold waves, ASSR, and CAP. Cochlea and brain tissue were immunohistochemically stained with specific markers for changes in excitation and inhibition.

Much to our surprise, we found that the central deletion of MR and/or GR in frontal brain regions exhibits a top down influence on peripheral auditory fibers. Therefore, peripheral auditory function of cochlear hair cells and neurons is most likely under control of centrally occurring responses to stressful damaging situations mediated by differential activation of GC receptor types.

Can tinnitus and tinnitus with co-occuring hyperacusis be reflected in high-frequency brain oscillations?

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Tinnitus (a constant phantom humming or ringing) and hyperacusis (the noisy or even painful perception of moderate sounds) presenting a major global burden with a preavalence of 10% to 20% in the population. These impairments in everyday life can strengthen or even evoke affective disorders such as depression, anxiety and insomnia.

Currently, conflicting views on the neural correlate of tinnitus hinder the discovery of curative therapies for tinnitus. Hyperacusis can co-occur with Tinnitus but until now hyperacusis is not considered with clinical routine, differential diagnostic and individualized therapies. We hypothesize that the variability of successful tinnitus therapy is to the most part generated by our insufficient knowledge about the neural correlate of tinnitus. The successful individualized therapy of both subentities of tinnitus requires differentiation, identification and classification of hearing disorders by objective tools.

Based on previous suggestions that link reduced and delayed auditory processing and reduced evoked and resting state BOLD fMRI responses with tinnitus ,(1,2) we here aim to investigate defined frequency bands of neural oscillations with a combined NIRS/EEG approach in different patient groups. Selected EEG frequency bands are discussed as objective tools to identify tinnitus subentities required for implementing successful therapeutic intervention strategies.

FUNDING
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Studying the hearing of bats in a minimally invasive way: ABRs and FFRs to simple and complex tones in the bat species *Carollia perspicillata*

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Recording techniques used to study the auditory system of animals are usually invasive and require a considerable amount of time and methodological efforts. In contrast to this, the procedure of measuring auditory brainstem responses (ABRs) and frequency following responses (FFRs) is an easy means to assess the functionality of the early stages of the hearing system, i.e. auditory nerve, brainstem and midbrain. In our study, we recorded ABRs and FFRs in the bat species *Carollia perspicillata*, a hearing specialist sensitive to a broad range of auditory stimulus frequencies and thus serving as an excellent model organism for auditory research. ABRs to simple pure tones as well as to more complex stimuli with more behavioural relevance were recorded with the aim to investigate which role the earliest stages of the auditory pathway play in encoding those stimuli. Additionally, FFRs to amplitude modulated (AM) tones were recorded by the same minimally invasive recording technique, providing evidence for the ability of the bat brain to follow AM frequencies of more than 1200 Hz. At AM frequencies around 100 Hz, the representation of the stimulus in the brain shifted from a time-locked to a frequency-locked coding of the AM frequency. To sum it up, we used a simple yet potent method to obtain data showing how auditory information is represented in the earliest stages of the auditory system of a hearing-specialised animal (bat).

Poster Topic

T19: Chemical Senses: Olfaction, Taste, Others

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Sudeshna Das Chakraborty, Hetan Chang, Bill S. Hansson, Silke Sachse

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Julie Carcaud, Virginie Larcher, Gabriela de Brito Sanchez, Martin Giurfa, Jean-Christophe Sandoz

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T19-5 Metabolic state dependence of olfactory cortex flavor representation during binge eating
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T19-11 Neuromodulator diversity in the central olfactory pathway of *Parhyale hawaiensis* (Dana, 1895)
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T19-16 Spontaneous spikes of mitral cells in a semi-intact preparation of the rodent olfactory bulb are modulated by intrinsic theta oscillations
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T19-17 Fast dynamics of the odour landscape are encoded in the mouse olfactory system and guide behaviour
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T19-18 Functional study of the queen pheromone receptor OR11 in honey bees within the genus Apis.
Julia Mariette, Amélie Noël, Virginie Larcher, Chloé Petitimbert, Julie Carcaud, Thomas Chertemps, Nicolas Montagné, Emmanuelle Jacquin-Joly, Frédéric Marion-Poll, Jean-Christophe Sandoz

T19-19 Humidity sensing in *Drosophila melanogaster*
Kristina Corthals, Anders Enjin

Odor representation by a glutamatergic subset of lateral horn neurons in *Drosophila melanogaster*

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Understanding stimulus representation and its progressive transformation from sensory level to the higher brain centers has been a main focus in the field of olfactory neuroscience. In *Drosophila*, the functional understanding of odor representation by third order neurons in the lateral horn, one of the higher olfactory centers implicated in innate behavior, is lacking. In this study, we focused on the glutamatergic subset of lateral horn neurons (LHNs). We could show that every odor evokes a reproducible and stereotyped response pattern in the LH. Along with the spatial pattern, the odors with positive valence (attractive odors) evoke stronger activity in these glutamatergic LHNs compared to the negative ones (aversive odors). The activity of these neurons to attractive and aversive odors correlates positively with their degree of behavioral response. While this differential level of activity is not emerging at the sensory level, we postulate that this valence-code results from processing mechanisms at the second order neuronal level. Morphological and functional studies provide clear evidence that these neurons receive the majority of the input from excitatory projection neurons (ePNs) and an odor specific inhibition from the inhibitory projection neurons (iPNs). iPN mediated inhibition onto these glutamatergic LHNs is necessary for odor discrimination. In summary, our study indicates that this glutamatergic subset of LHNs might be involved in coding of odor valence and in maintaining odor identity.

Anatomy of central gustatory circuits in the honyebee brain

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Taste allows animals and humans to discriminate edible from non-edible items and is, therefore, crucial for survival. How taste information is encoded and modulated in the central nervous system is important both for the field of neurosciences and for managing food intake in species that play a major role in agriculture and food production. In the honey bee, an insect that is both a well-established model for neuroscience studies and a key species for crop pollination, the sense of taste has remained largely unexplored despite intensive studies on this insect' other sensory modalities (olfaction, vision).
Electrophysiological recordings showed that gustatory receptors, located on the antennae, the mouthparts and the tarsi, respond with varying sensitivity to sugars, salts, and possibly amino acids, proteins and water. However, the sequencing and annotation of the honey bee genome revealed a surprising scarcity of gustatory receptor genes (GRs) compared to other insect species, which possess several dozens of GRs. In the honey bee, only 10 functional GRs have been identified so far. How taste sensations arise from such a reduced number of gustatory input channels remains unknown and calls for a thorough analysis of central taste processing in the bee brain.
Here we report neuroanatomical reconstructions of central gustatory circuits of the honey bee. We described the output neurons of the subesophageal zone (SEZ), the primary gustatory center, and confirmed the Subesophageal-Calycal Tract (SCT) as the main output of this structure. We also performed retrograde staining of the SCT coupled to anterograde staining of the antennae or proboscis, which allowed us to draw up a map of sensory inputs to the SEZ. We show that SCT dendrites overlap with sensory input from the proboscis but not the antennae in the SEZ. Using specific staining with calcium-sensitive dyes (Oregon-Green dextran), we are currently developing a preparation to perform optophysiological recordings of the SEZ during gustatory stimulations.

Female Drosophila respond to ejaculate with copulation song

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In most animal species, males and females communicate during sexual behavior to negotiate reproductive investments. Pre-copulatory courtship may settle if copulation takes place, but often information exchange and decision-making continue beyond that point.
We have shown that female Drosophila sing by wing vibration in copula. This copulation song is distinct from male courtship song and requires neurons expressing the female sex determination factor DoublesexF. Copulation song depends on transfer of seminal fluid components of the male accessory gland. Playback of female copulation song to a mating couple increases the time the female takes to remate with subsequent males and thereby increases the reproductive success of the first male. This suggests that auditory cues from the female modulate male seminal fluid transfer (strategic ejaculate allocation).
We hypothesize that female copulation song serves as a signal in mate choice, giving the female the opportunity to influence the composition and postmating effect of ejaculate.
Our findings reveal an unexpected fine-tuning of reproductive decisions during a multimodal copulatory dialog. The discovery of a female-specific acoustic behavior sheds new light on Drosophila mating, sexual dimorphisms of neuronal circuits and the impact of seminal fluid molecules on nervous system and behavior.

Current efforts are directed at identifying which seminal fluid components affect female song (mass-spectrometry), as well as uncovering the receptors and sensory neurons in the reproductive tract necessary for rapid ejaculate evaluation by the female.

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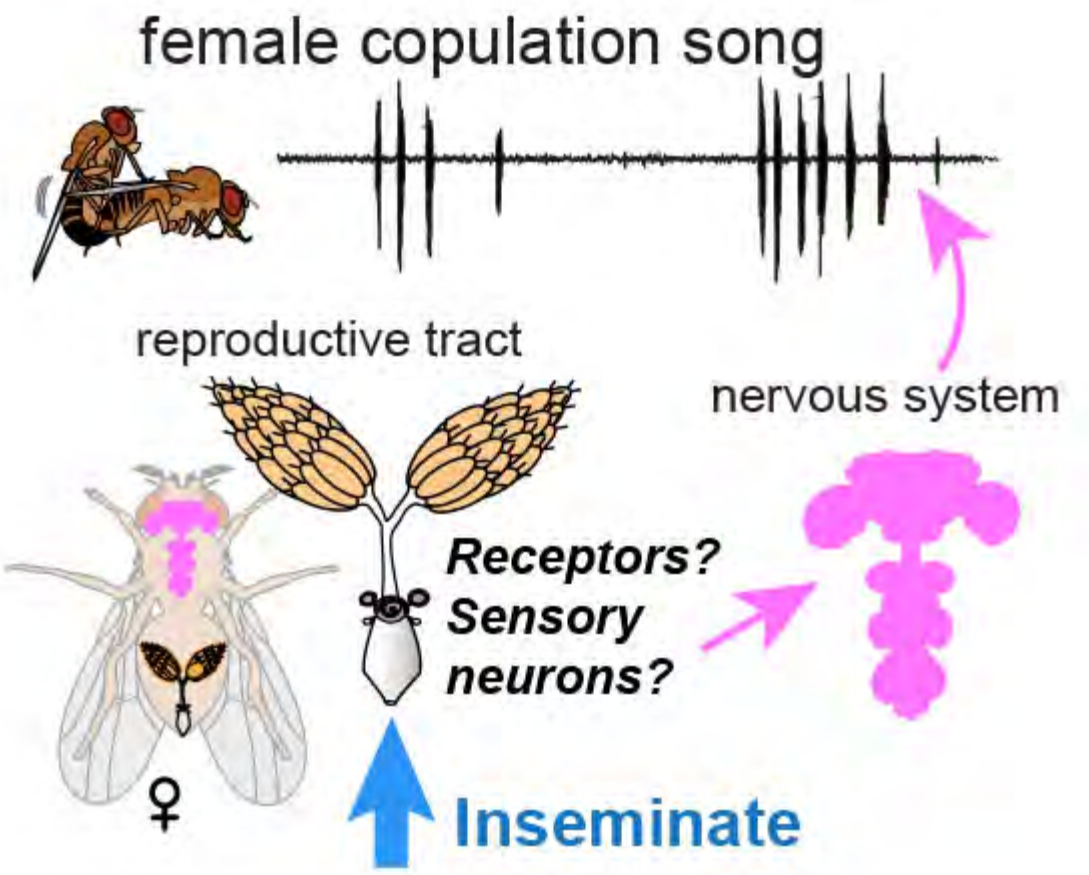
female copulation song
reproductive tract
nervous system
Receptors?
Sensory
neurons?
Inseminate

Cholinergic calcium responses in cultured antennal lobe neurons of the migratory locust.

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The antennal lobe is the primary olfactory center in insects, similar to the olfactory bulb in mammals. Sensory input is provided by the olfactory receptor neurons that synapse with projection neurons and local interneurons in neuropilar compartments termed glomeruli. Most investigated insects, like for example the genetic model Drosophila, employ an uniglomerular wiring strategy, in which olfactory receptor neurons expressing the same olfactory receptor genes converge within the same glomerulus in the antennal lobe. In contrast, locusts use multiglomerular wiring, arranging axonal branches of single olfactory receptor neurons to innervate multiple glomeruli. The dendrites of each projection neuron, in turn, sample input from several glomeruli.
In the species Locusta migratoria, several quantitative neuroanatomical features of the antennal lobe, like number of cells and the percentage of local neurons have not been reported in the literature. We processed tissue sections of Locusta for GABA immunocytochemistry and counted the total number of cells in the antennal lobe. Under the assumption that somata/nuclei of all local neurons are of similar size as those of GABAergic local neurons, we estimated that 24% of antennal lobe neurons are local neurons. Moreover, we established a binary classifier for a discrimination between local and projection neurons.
So far, cellular properties of neurons isolated from the circuitry of the olfactory system, such as transmitter-induced calcium responses have not been reported. Based on immunocytochemical investigations of several working groups, the majority of antennal olfactory receptor neurons are thought to use acetylcholine as classical transmitter. We performed calcium imaging of dissociated antennal lobe neurons in cell culture by superfusing cholinergic agonists and antagonists. Inhibitory local neurons show a higher responsiveness to muscarinic stimulation than projection neurons, which has also been found in the intact Drosophila brain (Rozenfeld et al., 2019). In contrast to Drosophila, we also observed calcium responses to muscarinic stimulation in projection neurons. Furthermore, we detected an increased nicotinic responsiveness after nicotinic/muscarinic co-stimulation in local neurons, lasting up to a few minutes. This cellular characteristic was not found in projection neurons. Our results indicate similarities and differences in transmitter responses between antennal lobe neurons of the two insect species, that show striking distinctions in the wiring pattern of the olfactory system.

Reference:
Rozenfeld, E., Lerner, H., and Parnas, M. (2019). Muscarinic Modulation of Antennal Lobe GABAergic Local Neurons Shapes Odor Coding and Behaviour. Cell Reports 29, 3253-3265.

Metabolic state dependence of olfactory cortex flavor representation during binge eating

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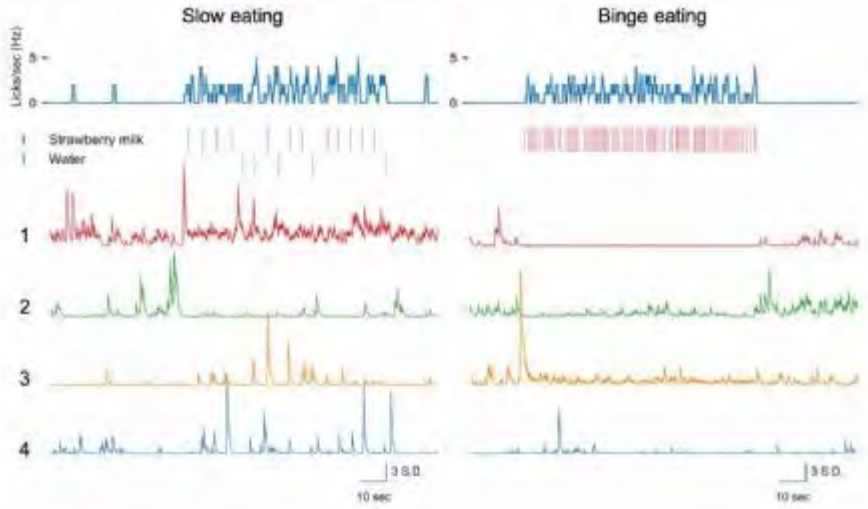
Energy homeostasis is achieved by the complex regulatory network that involves a feedback circuit between dietary intake behavior and nutritional state. The uncoupling of dietary intake and nutritional state results in overeating. Hedonic overeating can escalate into binge eating, a heritable trait associated with binge eating disorder and bulimia. One factor that modulates this feedback loop is the sensory perception of flavors. Olfaction is a critical element of flavor perception and greatly influences the qualitative and hedonic evaluation of food. Patients with olfactory chemosensory deficits commonly report a reduced appreciation of food items. Current evidence suggests that upon fasting, the olfactory system is necessary for triggering fasting-induced overeating. However, it is unclear how adaptations related to binge eating are reflected by the olfactory flavor responses.

To understand the relationship between olfactory perception and binge eating, we developed a hedonic feeding paradigm that repeatedly delivers hedonic food (flavored milk) to mice. We controlled the duration of inter-trial-intervals so that mice can only activate the lick sensors and feed themselves with slow or fast paces. Implementing this paradigm with a head-mount miniaturized microscope allows us to measure neural responses upon slow eating or binge eating in the olfactory cortex (anterior piriform cortex, aPC) in freely moving mice. We found very distinct neuronal responses in the aPC upon slow eating and binge eating. During slow eating, we observed different populations of excitatory piriform neurons responding to flavored milk and water. However, during binge eating, the majority of excitatory neurons are suppressed in the aPC. To decipher whether inhibitory interneurons are involved in this binge-induced global suppression, we performed recordings in interneurons. Results from PV^+ interneurons suggested that they are not a major contributor to this global suppression. Whether this implies the involvement of other inhibitory neurons during binging and/or whether the flavor representation adapts during the escalation of binging are the next steps of our examination.

Freely-moving Ca^{2+} imaging in the anterior piriform cortex during eating and binge eating



Neuropeptide profiling of the *Manduca sexta* antenna

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[2]authors contributed equally to this work

Hawkmoth *Manduca sexta* females attract their mates via release of a species-specific sex pheromone blend that the males detect via pheromone-sensitive trichoid sensilla type 1 forming a V-shaped phalanx on each annulus of their antennal flagellum. Located between the field of trichoid sensilla there are other chemosensory sensilla such as sensilla basiconica that detect general food odors. While trichoid sensilla are innervated by two olfactory receptor neurons (ORNs), sensilla basiconica are mostly innervated by three ORNs. In all antennal chemosensory sensilla non-neuronal supporting cells form the cuticular hair shaft and the sensillum-lymph space. The supporting cells also secrete pheromone/odorant-binding proteins into the sensillum lymph for the sensitive detection and transport of lipophilic odorants through the aqueous sensillum lymph to odor receptors on the ORN´s cilia. With immunocytochemical, biochemical, and electrophysiological techniques we examine whether neuropeptides are present in antennal cells that might modulate pheromone/odor transduction in hawkmoths. Recently, an extensive set of gene families involved in olfaction were identified by transcriptomic analysis of *M. sexta* antennae from both males and females. However, expression information of neuropeptides was missing. As prerequisite to a functional analysis of neuropeptides in olfaction, we started to identify the neuropeptidome of the hawkmoth antenna by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) combined with immunocytochemistry. Immunocytochemical stainings employing antisera against myoinhibitory peptides (MIPs), and Mas-allatotropin (AT) showed immunoreactivity in different antennal cells. Anti-MIP antisera stained subgroups of ORNs of pheromone-sensitive sensilla and larger accessory cells at the area of sensilla basiconica.In contrast the AT-like immunoreactivity appeared to be confined to the larger accessory cells, but not to the smaller ORNs of a subgroup of sensilla basiconica. With direct antennal tissue profiling by MALDI-TOF MS we detected an ion signal corresponding to AT which confirmed our immunochemical data, while so far no ion signals for MIPs was located to hawkmoth antennae. Furthermore, preliminary BLAST searches in the *M. sexta* antenna transcriptomes (accession no. GSE27470) yielded the expression of *ion transport peptide-like* and *ion transport peptide* genes. Further immunocytochemical and electrophysiological studies test the location and function of these neuropeptides in the olfactory system of hawkmoths to determine whether physiological state or circadian rhythms modulate olfaction neuropeptide-dependently already at the periphery. [Supported by DFG grants STE531/20-1,2 of the SPP1392 to MS and by the graduate college “cellular clocks” P/879 of the University of Kassel to MS]

Activity patterns in a wild population of the electric fish *Apteronotus macrostomus* and *Eigenmannia sp.*

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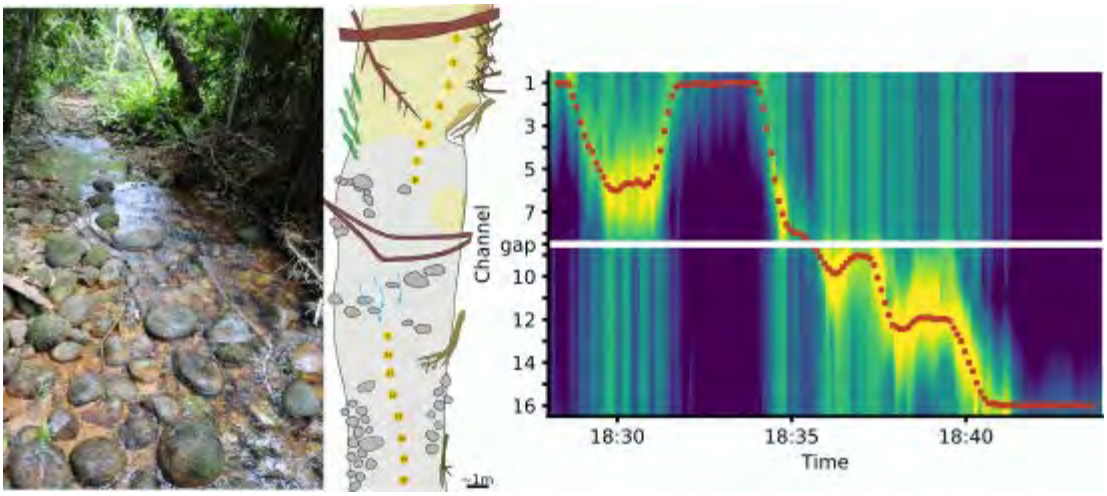
Characterizing both the statistics of natural sensory stimuli and of their behavioral relevance is crucial for understanding functional adaptations of neural systems. While laboratory studies allow for very precise characterizations, field observations are needed to put potential stimuli into the context of the habitat where the organism of interest evolved to. Electric fish are well suited for observations in their natural habitats, because they continuously send out an individual specific ID with their electric organ discharges. These self generated fields can be measured by arrays of electrodes placed in their habitats. This way, both movement patterns and electrocommunication signals can be tracked for each fish present in the array. In this study we were interested in movement and activity patterns over a longer section of a little neotropical stream. For this we distributed 16 electrodes along a line within a 20 m section of a side arm of the Rio Canocamoa near San Martin (Department Meta, Colombia), covering a variety of micro habitats including stones as well as roots as suitable resting sites and sections of sandy river beds void of hiding places (Fig. 1). We continuously recorded the activity of the two species *Apteronotus macrostomus* (of the *Apteronotus leptorhynchus* species group) and *Eigenmannia sp.* for 4 consecutive days outside of breeding season (October 2019). Species affiliation was determined using EOD frequency and waveform. From the recordings we were able to track electric signals of individual fish over more than 12 hours. The majority of the fish we detected in the recordings were of the species *A. macrostomus* (n=154). All of them were resting between stacked stones in the shallow part of the stream for most of the time (84.32 %) and only for short periods of time (18.9 min ± 37.9 min) they explored the vicinity of their resting site within 4.4 m ± 1.8 m. Consequently, the overall movement activity is low, but increases in the evening and is highest during the night. At dawn the population activity decreases again and is significantly lower during the day than during the night. In contrast 3 fish, of the 5 *Eigenmannia sp.* we observed, were crossing the entire 20 m river section within 5 – 15 min during the night (Fig. 1). All *Eigenmannia sp.* were solitary, we did not observe them in groups. We conclude that outside their breeding season the stone microhabitat provides sufficient shelter as well as enough food for the quite abundant *A. macrostomus*. There is no need for them to leave these habitats as long as they are not searching for mating partners. Further analysis needs to show whether the fish compete and interact for food or whether electrosensory stimuli are restricted to stationary electric fields of the neighboring fish and to electrolocation signals.


Channel
1
3
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7
gap
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16
18:30
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18:40
Time

The significance of EOD frequency rises during dominance establishment in the electric fish *Apteronotus leptorhynchus*

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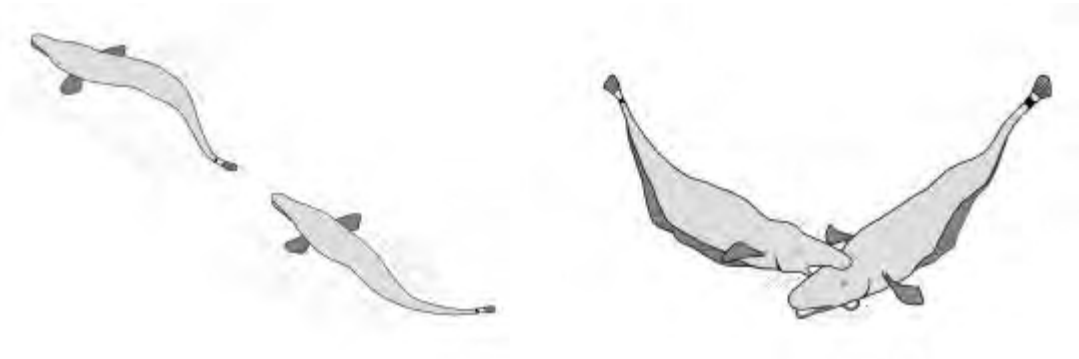
For the understanding of functional adaptations of sensory systems it is crucial to consider and characterize an animals natural sensory scene. For many animals, especially social species, interactions with con-specifics represent an important part of their sensory world, since the respective sensory cues and signals are an integral component of social interactions. Social animals often form dominance hierarchies, whereby more dominant individuals have priority access to limited resources, including food, shelter and mates. To avoid costly, repetitive fights for resources animals use cues and signals to assess the dominance of other individuals or signal their own. Thus, these signal are an important part of their natural sensory world. The electric fish *Apteronotus leptorhynchus* produces an electric field through discharges of an electric organ used for electrolocation and communication. In staged competitions these fish compete for a superior shelter and use agonistic interactions and electrocommunication signals to form a dominance hierarchy (Fig. 1). In this setting the best proxy for dominance is body-size. However, motivational and behavioral differences , as well as the discharge frequency of the fish's electric organs (EODf) may have an influence on the outcome of competitions. We suggest EODf to be used as proxy for dominance, as has been suggested by previous studies, but only in more natural situation, where fish primarily perceive each other electrically because of larger distances compared to laboratory settings. Evolutionary adaptations to trust this sensory cue for assessing dominance could influence competitions, even when fish can use more reliable sensory information to estimate an opponent’s size and dominance when in each others proximity. During competitions EODf rises, specific electrocommunication signals, are almost exclusively produced by subordinates. We propose EODf rises to be produced to improve access to limited resources by signaling a partial claim. These communication signals are more frequently produced by subordinates when dominated by a female. The less territorial and aggressive females could be more tolerant towards a subordinates claim. In trials won by male, EODf rises are more frequently produced by subordinates when the size difference between competitors is small. In these cases the motivation of subordinates to signal a partial claim is increased, because of potential higher chances of success. Frequently dominants initiate agonistic attacks in response to EODf rises. EODf rises of subordinates and agonistic attacks initiated by dominants in response to them could be used to define the skewness of access to limited resources in *Apteronotus leptorhynchus*.



Multisensory integration of carbon dioxide and body heat for tick host-seeking behavior

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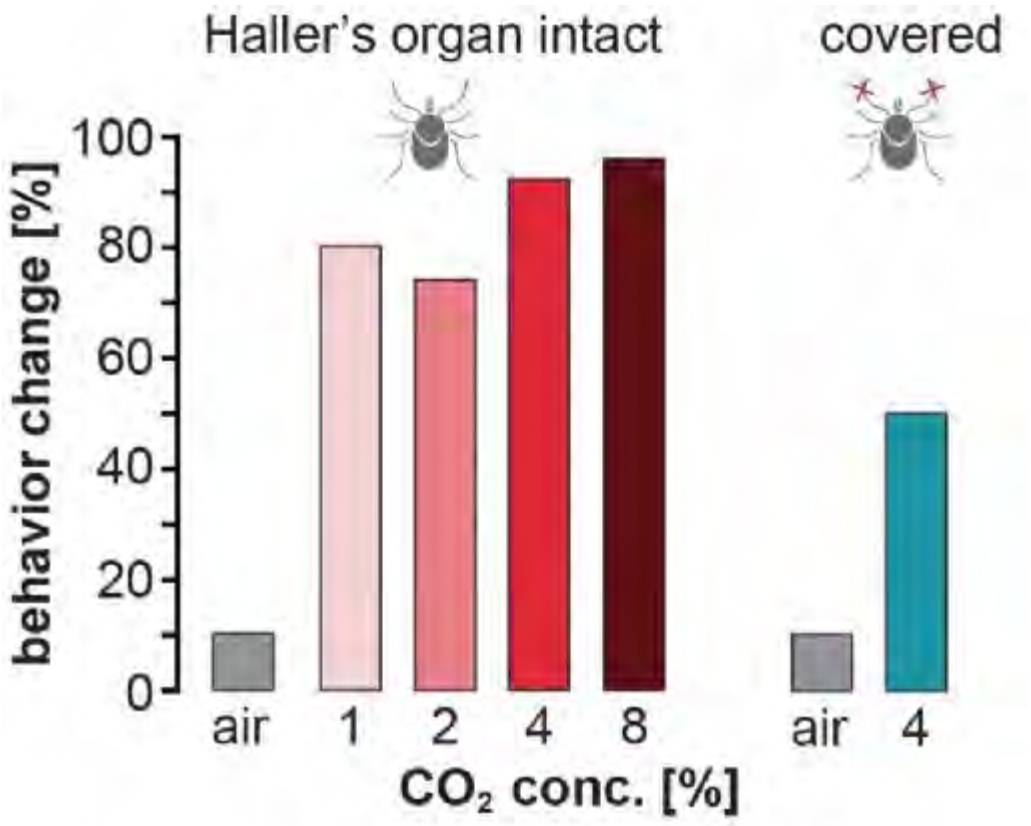
Tick males and females are obligatory blood-feeders and must eat blood in each of their three life stages to survive. While of the ~900 tick species only a few species (10-20) transmit human pathogens, ticks transmit a greater diversity of infectious agents than any other group of blood-feeding arthropods. A multitude of sophisticated sensory abilities designed to locate and infest hosts efficiently have been proposed to be used by ticks, including detection of body odor, carbon dioxide (CO_2), moisture, heat, vibrations, and visual contrast. However, despite extensive research on ticks, we have no good understanding of what makes a tick tick, and our current knowledge of tick sensory abilities and neurobiology, in general, is very reduced.

Generally accepted is that ticks are attracted to breath-like CO_2 concentrations (4%) and body-heat. However, different tick species with different host-preferences and hunting strategies have been studied over the last century. Quantitative and qualitative studies that describe CO_2 responses and heat-sensing for many economically relevant tick species are absent. Furthermore, anatomical structures and receptors involved in tick CO_2 detection and heat-sensing have yet not been identified.

This study focuses on *Ixodes scapularis*, the main vector for Lyme disease in the U.S. and one of the economically most important tick species. Here, we test *I. scapularis'* ability to sense CO2 and heat in a behavioral assay. So far, our preliminary data indicate that *I. scapularis* do not show behavioral changes to heat stimuli, including 37 degree C body heat. In contrast, *I. scapularis* sense and respond to CO_2. An increase in ambient CO_2 concentration from 0.06% (air) to 1% was sufficient to elicit behavioral responses in 80% of the animals (50 ticks; males and females alike). CO_2 thus is a potent stimulus for *I. scapularis* with a low threshold. In 4% CO_2 (respiratory concentration), 95% of the animals tested showed a behavioral response in a state-dependent manner (50 ticks). In ambient air, ticks are either walking or quiescent. About 80% of the animals that were quiescent in ambient air remained stationary but displayed horizontal questing, where they raise and start waving their forelegs. By contrast, 75% of the walking animals continued to walk but with increased walking speed and raised forelegs. The first tarsal segment of tick forelegs contains a specialized sensory organ called Haller's organ that has long been suspected to contribute to tick host-seeking. With disabled Haller's organ, *I. scapularis'* response to CO_2 was strongly reduced. Only ~40% of the animals showed behavioral responses. Our results indicate that while the Haller's organ contributes to CO_2 detection, it may not be the only sensory structure. In conclusion, our data show that *I. scapularis* show no response to heat stimuli, but CO_2 initiates host-seeking behavior with foreleg waiving. However, heat and CO_2 processing are synergistic in other arthropods. We are thus currently testing whether CO_2 detection potentiates and activates heat-sensing.


Haller's organ intact
covered
behavior change [%]
100
80
60
40
20
0
air
1
2
4
8
air
4
CO2 conc. [%]

Neuronal principles underlying internal state dependent decision-making revealed by pan-neuronal volumetric imaging

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Animals need to adapt food intake to their current nutritional needs which change according to their physiological state. Flies, like other animals, develop nutrient specific appetites in response to changes in internal states. When deprived of protein, flies selectively increase the intake of yeast, their main protein source, but not sugar. Mating has a similar effect, also leading to increased yeast feeding. These adaptations in feeding decision-making are well documented behaviorally, but we lack an understanding of the underlying neuronal processes.

We sought to tackle the neuronal basis of internal state dependent changes in taste processing and feeding related decision-making. As this highly integrative process was likely to be a distributed process involving several neuronal circuit layers, we developed a pan-neuronal volumetric imaging approach. We recorded neuronal activity from all neurons across the complete subesophageal zone (SEZ), the taste processing center of the fly brain, while stimulating flies' taste sensory neurons with different taste solutions. By aligning all data in 3D and performing a functional brain segmentation, we were able to compare taste induced activity between flies in different metabolic and reproductive states.

We found taste induced activity elicited by different tastants to be distributed across the SEZ. Besides known sensory projections activity also spanned higher order- and motor regions. Metabolic state modulated neuronal activity in regions of all sensory motor processing layers. Importantly, modulations were nutrient specific, correlating strongly with the observed shift in behavioral decision-making. Interestingly, reproductive state had much more discrete effects affecting mostly the output level. Thus, by assessing neuronal activity across a full sensory-motor neuropil, we gained novel insights into the neuronal principles underlying internal state dependent taste processing and highlighting the distinct processing leading to seemingly similar behaviors.

Neuromodulator diversity in the central olfactory pathway of *Parhyale hawaiensis* (Dana, 1895)

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The primary olfactory centers (olfactory lobes) in the brain of malacostracan crustaceans consist of synapse-dense regions, called glomeruli (Harzsch and Krieger 2018 Prog. Neurobiol. 161:23-60). Within the glomeruli, afferents, local interneurons, and projection neurons interact on a synaptic level. The glomeruli receive input from the afferents of olfactory sensory neurons (OSN) which are associated with specialized sensilla, called aesthetascs, on the first antennae. The glomeruli are radially arranged around a core of non-synaptic fibers. The wiring pattern of the glomeruli of crustaceans and hexapods are suggested to be homologous because of the pattern of connectivity of afferents, local interneurons, and projection neurons (Schachtner at al. 2015 Arthropod Struct Dev 34: 257–299, Derby et al. 2016 Chem. Senses. 41:381-398). Our collaborative study aims at gaining new insights into the evolution of olfactory core circuits by studying the olfactory pathway of one representative of crustacean (our lab) and hexapods (MPI for Chemical Ecology Jena) each. Maintaining nervous tissue in a physiologically active state requires much energy, which is why energetic constraints are a major selective pressure on the evolution of sensory and nervous systems (Sterling and Laughlin, 2015 MIT Press, Principles of Neural Design). Therefore, the body size of a given species may play a crucial role in the evolutionary elaboration of its nervous system. Considering this, we will investigate the amphipod crustacean *Parhyale hawaiensis* (Dana, 1853) which is of similar size to many hexapods (around 1 cm) and, therefore, may have similar metabolic constraints. Additionally, this species is easy to rear in the lab, its brain anatomy is already described in detail, and protocols for immunohistochemistry are well established (Wittfoth et al. 2019 Front. Zool. 16:30).

In order to identify distinct subsets of glomeruli, we chose different marker sets for immunohistochemical labeling of the brain including anti-serotonin, anti-RFamide, anti-allatostatin, anti-orkokinin and anti-synapsin. Furthermore, we aim at gaining deeper insights into the peripheral olfactory pathway. Thus, we investigate the first antennae using different fluorescent markers for nuclear staining and labeling of neuronal structures. The olfactory lobe consists of around 30 to 50 glomeruli, which do not show a well-defined outline and only a slight differentiation into cap and base subregions. Every antennae consists of 3 antennomers with a variable number of flagellomeres. All flagellomeres except the two most distal ones have two aesthetascs. Since the nuclear marker do not allow a distinction of the OSN from other cell types, we expect to identify the OSN somata with a combination of nuclear marker, cytoskeletal markers and antisera against neuromodulators.

This study is supported by DFG grant HA2540/20-1 in PP 2205 “Evolutionary Optimization of Neuronal Processing”

Evolution of Odour Coding in Drosophilids

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[3]Max Planck Institute for Chemical Ecology, Germany
[4]University of Sussex,UK

Sensory systems process information in a near-optimal way. However, it is unclear to which extent this optimisation takes place across closely related species adapted to different environments.

We are addressing this question by using, as models, the larval olfactory system of different *Drosophila* species spanning a wide range of phylogenetic distances and ecological niches. We employ an interdisciplinary approach combining behavioural, functional, and chemical analyses.

We first show that recently born larvae of specialist species- those that feed and breed on only a particular fruit- exhibit differential odour-guided behaviours in the presence of their host fruit smells, a novel result showcasing innate host preference in these species. Then, to address whether sensory neurons differentially encode environmental olfactory information across species, we are developing genetic tools in non-model Drosophilids to record population calcium activity from the entire complement of olfactory sensory neurons- in intact larvae- responding to volatiles. Finally, by combining field work with chemical analyses of each species' natural environments, we can provide an ecological context to behavioural and functional changes, as well as a detailed description of each species' olfactory stimulus landscape.

Investigation of adult neurogenesis in the mouse accessory olfactory system

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In the rodent accessory olfactory system (AOS), neurogenesis occurs throughout life. Vomeronasal sensory neurons (VSNs) as well as inhibitory interneurons neurons (granule and periglomerular cells) in the accessory olfactory bulb (AOB) are constantly renewed. Therefore, AOS output is continuously (re)shaped. So far, however, the physiological function of adult neurogenesis in the AOS remains unclear. To address this question, we present a novel genetic approach, labelling newly generated neurons in the peripheral and central AOS. After tamoxifen injection, neuronal stem cells in *Id2CreERT2+ : Rosa26R-XXX* mice express fluorescent reporter proteins upon coincident Id2 promoter activity. Neuronal descendants of AOS stem cells can thus be labelled with suitable fluorophores for both neuroanatomical and physiological analysis. Introducing the *Id2* stem cell marker as an AOS lineage tracer, we show horizontal and tangential migration of VSN precursors. We demonstrate that differentiated VSNs appear two days after tamoxifen treatment. Finally, we provide insight into the lifespan of VSNs. Ongoing experiments aim to both pinpoint Id2 expression at various stages of VSN differentiation and analyse the physiological properties of adult-born AOS neurons.

Electrophysiological studies of the modulation of olfactory receptor neurons of the hawkmoth *Manduca sexta*

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Insect olfactory receptor neurons (ORNs) detect pheromones and general odorants at astounding sensitivity and over a large range of concentrations, covering more than 4 log units. Furthermore, they can tune into rhythms of pulsed odor stimuli over a broad frequency range with interstimulus intervals at the scale of hours to milliseconds. How this astounding range of gains and kinetics is accomplished is not known. Previously, experimental evidence proved that insect ORNs are peripheral circadian clocks that rhythmically modulate odor sensitivity and kinetics over the 24 h day. Furthermore, it was shown that second messengers such as intracellular Ca2+, cAMP, and cGMP are involved in the modulation of sensitivity and kinetics of ORNs at different time scales. Daytime-dependent antagonistic rhythms of cAMP and cGMP expressed peaks of cAMP and minima of cGMP during the insect´s activity phase while during rest levels of cAMP were minimal and of cGMP maximal. Accordingly, while cAMP sensitized, cGMP adapted pheromone responses of ORNs *in vivo*. Furthermore, the ORNs circadian clock regulated cAMP levels in circadian rhythms also under constant conditions. To further evaluate second messenger-dependent modulation of odor transduction, with patch clamp studies we examined ionic currents of hawkmoth ORNs in primary cell cultures in vitro. Second messenger-dependent ionic currents were antagonistically modulated via cAMP, cGMP, Ca2+, and protein kinase C (PKC). Interestingly, the cAMP-dependently activated currents had faster kinetics and maintained ORNs at hyperpolarized membrane potentials with lower intracellular Ca2+ baseline levels. In contrast, the cGMP-dependent currents expressed slower kinetics and stabilized ORNs at more depolarized membrane potentials that allowed for higher intracellular Ca2+ concentrations. Thus, we hypothesized that relative concentration levels of Ca2+, cAMP, and cGMP determine which ion channels are available for odor/pheromone-detection at different times of the day, at different physiological states of the insect, and during/after exposure to different concentrations of odor stimuli. To challenge our hypothesis and to focus on the modulation of the kinetics of pheromone transduction, we performed tip recordings from long trichoid sensilla on male hawkmoth antennae. Male moths locate their pheromone-emitting females with pheromone-guided anemotaxis that depends on intermittent pheromone stimulation. The frequency rather than a concentration gradient guides the males to their mates. Since it was known from mammalian brains that spontaneous membrane potential oscillations at different ultradian frequencies specific for sleep, wakefulness, or attention shape the detection of environmental stimuli we first focused on the analysis of spontaneous activity of ORNs in vivo. Indeed, pheromone-sensitive ORNs express circadian rhythms in spontaneous activity that appeared to affected pheromone-, octopamine-, and second messenger level-dependently. Further experiments examine whether resonance properties of ORNs are specifically different for different physiological states of the hawkmoth and whether they are controlled/entrained via Zeitgeber-dependent changes in second messenger levels. [Supported by DFG grants STE531/20-1,2 of the SPP1392 to MS and by the graduate college “cellular clocks” P/879 of the University of Kassel to MS]

Neuromodulator diversity of local olfactory interneurons and regionalization of olfactory glomeruli in crustaceans

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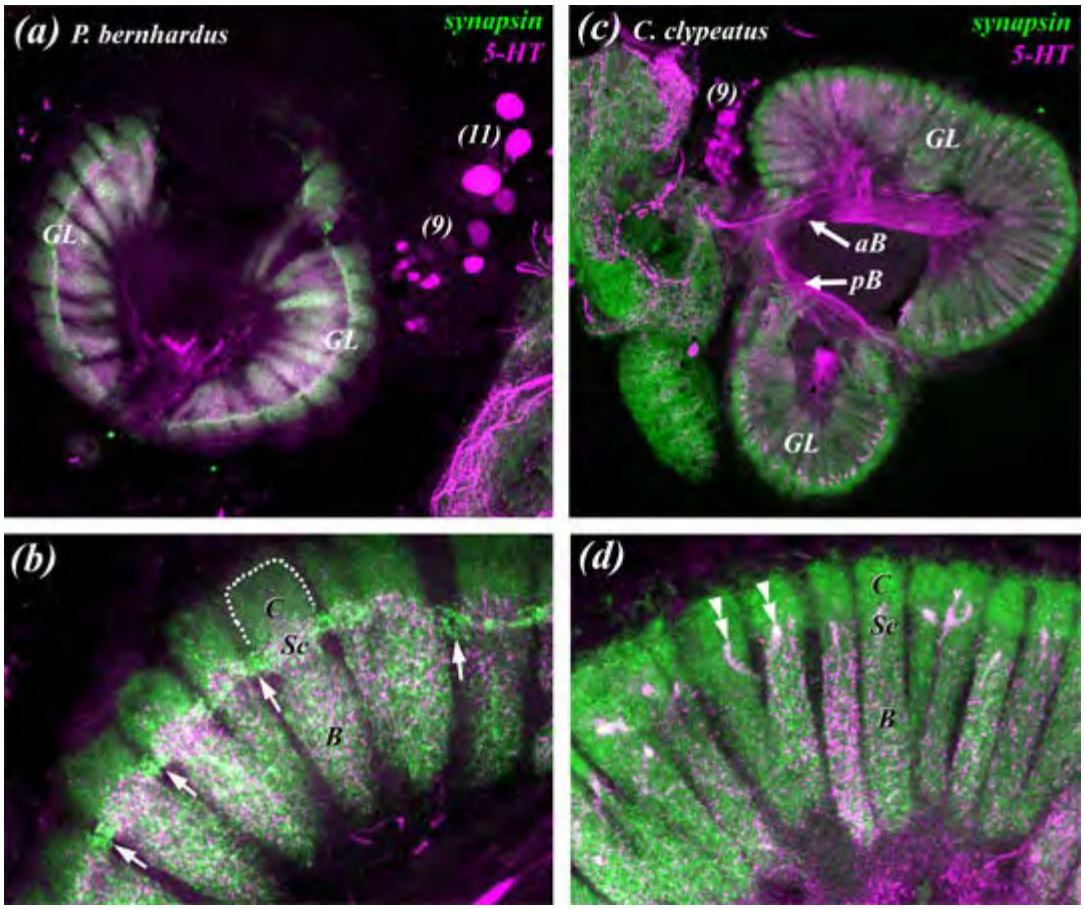
[2] Max Plack Institute for Chemical Ecology, Dept. of Evolutionary Neuroethology, Hans-Knoell-Strasse 8, D-07745 Jena

In malacostracan crustaceans, the primary olfactory centers (olfactory lobes) in the brain consist of an array of fields of dense synaptic neuropil, the olfactory glomeruli (review Harzsch & Krieger, 2018, Prog. Neurobiol. 161:23-60). These fundamental units of olfactory processing are sites where axons from olfactory sensory neurons synapse with local olfactory interneurons and olfactory projection neurons. The glomeruli are radially arranged around the periphery of a core of non-synaptic fibers in a highly geometrical constellation. In higher crustaceans, glomeruli are elongate and are regionalized into functional compartments along their long axis, the cap, subcap and base regions, each with a distinct neurochemistry. This study sets out to understand the neurochemical basis of glomerular regionalisation in two crustacean representatives that in previous studies we had found to feature highly complex central olfactory systems, the hermit crabs *Coenobita clypeatus* and *Pagurus bernhardus* (Harzsch and Hansson, 2008, BMC Neurosci. 9, 58; Krieger et al. 2012, Cell Tissue Res. 348, 47–69; Polanska et al. 2020, Cell Tissue Res. 380:449-467). To that end, we explored the neurochemical diversity of local olfactory interneurons using antisera against serotonin, allatostatin, and RFamide in combination with anti-synapsin immunohistochemistry. These interneurons can be broadly subdivided into two morphological classes, one of which primarily targets the cap and subcap regions of the glomeruli (“rim” interneurons), and a second class which primarily invades the base of the glomeruli from inside of the lobe (“core” interneurons). Serotonergic interneurons belong to the ”core” type in both species, and in *C. clypeatus* (but not in *P. bernhardus*) give rise to a rod-like central domain of strongly immunoreactive material in the centre of the glomerular subcap region. RFamide-like immunoreactive interneurons also belong to the “core” type and in *P. bernhardus* (but not *C. clypeatus*) provide distinct lateral connections between neighbouring glomeruli, possibly mediating lateral inhibition. Allatostatin-immunoreactive interneurons belong to the “rim” type and densely innervate the glomerular subcap region by beaker-shaped (*C. clypeatus*) and baton-shaped (*P. bernhardus*) domains. Our data provide evidence for 1. a small-scale subdivision of crustacean olfactory glomeruli into regional compartments, and 2. for distinct differences in the innervation pattern of local olfactory interneurons even between closely related crustacean species. This study was supported by DFG grants Ha 2540/13-1 (SH), Ha 5871/5-1 (BSH), and the Max Planck Society.


(a) P. bernhardus
synapsin
5-HT
(11)
(9)
GL
GL
(c) C. clypeatus
synapsin
5-HT
(9)
GL
aB
pB
GL
(b)
C
Sc
B
(d)
C
Sc
B

Spontaneous spikes of mitral cells in a semi-intact preparation of the rodent olfactory bulb are modulated by intrinsic theta oscillations

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Both spontaneous and odor-evoked network activity in the rodent olfactory bulb (OB) are strongly patterned by respiration, resulting in oscillations within the theta regime. Previously, we observed theta rhythms also in local field potential recordings (LFP) in a semi-intact nose-brain preparation of the juvenile rat that is uncoupled from respiration (*Perez, ...Dutschmann, Egger, J. Neurophysiol. 2015*). Therefore we hypothesized that the bulbar respiratory theta rhythm taps into an intrinsic theta resonance of the bulbar network.

Here we further investigated the properties of this intrinsic theta rhythm in our preparation. First, we frequently observed harmonics of theta, in line with recent reports on theta in hippocampal LFP (e.g. *Sheremet et al., J. Neurosci. 2016; Scheffer-Texeira & Tort, eLife 2016*). With regard to stability, we observed that both the theta oscillation frequency and its power persisted for at least 60 min (average frequency 2.1 ± 0.3 Hz, n = 10 preparations). Thus the investigation of intrinsic theta in this preparation should be amenable to pharmacological manipulations and imaging approaches.

We also tested for a spatial dependency, i.e. whether a recorded theta oscillation frequency would vary across the OB. We mapped LFPs across 9 different locations on the dorsal OB (within a 3 x 3 grid) and found that the very same oscillation frequencies persisted across all sampled locations (in n = 9 preparations), suggesting that the observed theta frequency is a property of the entire OB network and not generated independently at several loci.

Since we were also able to pick up multi-unit activity from putative mitral cells within the LFP recordings, we asked whether this spontaneous spiking activity could be correlated with the ongoing theta rhythm. We developed an algorithm to filter and isolate the spikes and to determine their phase relative to the LFP theta, and applied circular statistical tests (Rayleigh and Kuiper tests) to check for preferred phases of firing.

In all recordings from the 6 preparations analyzed so far, we always observed significant periodicity of spike distributions relative to the phase of LFP theta within subsets of 1 minute recording intervals. Such correlations did not persist throughout the entire recordings but were observed within an average fraction of 0.4 ± 0.1 of the total number of recorded intervals. We also tested for the robustness of these results by using various spike detection thresholds and analysis intervals.

This preliminary finding serves as an additional control that the observed intrinsic theta rhythm is not a recording artefact. If that was actually the case, mitral cells should fire randomly with respect to the recorded theta rhythm. Moreover, our results align well with *in vivo* recordings showing a correlation between mitral cell spontaneous activity and breathing (*Fukunaga et al., Neuron 2012*) and thus prove that the semi-intact nose brain preparation allows to investigate rhythmic bulbar activity at the single cell level.

Fast dynamics of the odour landscape are encoded in the mouse olfactory system and guide behaviour

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[3]These authors contributed equally

Odours are transported in turbulent plumes resulting in rapid concentration fluctuations that contain rich information about the olfactory scenery, such as odour source composition and location. Yet whether the mammalian olfactory system has access to the underlying temporal structure to extract information about the environment remains unknown. Here, we show that odour pulses separated by as little as 10 or 25 ms result in distinct responses in olfactory receptor neurons. In operant conditioning experiments mice can discriminate the temporal correlation of rapidly fluctuating odours at frequencies of up to at least 40 Hz. In imaging and electrophysiological recordings, we find that such correlation information can be readily extracted from the activity of mitral and tufted cells, the output of the olfactory bulb. Furthermore, we show that temporal correlation of odour concentrations reliably predicts whether odorants emerge from the same or different sources in normal (turbulent) environments. Training mice on such source separation tasks and probing with synthetic correlated stimuli at different frequencies suggests that mice can indeed use the temporal structure of odours to extract information about space. Our work thus demonstrates that the mammalian olfactory system has access to unexpectedly fast temporal features in odour stimuli, at timescales of 25 ms. This in turn endows animals with the capacity to overcome key behavioural challenges such as odour source separation, figure-ground segregation and odour localisation, by extracting information about space from temporal odour dynamics.

Functional study of the queen pheromone receptor OR11 in honey bees within the genus Apis.

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In honey bees, olfaction is crucial for cohesion of the colony, foraging and reproduction. Those fascinating social insects employ a rich repertoire of pheromones to ensure intraspecific communication. They produce more than 50 different pheromones and some of them are still unknown. Moreover although bees' olfactory system has been extensively studied, peripheral processes are still poorly known, like for instance the sensitivity and response spectra of individual ORs (olfactory receptors). This is mainly due to difficulties to record activity from individual OSNs within honeybees' sensilla that contain as many as 35 OSNs. To overcome this problem, we chose to express honeybee ORs within the empty neuron system of *Drosophila melanogaster*. The western honey bee *Apis mellifera* is the better known honey bee within Apis genus. The western honey bee's genome presents ~170 functional ORs. Until now, only 3 of them have been deorphanized (i.e. their ligands identified): AmOR11, AmOR151 and AmOR152. Here in this study, we focus on AmOR11 which was shown to respond specifically to the 9-ODA, the major component of the queen pheromone. Indeed, honeybees mating takes place high in the air at so-called drone congregation areas were thousands of drones gather and mate with virgin queens. This behaviour is mainly driven by olfaction, but little is known about the olfactory signals involved, except for the 9-ODA involved in the attraction of drones toward queens. Interestingly, 9-ODA is produced by other honeybee species in the genus Apis and is attractive for males. Moreover, those species also present orthologs of the olfactory receptor AmOR11 in their genomes. We choose to study the OR11 responses to 9-ODA of three other Apis species: the giant honeybee *Apis dorsata*, the dwarf honeybee *Apis florea* and the Asiatic honeybee *Apis cerana* and analysed their specificity by screening a panel of other odorants. We confirmed that AmOR11, AdorOR11, AcerOR11 and AflorOR11 primarily responds to 9-ODA. Nevertheless, we also recorded lower responses for the four ORs to different odorants such as hexanoic acid, heptanoic acid, sulcatone, oxovaleric acid, trans-2-hexenol, among others. Dose-response analyses show that these are secondary ligands. These first results describe the same sensitivity of the four different orthologs of OR11 for the queen pheromone and a broader response profile for the OR11 within the genus Apis then previously thought.

Humidity sensing in *Drosophila melanogaster*

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Humidity is a crucial and omnipresent environmental factor influencing fitness, reproduction and even the geographic distribution of animals and is closely tied to temperature. The ability to sense changes in humidity levels, which in turn can affect behaviour, is called humidity sensing or hygrosensation. In *Drosophila melanogaster*, humidity sensing is mediated by the hygrosensory receptor neurons (HRNs), located in the sacculus, an invagination on the dorsal side of the antenna. Each sensilla harbours a triad of neurons, consisting of a dry, a moist and a hygrocool cell. Four ionotropic receptors involved in humidity sensing have been identified: IR40a, IR68a, IR9a andIR25a. Genetic inactivation of either receptor leads to disrupted humidity sensing behaviour in adult Drosophila. IR68a and IR40a are expressed in the moist neuron and dry neuron, respectively whereas IR25a and IR93a, expressed in the hygrocool cell, presumably acting as co-receptors.

However, the role of other cells surrounding the HRNs and the neuronal mechanism mediating hygrosensation remain unknown. To identify further receptors and co-receptors involved in humidity sensing we are using single nuclei RNA sequencing. snRNA seq examines the transcriptome of individual cells allowing for an in-depth characterisation of a given group of cells. Compared to conventional sequencing techniques, snRNA seq can be used to gain information from a small group of cells. In this project the analysis of snRNA seq data allows the characterization of the hygro- and thermosensory neurons located in the *Drosophila* antennae. By using a clustering algorithm, we describe different groups of neurons within the hygro- and thermosensory population. Genes that are predominantly expressed within each of these groups can be defined as new marker genes for a certain sub-group of neurons and serve as candidate genes for the identification of new players in hygrosensation. Furthermore, this data is used to describe the expression of neurotransmitters, neurohormones or neuromodulators in these groups of neurons.

By pairing these results with multi-colour flip-out staining of HRNs in both antenna and brain we can make a step towards understanding the neuronal processes mediating hygrosensation.

Poster Topic

T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

T20-1 Ascending neurons in *Drosophila melanogaster*
Erica Ehrhardt, Ryo Minegishi, Tatsumi Nagashima, Masayoshi Ito, Barry Dickson, Jim Truman, Ansgar Büschges, Kei Ito

T20-2 Selective blood-nerve-barrier leakiness with claudin-1 and vessel-associated-macrophages loss in diabetic polyneuropathy
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production in TRPA1 or TRPV1 expressing sensory neurons
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T20-11 Input of antennal proprioceptors to an identified descending interneuron in the stick insect
Bianca Jaske, Gaëtan Lepreux, Volker Dürr

Ascending neurons in *Drosophila melanogaster*

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Somatosensory feedback is necessary in order for motor flexibility, for the nervous system to adjust motor commands based on the behavioral situation. Our aim is to investigate the role of ascending signals in fruit fly locomotion. To lay the groundwork for this project, we have mapped and annotated hundreds of ascending neuron types based on their innervation in the somatosensory layers of the ventral nerve cord and regions of the fly brain. We used split GAL4 intersections to create cell type specific driver lines for approximately one hundred of these ascending neuron types. We used confocal microscopy to image ascending neurons, and then searched the published EM volume of the Drosophila brain for matching images, in order to gain an understanding of the connections of the ascending neurons. Our results map the anatomy of ascending pathways in flies and provide tools for investigating ascending feedback.

Selective blood-nerve-barrier leakiness with claudin-1 and vessel-associated-macrophages loss in diabetic polyneuropathy

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Background: Painful diabetic neuropathy (pDN) is a common complication of diabetes and many questions in the pathophysiology remain open. Traumatic peripheral nerve injury is associated with increased permeability of the blood-nerve-barrier (BNB) [1] as part of the neurovascular unit. Increased permeability involves tight junction protein downregulation. These tight junctions are also altered in freeze-fracture sections of diabetic human sural nerve biopsies [2]. Similarly, in preclinical models of type II diabetes, the tight junction protein claudin-5 is downregulated in BNB [3]. However, the involvement of barrier breakdown in pDN remains controversial and deserve more attention. Using new tools and a systematic analysis, we hypothesize that breakdown of BNB could participate in pDN development.
Methods: Type I diabetes was induced by a single intravenous injection of 40 mg streptozocin to male Wistar rats. We tested pain behavior by measuring mechanical and thermal thresholds, using the von-Frey and Hargreaves test, respectively. We assessed rats' motor coordination on a rota rod. Then, we tested permeability of the BNB by intravenous injection of Evans blue albumin (EBA) and sodium fluorescein (NaFlu) or the immigration of CD68+ macrophages by immunostaining. Tight junction proteins expressed in the whole sciatic nerve or capillaries and perineurium - isolated by laser microdissection - were analyzed by RTPCR.
Results: Streptozocin induced hyperglycemia, weight loss, mechanical allodynia, decreased motor performance but no thermal hypersensitivity. Perineurial and capillary permeability did not change for EBA (68 kDa), but significantly increased for NaFlu (368 Da) 8 weeks after streptozocin. Tight junction transcript signatures of whole sciatic nerve did not change. However, when we specifically analyzed perineurium and capillaries Cldn1 and Cldn5 were reduced, respectively. At the proteomic level, claudin-1 immunoreactivity was reduced in perineurium but claudin-5 in endoneurial vessels was unchanged. But, we observed a lower number of blood-vessel-associated-macrophages – known to be responsible for BNB sealing.
Conclusion: Persistent streptozotocin-induced type I diabetes results in a BNB leakiness for small molecules. This is in accordance with the known microangiopathy in diabetes. Selective downregulation of perineural Cldn1and blood-vessel-associated-macrophages, points towards a TJ involvement in the neurovascular unit and supports macrophages role in sealing endoneurial blood vessels. Sealing both perineural and endoneurial barrier might allow for disease modification and prevent further damage.

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Prox2+ vagal neurons mediate digestive and breathing behaviors

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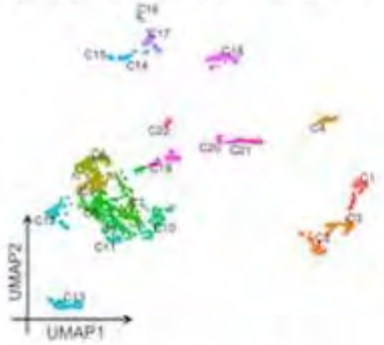
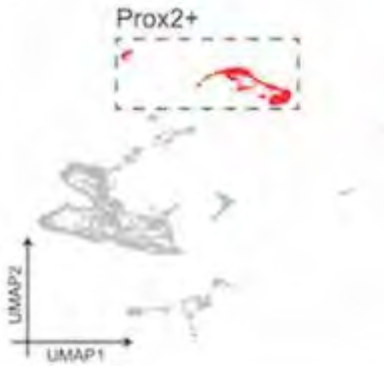
Viscerosensory neurons of the vagal ganglia are important for maintaining body homeostasis. They are pseudounipolar cells, sending afferents to innervate the inner organs and projecting centrally to the nucleus of the solitary tract in the hindbrain. These neurons monitor diverse chemical and mechanical stimuli such as nutrients passing through the intestine, blood pressure in the aortic arch, and irritants in the larynx. Recently, numerous groups have begun to apply bulk and single cell RNA sequencing to the vagal ganglia and have found an incredible heterogeneity of cell types. Although much work has been done to elucidate the identity and function of vagal subtypes, many open questions remain. Advances in the field depend on the availability of cluster markers and transgenic mice. In order to better resolve vagal neuron heterogeneity, we performed single cell RNA sequencing of the vagal ganglia and combined our data with two previously published data sets into a meta-analysis. This combined data set allowed us to describe the mechanosensory-like vagal neurons as belonging to one of three superclusters, each containing multiple clusters that shared expression of an exclusive marker gene. One of these superclusters was defined by the expression of Prox2, a homeobox domain transcription factor expressed in viscerosensory, but not somatosensory neurons. We then generated a Prox2FlpO mouse and found that Prox2 neurons segregate into five transcriptionally unique clusters, which together encompass about 17% of vagal neurons. First we examined the development of these neurons. We found that at embryonic (E) day 11.5 they already project centrally to the nucleus of the solitary tract, and by E14.5 have innervated their target organs in the periphery. Next, we utilized lightsheet imaging to investigate the peripheral targets of Prox2+ vagal neurons and found that they innervate the proximal digestive organs, including the esophagus and stomach, as well as respiratory organs such as the larynx and lungs. In order to obtain a better understanding of the physiological roles of these neurons we analyzed their end organ morphology using immunofluorescence. This allowed us to uncover two types of endings in the digestive organs: intraganglionic laminar endings that innervate enteric neurons, as well as intramuscular arrays that innervate interstitial cells of Cajal. These two types of endings are suggested to detect tension and stretch, respectively, suggesting that Prox2 vagal neurons are important for mediating digestive behaviors. Future work will include tracing from the various target organs to resolve which cluster they are innervated by, as well as analyzing Prox2 mutant mice *in vivo* to determine the physiological role of these neurons in digestive and respiratory behaviors.

1) Vagal Ganglia Atlas at P4



2) Meta-analysis



3) Generation of Prox2 Mouse

4) Lightsheet imaging of target organs

Prox2FlpO; tdTomato - Esophagus

Neuroanatomy and postembryonic development of the subgenual organ complex in stick insects

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Mechanosensory organs in insects function in different biological contexts to detect body movements, touch, gravity, sound, or substrate vibrations. They are often chordotonal organs consisting of scolopidial sensilla, and the different organs vary considerably in their functional morphology and the number of sensilla. The physiological function depends on the suspension or attachment of sensilla to e. g. tendons, joints, trachea, or tympanal membranes. Especially the linkage to other structures determines the sensory function of the organs.
In the proximal tibia of most insect occurs the subgenual organ, an important vibration receptor organ. Notably, additional organs occur together with the subgenual organ in orthopteroid insects (including crickets, cockroaches, or stick insects), and form the so-called subgenual organ complex. The sensory organs in the stick insect *Sipyloidea sipylus* were investigated for their neuroanatomy and their development.

The subgenual organ complex consists of two chordotonal organs, the subgenual organ and the distal organ. The approximately 20 sensilla of the distal organ occur in a linear organisation and extend distally in the tibia. This combination of sensory organs and the elaboration of the distal organ is unique to stick insects. However, the function of the distal organ in stick insects is so far not investigated specifically, while the entire subgenual organ complex is sensitive to sinoidal vibration stimuli. A similar neuroanatomy with linear sensilla is known for the tympanal hearing organs in crickets, bushcrickets, and other insects, which have auditory sensilla in close association with the thin tympanal membranes and the auditory trachea in the leg. Using histological and microcomputed tomography shows the position of the distal organ in the hemolymph channel, and investigates the attachment structures and connection to cuticle and leg trachea. Stick insects have no tympanal membranes in the tibia, which are relevant in the sound detection in crickets or bushcrickets. The sensilla in the distal organ also have long accessory cells, which could be involved in detecting hemolymph oscillations in the tibia caused by substrate vibrations. Covering the neuroanatomy of first and second larval instars shows the presence of both organs in these developmental stages, and the full set of scolopidial sensilla in the tibia after hatching. The sensory organs likely differentiate during embryogenesis, which indicates that they are presumably physiologically functional after hatching.

Studying multisensory external context and internal state integration for the modulation of neuronal circuit activity in *Drosophila* larva

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In a given external context, the internal state of the individual modulates decisions so that behavior that best meets its current needs is promoted. Neuropeptides represent a wide family of molecules that are known to act as modulators of neuronal activity. Their expression patterns are various and may change in multiple conditions. For one, neuropeptide Y (NPY) enhances feeding-related behaviors in starved mammals. However, its role in non-feeding related behaviors is poorly documented. In flies, short neuropeptide F (sNPF) - a member of NPY family – and its receptor (sNPF-R) are widely expressed in the nervous system and might thus modulate various behaviors depending on the internal state. To define sNPF impact on internal state-dependent choices, we use genetic manipulations combined with high-throughput behavior tracking in Drosophila melanogaster larva. We exploit one decision-making circuit we have previously characterized in detail (Jovanic et al., 2016) to determine whether sNPF could affect its activity according to the internal state.

To this aim, we downregulate sNPF-R expression in specific neurons of the described decision circuit to study the impact of sNPF signaling in larvae in different feeding states. We then quantify the impact of downregulating sNPF-R on the behavioral output of the circuit (in response to a mechanical stimulus) using an automated behavior classification algorithm and on single neuron activity using calcium-imaging.

Our preliminary results thus show that sNPF signaling modulates neuronal response to mechanical stimuli as well as the final behavioral output of the circuit differently depending on the feeding state. In addition, connectome electron-microscopy data of air-puff sensing mechanosensory neurons suggest they receive olfactory and gustatory information from long-range descending neurons which may participate in the modulation of mechanosensory neurons depending on the feeding state.

In order to study how appetitive odors may modulate the activity in a mechanosensory network depending on the feeding state, we are developing a setup for automated delivery of odors and air-puff combined to the larvae under our behavior tracking apparatus, allowing us to impose a conflicting choice to the larvae: either move away from a noxious air puff or move towards an appetitive odor source. The ultimate aim of this study is to determine how internal-state information and external multisensory context are integrated at the level of neuronal circuits in order to select the appropriate behavior.

Reference: Tihana Jovanic et al., “Competitive Disinhibition Mediates Behavioral Choice and Sequences in Drosophila,” Cell 167, no. 3 (October 2016): 858-870.e19, https://doi.org/10.1016/j.cell.2016.09.009.

The effect of high-sugar diet: from neuronal circuit modulations to behavioral changes

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A growing body of evidence suggests that a balanced diet is of particular importance for proper brain function and psychological wellbeing[1–4]. For instance, a poor diet rich in sugar is a risk factor for neurodegenerative diseases such as Alzheimer's disease[5–7], but also for psychological pathologies as schizophrenia[8] and depression[9,10]. However, the mechanisms underlying unbalanced diet effects on neural function and behavior remain poorly understood.

In order to study the mechanisms by which a high-sugar diet modulates neuronal and how this impacts behavioral choices, we take advantage of an already described decision-making circuit in the Drosophila larva[11,12]. This circuit controls the behavioral decision between a startle behavior and an escape-like behavior following a mechanical stimulus. Even though the building-blocks of this circuit are located exclusively in the ventral nerve chord, the equivalent of the vertebrates spinal cord, some neurons from this network extend projections towards the brain, and receive projections from the brain. We thus hypothesized that integration of other information, such as the internal state, might occur through these top-down projections, and/or from other distant regions of the nervous system.

In our study, we first characterize how high-sugar diet modifies decision-making following a mechanical stimulus. Then, we describe how the activity of the already known neuronal substrates of decision making are modulated by high-sugar diet in order to differentially shape behavioral decisions. To this end, we developed a technique allowing live 2-photon imaging of neuronal calcium levels in intact larvae.
Understanding how a high-sugar diet modulates the activity of a decision-making circuit in the Drosophila larva, where we can study neural circuit mechanisms with single cell and synaptic resolution across the nervous system, might shed light on the molecular and neural circuit bases of the effect of an imbalanced diet on human cognition and behavior.

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Sensory organs of *Drosophila* larve: morphology and ultrastructure

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The *Drosophila melanogaster* larva allows to study various aspects of behavior including taxis, kinesis and even learning and memory. All behavior that the animal exhibits is based on processing of sensory information from environmental stimuli, the internal state and acquired experience. But how do larvae actually perceive their environment? What sensory organs are they equipped with? Does the ultrastructure of these sensory organs allow for conclusions about their function? *Drosophila melanogaster* larvae possess external sense organs on their head, thoracic, and abdominal segments specialized to receive diverse sensory information. As in humans, most of the sensory organs are concentrated at the head of the *Drosophila* larva. There are four major external organs, the terminal organ (TO) the dorsal organ (DO), the ventral organ (VO) and the labial organ (LBO). The 3D ultrastructure of the latter 3 is described in this study using focus-ion-beam scanning electron microscopy (FIB-SEM) and serial section transmission electron microscopy (ssTEM). In addition, all external sensillum types of the trunk are described. We provide data on the subcellular configuration and morphology of all individual sensilla and link them to putative sensory function. The ultrastructural description of the main larval sense organs of *Drosophila* will serve as a basis for further molecular and functional examination.

Disinhibitory Circuit Motifs in Mouse Primary Somatosensory (Barrel) Cortex

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GABAergic interneurons play a crucial role in information processing in the rodent neocortex and are intricately integrated into the circuitry of the primary somatosensory (barrel) cortex. Martinotti cells (MC) are a well-described cell type of GABAergic interneurons thought to target the apical dendritic tufts of pyramidal neurons, thereby effectively controlling the main excitatory cell population of the barrel cortex. However, MCs are also targeted by other inhibitory interneurons, forming a circuit motif which is called disinhibition. For layer 2/3 (L2/3) MCs it was reported that they are targeted by vasoactive intestinal peptide (VIP) as well as parvalbumin (PV) expressing inhibitory interneurons. Nevertheless, little is known about the disinhibitory circuit motifs of layer 5 (L5) MCs, with L5 being the main output layer of the primary somatosensory (barrel) cortex.

Therefore, we performed intralaminar (L5 to L5) as well as translaminar (L2/3 to L5) paired patch clamp recordings of VIP neurons to L5 MC in barrel cortex of mice using acute thalamocortical brain slices. By using caesium-based intracellular solution and a holding potential of 0 mV in the postsynaptic MC, we increased the driving force of inhibitory currents to visualise inhibitory currents also from more distal dendrites. The internal solutions, also the potassium gluconate-based for the presynaptic VIP cells, contained biocytin to stain, reconstruct and identify putative contacts of connected cells in successfully recovered pairs.

Paired patch clamp recordings resulted in a connection probability of 30 % for L2/3 VIP to L5 MC and 26 % for L5 VIP to L5 MC. Analysis of the synaptic short-term plasticity using presynaptic 1 Hz, 8 Hz and 40 Hz train stimulation resulted in facilitation for translaminar as well as intralaminar connections, but only at 40 Hz. Single action potentials led to postsynaptic currents in >50 % of trials with similar latencies but lower amplitudes in L5 VIP to L5 MC (5,67 ± 0,26 pA) than in L2/3 VIP to L5 MC (9,87 ± 0,57 pA). Testing the reverse MC to VIP connection showed a connection probability of 24 % for L5 MC to L2/3 VIP and 23 % for L5 MC to L2/3 VIP including reciprocally connected pairs. However, for both tested connections, individual pairs with larger amplitudes (11,22 pA for L5 VIP to L5 MC and 21,33 pA for L2/3 VIP to L5 MC) and lower synaptic failure rates (<5 %) were found. Confocal laser scanning microscopy of putative contacts suggests a soma or proximal dendrite targeting of VIP axons of those pairs.

We were able to demonstrate that L2/3 as well as L5 VIP neurons effectively target L5 MC, thereby confirming and extending the VIP cell to MC connection as a prominent disinhibitory circuit motif in mouse primary somatosensory cortex. Synaptic currents of VIP cells to L5 MC were facilitating but only at 40 Hz presynaptic train stimulation. Since the group of VIP-expressing neurons is the most diverse group of inhibitory interneurons in terms of marker expression, morphology, membrane properties and firing pattern, more work is needed to investigate whether MCs are targeted by specific subpopulations of VIP neurons.

Disentangling a L2/3 VIP cell to L4 SST cell motif across primary somatosensory and visual cortices of mouse

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Complex animal behaviors can be attributed to a myriad of neuronal networks working synchronously. One motif that has been well-studied in circuit neuroscience, particularly in sensory areas of mouse, is the vasoactive intestinal polypeptide (VIP) cells targeting somatostatin (SST) cells. The inhibition exerted by VIPs onto SSTs opens a window of disinhibition, leading to increased dendritic excitability in excitatory cells. Although this motif has been functionally attributed to many sensory capacities in mouse, it has largely been studied in L2/3 of the cortex. This is understandable considering that a majority of VIP cells are localized in L2/3, where they receive inputs from many different brain areas. Morphologically, L2/3 VIP cells project robustly into the home layer, as well as into deeper layers, including the main cortical input layer, L4. However, the effects of L2/3 VIP cells onto L4 SSTs are yet to be uncovered. This missing link in cellular connectivity is particularly interesting to study, considering that recent evidence indicates vast differences between SST cells of L4 in primary somatosensory (S1) and visual (V1) cortices. L4 of S1 is considered to house many non-Martinotti cells (nMCs) with dense local axonal arborizations, while L4 of V1 is shown to primarily feature Martinotti cells (MCs), characterized by their L1-reaching axonal arbor. It is unclear if these morphological differences between cortices also manifests as functional differences in the VIP to SST motif. Therefore, we aim to study the L2/3 VIP to L4 SST motif in S1 and V1 sensory cortices of mouse.

We used transgenic mouse lines with fluorescence expression in VIP and SST cells for reliable targeting. We then employed dual whole cell patch clamp recordings in mouse brain slices to ascertain functional connectivity. VIP cells were studied with standard potassium gluconate intracellular solution. SST cells were patched with cesium methylsulfonate-containing intracellular solution and held in voltage clamp at 0mV, to reduce the space clamp issue, and to increase the driving force for VIP cells synapsed on SSTs in ~50% of the pairs (12/25 in V1 and 8/18 in S1), with an average amplitude of roughly 8pA and 6pA, respectively. Therefore, in terms of unitary synaptic connectivity, VIP cells seem to target the putative nMC and MCs in a similar manner. Short-term plasticity (STP) effects were also studied, wherein VIP cells were entrained to fire 5 action potentials at frequencies of 1, 10 and 50 Hz, and response patterns of downstream SST cells were analyzed. While we observed no visible STP in the inputs of VIP cells onto SSTs at lower frequencies, a strong difference was observed at 50Hz between V1 and S1. In V1, VIP cells of L2/3 show robust short-term facilitation onto SST cells of L4. On the other hand, in S1 we observed no facilitation, but rather one population of VIP cells that showed no STP onto L4 SSTs, and another that showed strong short-term depression. Therefore, the same circuit motif shares some properties like unitary and low-frequency response features, while some others, like high-frequency responses, are different between S1 and V1 cortices. How these functional differences contribute to sensory processing across areas will be an important addition to our knowledge in circuit neuroscience.

Platinum-based chemotherapeutics induce mitochondrial dysfunction and increase ROS production in TRPA1 or TRPV1 expressing sensory neurons

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Cisplatin and oxaliplatin are treatment options for a variety of cancer types. While highly efficient in killing cancer cells, both chemotherapeutics cause severe side effects as peripheral neuropathies, often accompanied by neuropathic pain syndromes. Consequently, chemotherapy-induced painful neuropathies (CIPN) are the main reason for limiting the dose during the anti-cancer treatment. Mechanisms leading to the genesis of CIPN are not fully understood until today. Several potential mechanisms involving the sensory neurons of the dorsal root ganglia (DRG) as DNA damage, loss of axons, cell death, disturbance of the calcium-homeostasis, or mitochondria dysfunction, are discussed.

Sensory neurons are critical players in transmitting pain sensations and can be divided into different subpopulations, expressing different membrane receptors as transient receptor potential ankyrin 1 (TRPA1) or transient receptor potential vanilloid 1 (TRPV1) channels. The TRPA1 channel reacts to noxious cold (<17°C) and chemical substances (e.g., allicin), whereas TRPV1 activates at high temperatures (>43°C), an acidic pH, and chemical substances (e.g., capsaicin). Both receptors are cation channels with high permeability for the second-messenger calcium.

In this study, we focused on cis- or oxaliplatin's influence on the mitochondrial function of TRPA1 or TRPV1 positive sensory neurons. Therefore, sensory neurons of Wistar rats were isolated, cultured, and exposed to cis- or oxaliplatin. Mitochondrial function was investigated using cell viability assay, mitochondrial stress assay, and live-cell imaging of reactive oxygen species (ROS) production and cytosolic and mitochondrial calcium concentration.

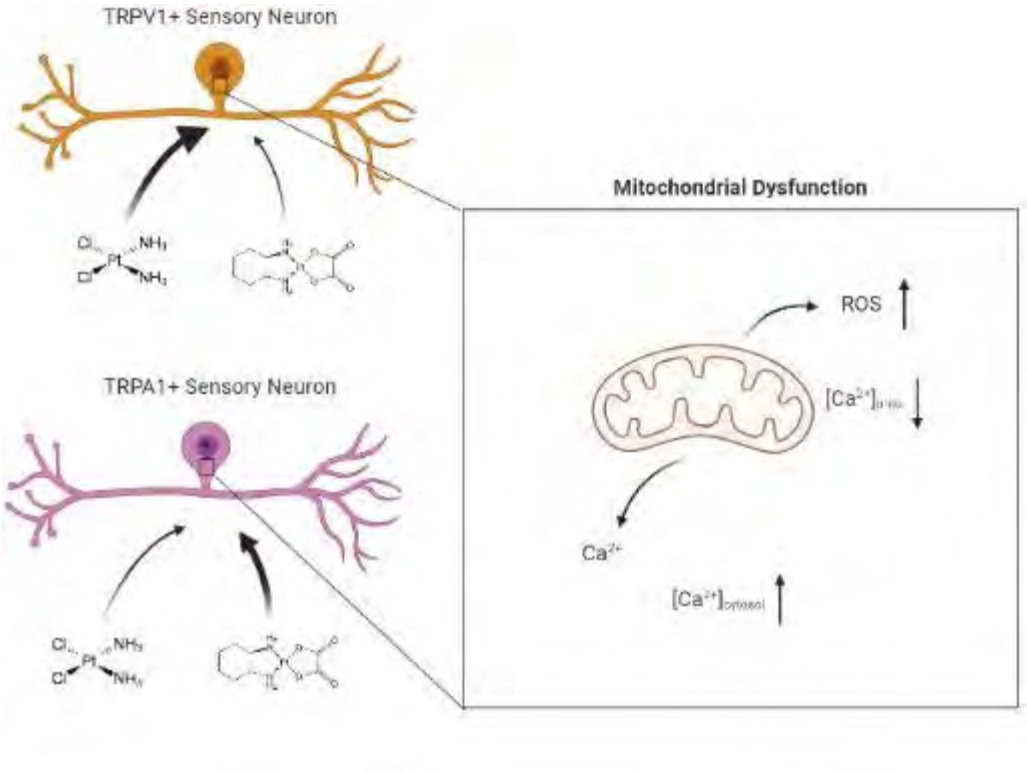
The mitochondrial stress assay determined that after exposure to cis- or oxaliplatin, the DRG neurons had impaired mitochondrial functions by inhibiting the respiratory chain complex I-III. The basal respiration (BR), spare respiratory capacity (SRC), and the adenosine triphosphate (ATP)-linked respiration (ATPR) were decreased after exposure to 10 µM cis- or oxaliplatin.

The ROS production showed an immediate increase, and after reaching the peak, ROS production dropped. The reason for the abrupt and significant decline could be the antioxidant defenses within the neuron to avoid ROS induced damage to the cell.

Calcium imaging showed an increase in the cytosolic calcium concentration during exposure to 10 µM cis- or oxaliplatin in TRPA1- or TRPV1-positive DRG neurons, while the mitochondrial calcium concentration continuously decreased (Fig. 1). The increased cytosolic calcium concentration could alter the electrochemical gradient for the calcium movement across the biological membrane by ion channels. It could also signal a change of cis- or oxaliplatin exposed DRG neurons' resting membrane potential. A depolarized resting membrane potential of neurons could lead to hyperexcitability and consequently, pain.

Our data demonstrate a significant effect of cis- and oxaliplatin on mitochondrial function as an early event of platinum-based drug exposure, suggesting mitochondria as a potential target for preventing

chemotherapy-induced neuropathy.



Input of antennal proprioceptors to an identified descending interneuron in the stick insect

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In insects the tactile sense is important for near-range orientation (Staudacher et al., 2005, Adv. Insect Physiol.) and is involved in various behaviours. Nocturnal insects such as the stick insect *Carausius morosus* continuously explore the surrounding by actively moving their antennae when walking (Dürr et al., 2001, J. Comp. Physiol. A). Upon antennal contact with objects, stick insects show a targeted front-leg movement (Schütz and Dürr, 2011, Phil. Trans. R. Soc. Lond. B). As this reaction occurs within 40 ms, descending transfer of information from the brain to the thorax needs to be fast. So far, a number of descending interneurons have been described that may be involved. One of these is the contralateral ON-type velocity-sensitive neuron (cONv). cONv was found to encode mean antennal joint-angle velocity during passive movement, as well as substrate vibration (Ache et al., 2015, J. Neurosci.). Here, we characterise transient response properties of cONv, as well as its dependence on joint angle range and direction. Further, Ache et al. (2015) showed that antennal hair field afferent terminals arborize close to cONv dendrites and are thus likely to make short-latency connections to cONv interneurons. Here, we use hair field ablations to test whether antennal hair fields contribute to the joint-angle velocity encoding of cONv. To address both research questions, we conducted bilateral extracellular recordings of both cONv interneurons per animal, using hook electrodes. Our results show that time courses of cONv responses are highly transient, though with slight velocity-dependent differences in delay (18 – 23 ms for 10 °/s and 50 – 65 ms 500 °/s), peak amplitude (20 – 25 Hz for 10 °/s and 40 – 60 Hz for 500 °/s) and steady state spike rate (5 – 5.5 Hz for 10 °/s and 17.6 – 25.5 Hz for 500 °/s). For all velocities tested, the steady activity level is maintained until the stop of antennal movement. The hair field ablation experiments show a moderate but significant reduction of movement-induced cONv firing rate by up to 28%. This indicates that proprioceptive hair fields contribute afferent input to cONv but that other antennal mechanoreceptor must be involved, too. Indeed, preliminary experiments indicate a second, movement-related afferent input to cONv comes from mechanoreceptors at the distal pedicel, and may be linked to passive deflection of the pedicel-flagellum junction.

Poster Topic

T21: Motor Systems

T21-11 Exploiting Low Dimensional Cortical Dynamics
To Speed Up Brain-Machine Interface Calibration
Michael Fauth, Michael Berger, Alexander Gail, Christian Tetzlaff

T21-12 Respiratory cycle timing during vocal exchanges in the squirrel monkey (Saimiri sciureus sciureus)
Udo Häusler

Role of CAMTA transcription factors in zebrafish cerebellar Purkinje cell neurons

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Purkinje cells (PC) signify the sole output neurons of the cerebellar cortex. Non-invasive *in vivo* Ca^{2+} imaging analysis in zebrafish has shown a regionalization of PCs in specific output domains that control discrete behaviors. For example, the caudal PC layer is involved in saccadic eye movements, while swimming behavior is controlled by the rostromedial PCs (1). However, the development of functional domains in PCs is only partially understood. *In vivo* Ca^{2+} imaging studies in our lab in larval zebrafish revealed that progressive refinement of PC layer regionalization occurs in correlation with PC differentiation and maturation. Optogenetic inhibition of neuronal activity during PC differentiation impaired dendrite maturation and spine formation and resulted in the absence of regionalization. However, the molecular cues associated with this activity-dependent maturation and regionalization in PCs remains unknown. Hence, we focus on the role of intracellular Ca^{2+}-signaling events in these processes at the transcriptional level. As candidate genes, we have focused on Ca^{2+} sensitive transcription factors - Calmodulin-binding transcription activators (CAMTAs) that are evolutionarily conserved across organisms from multicellular eukaryotes to humans (2) and are expressed in the mammalian cerebellum where their loss of function results in PC degeneration.

Two orthologous genes *camta 1a* and *camta 1b* of zebrafish have been identified as homologs to human *CAMTA1*. By fluorescent *in situ* hybridization followed by immunostaining in both adult and larval zebrafish, we revealed the expression of *camta 1a* in cerebellar cells that were confirmed to be PCs. CAMTAs contain a structurally conserved DNA binding domain termed CG-1 (2), which we used to establish dominant active and dominant negative variants for zebrafish *CAMTA1* transcription factors. The expression and function of these variants was validated by dual reporter luciferase assay. Subsequently, we established PC specific expression vectors that coexpress fluorescent proteins as morphology reporters together with the *CAMTA1* variants. Currently, the effect of altered *CAMTA1* onto PCs is under investigation. Furthermore, loss of function mutants of *camta 1a* and *camta 1b* were established with the help of the CRISPR/Cas9 system in zebrafish. These mutants and their phenotypes are currently being analyzed with respect to PC morphology and function. This model system accentuates the *in vivo* investigation of *CAMTA1* interference with PC neuronal activity, maturation, and functional regionalization in zebrafish cerebellum.

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Integrating multimodal proprioceptive feedback - influence of load on movement signal processing in the insect leg muscle control system

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Skilled motor control of body posture and movement strongly depends on proprioceptive feedback from the limbs. In legged animals, sense organs located on or within the limbs provide information about limb and joint posture and movement (muscle spindles (vertebrates); chordotonal organs (insects)), and load (Golgi tendon organs (vertebrates); campaniform sensilla (insects)). These proprioceptive signals are processed by local premotor networks in the spinal or ventral nerve cord, respectively, and used to generate the appropriate motor output such as corrective movements for posture maintenance, resistance or assistance reflexes. This requires the integration of proprioceptive signals from multiple sources into a single perceptual framework. In this study, we asked how the processing of different proprioceptive signals affect each other in the generation of the final motor output.

We combined intracellular sharp electrode recordings of sensory afferents, nonspiking interneurons (NSIs), and motor neurons (MNs) with extracellular nerve recordings and mechanical load and movement stimuli to investigate the effects of load signaling on movement feedback processing in the control loop of the stick insect femur-tibia joint.

Proprioceptive signals from the femoral chordotonal organ (fCO) and tibial campaniform sensilla (tiCS) induce reflex activation of tibial MNs. We tested the effect of combined load and movement stimuli on the gain of MN responses to altered movement (fCO) stimulus parameters (position / velocity). Simultaneous stimulation decreased the gain of positional feedback in the slow extensor tibiae MN (SETi), whereas the opposite was observed in the fast MN (FETi). In contrast, the gain of the effect of movement velocity signals in SETi and FETi was not consistently changed, while an increase was observed in the common inhibitor 1 MN (CI_1). Thus, the gain of proprioceptive movement feedback in tibial MNs was altered by load signals in a parameter- and neuron-specific way.

Both load and movement signals are integrated by a common network of local premotor NSIs which drive or inhibit the initially investigated extensor tibiae MNs. Concurrent load and movement stimulation resulted in nonlinear summation of the two signals in individually identifiable NSIs.

We then investigated where signals from tiCS and the fCO interact. We found interaction at the earliest neuronal stage, via presynaptic afferent inhibition. Activation of tiCS depolarized fCO afferents and reduced the amplitude of coinciding fCO action potentials. Presynaptic inhibition was instrumental for the observed influence of load on the processing of movement feedback, since its pharmacological removal by bath application of picrotoxin abolished the observed influence of load on movement responses in tibial MNs.

We conclude that fCO movement signal processing in the local premotor network is under the control of load feedback from tiCS. This provides a mechanism that could explain how the nervous system implements context-specificity in its computations at a local level, e.g. to alter signal processing and motor output between swing and stance phase during walking. Future experiments will focus on the behavioral effects of the control of movement gain via load feedback and on a more detailed understanding of presynaptic afferent interactions.

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Synaptic drive from central pattern generating networks to leg motoneurons in the deafferented walking system of the stick insect

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Rhythmicity of motor activity during walking in the stick insect is supported by the activity of central pattern generating networks (CPGs). CPGs rhythmically drive the motor neurons innervating antagonistic muscle pairs that are responsible for movement of the leg segments. Flexor tibiae and extensor tibiae motor neurons, innervating the muscles of the femur-tibia-joint, receive phasic alternating inhibitory synaptic drive from the premotor CPGs (Büschges, 1998, Brain Res 783:262; Büschges et al. 2004, Eur J Neurosci 19:1856). However, the synaptic drive from CPGs to the motor neurons innervating the muscles of the other leg joints still remains elusive. We sought to answer this question for the motor neuron pools of the two most proximal leg joints, i.e. protractor coxae and retractor coxae as well as levator trochanteris and depressor trochanteris motor neurons. For this, we activated the central networks and thereby elicited rhythmic activity in antagonistic leg motor neuron pools in the mesothoracic ganglion by topical application of the muscarinic acetylcholine receptor agonist pilocarpine (Büschges et al. 1995; J Exp Biol 198:435), while recording intracellularly from their neuropilar arborizations. Synaptic inputs to motor neurons were examined by analyzing membrane potential modulations and changes in their membrane resistance during rhythmic activity. In addition, we aimed at determining the synaptic drive leg motor neurons receive during the occurrence of so-called spontaneous recurrent patterns (SRPs) of coordinated motor activity, which resemble fictive step-phase transitions. Our results so far have shown that rhythmic activity of retractor coxae and protractor coxae motor neurons is generated by phasic inhibitory synaptic inputs, similarly to the flexor tibiae and extensor tibiae motor neurons. The amplitude of this inhibition increased upon depolarizing current injection and decreased upon hyperpolarization of the motor neurons.

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Motor skill learning and execution in a distributed brain network

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The remarkable capacity of the brain to acquire and execute motor skills depends on a distributed motor network. While many components have been identified, less is known about their specific roles and interactions during skill learning and execution. Here we probe this network through the lens of complex, spatiotemporally precise motor sequences we train in rats. We focus on the basal ganglia and the contributions made by their main inputs, from motor cortex and thalamus, respectively. Using electrophysiological recordings, we find that the dorsolateral striatum (DLS), the main motor-related input nucleus of the basal ganglia, encodes the detailed kinematic structure of the learned motor sequences. We further show that a loss of the DLS renders animals unable to execute the learned idiosyncratic motor patterns, causing them to revert to simple species-typical behaviors. In addition, we find that not only the DLS, but also its motor cortical inputs are necessary for learning the skills we train. This very same pathway, however, becomes dispensable after the behaviors are acquired. In line with this, the loss of motor cortical inputs leaves the DLS activity encoding the kinematic structure of the behavior largely unaffected. In contrast, thalamic inputs to the DLS remain crucial for the generation of the learned skills and loss of these inputs disrupts performance akin to DLS lesions, causing a reversion to the same species-typical behavior. Together, our results suggest that the basal ganglia can play a role in the control of complex learned behaviors which goes beyond traditional models of basal ganglia function. They further suggest that motor cortex ‘tutors’ sub-cortical motor circuits during learning, potentially by guiding plasticity at thalamostriatal synapses. Such adaptive reprogramming of lower-level motor circuits may broaden their flexibility and allow them to store and generate complex learned motor skills.

Reconfiguration of neural population dynamics for sensorimotor adaptation

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Sensorimotor adaptation refers to the ability to flexibly modify behaviors to a changing environment. One of the brain areas associated with sensorimotor adaptation is the posterior parietal cortex. In this study, we ask if population-level neural dynamics characterize visuomotor remapping behavior differently if associated with different levels of intrinsic sensorimotor adaptation.

We compared the dynamics of spiking neuronal activity in parietal reach region (PRR) when monkeys performed delayed center-out reaching tasks guided by two different visuomotor configurations. 1) Mirror-reversal reaching: A prism triggered sensorimotor adaptation to reversed visual feedback. Motor skill learning is required in this task for adapting movements to environmental changes. 2) Anti-reaching: An arbitrary spatial task rule requires to link spatial sensory inputs to pre-existing spatial motor patterns. Motor association learning is required for the cognitive spatial remapping. Under mirror-reversal, the spatial representation of a visual cue needs to be translated into an opposite-side physical goal representation during the motor planning period. If monkeys by default initially plan a reach towards the visual cue location, then a spatial motor-goal remapping is required under mirror-reversal, equivalently to anti-reach planning. We hypothesized that PRR employs two different neurocomputational strategies for motor-goal remapping in these two tasks, despite the fact that the overt visuomotor behaviors are similar. First, neurons may alter their intrinsic direction selectivity for planning. This scenario changes the structure of neural activity across the population, leading to different covariance structures and expanded dimensions in neural state space that is explored by the population activity. Second, the intrinsic direction selectivity of each neuron may be preserved and remapping achieved by selectively activating neurons with opposite selectivity. In the second case, we expect population activity to occupy the pre-existing neural manifold.

Results show that during reversed-vision reach planning the subspace capturing the neural dynamics best in the normal-viewing captured only little variance of activity in mirror-reversal reaching. This means, in this task which is associated with sensorimotor adaptation, neural activity patterns were characterized by a misaligned neural manifold and spanned a new set of neural dimensions. During anti-reach planning, instead, neural dynamics were characterized by a much higher alignment of the corresponding subspaces, indicating the utilization of a pre-existing neural manifold.

Our results emphasize the importance of the parietal region in embedding both context-dependent spatial remapping, as well as sensorimotor adaptation to changing sensorimotor environments. While the former can be achieved within existing neural dynamic manifold, the later requires reconfiguring neural population responses.

Movement force or effort encoding in the fronto-parietal reach network in primates?

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When choosing among multiple actions, according to the affordance competition hypothesis, several areas of the brain generate competing neural representations of these actions, which are modulated by the desirability of each action outcome prior to decision. Correspondingly, neuronal activity in the primate fronto-parietal reach network co-encodes the directions of two reach goals in parallel, before a reach movement is chosen, and this direction-related neuronal activity can be modulated by benefits, e.g. reward value assigned to an action. Here we ask whether direction-related neuronal activity in the fronto-parietal reach network can be modulated by action costs, namely, the physical effort associated with a movement.

Two rhesus monkeys were trained to perform a memory-guided goal-directed center-out reach task on a robotic manipulandum. We experimentally controlled physical effort by varying resistive movement force (0N, 3N, 6N). pseudo-randomly every 32 successful trials. Except from experiencing the force haptically while reaching, and expecting a certain force level based on the previous trials, the animals were given no further information about the forces encountered during a trial. We recorded simultaneously from populations of single neurons in dorsal premotor cortex (PMd), the primary motor cortex (M1) and parietal reach region (PRR).

Our results show that the average direction-related population activity in PMd, M1 and PRR does encode movement force during the movement, but not during movement planning. We additionally decomposed direction- and force-related neural population activity using a support vector machine. Directional information can be decoded from the neuronal population activity during movement planning and during movement, whereas force information only during the movement, when the resistive forces were actually physically present.

We conclude that the primate fronto-parietal reach network encodes information about the movement force when performing a reach movement against a resistive force, but not when planning such movement unambiguously. Our conclusions are in line with previous studies that indicate that direction-related neuronal activity in the fronto-parietal reach network during action planning is only modulated by action benefits in a decision context, i.e. when the directions of two reach goals are encoded in parallel and compete against each other. We hypothesize and currently test if movement force modulates planning activity also only in a decision contest, which then would have to be interpreted as encoding of movement effort, rather than physical force. So far the results suggest that the primate fronto-parietal reach network supports selection of action in decision making, by integrating the corresponding subjective benefits and subjective cost associated to these actions, rather than planning the physical properties of a selected action.

Tactually induced Changes in Walking Direction and Antennal Movement in Stationary Walking Stick Insects

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To explore their near-range environment many animals make use of so-called active tactile sensing [1]. Rodents like rats use whiskers on their snout, whereas insects and crustaceans use a pair of antennae on their head. Rhythmic movements of these sensory probes allows them to obtain a haptic picture of their surroundings. In insects, for example, antennal tactile information has been shown to underlie tactually guided turning and wall-following in cockroaches [3], or induce reach-to-grasp movements in climbing stick insects [2]. Cockroaches and stick insects are widely used study organisms in research on hexapedal locomotion. Therefore, their use of the tactile sense in their locomotor behavior is of particular interest. A common problem in research of active tactile exploration behavior is the control of stimulus presentation during locomotion. Here, we use a setup that allows us to control the position of a tactile stimulus in the antennal beating field of tethered walking stick insects, while motion-capturing the movement of the head, front legs and both antennae, as well as recording the speed and heading of the walking animal.
The antennae of the Indian stick insect *Carausius morosus* have approximately the same length as their front legs, which allows them, for example, to find suitable foothold within reach of the front legs [4]. Our objective was to assess the dependency of the overall turning response and of tactile sampling behaviour on stimulus position. To this end, we positioned a vertical metal rod within the contact range of the antennae while vision was occluded. Rod positions were randomized in steps of 10° within an azimuthal range of ± 40° relative to the head midline, with constant distance to the head.
We find that stick insects frequently contact the vertical rod with their antennae within 0.5 s after its introduction into the antennal range. For the entire tested stimulus range we find that both antennae make contact with the object, though with higher contact frequency of the ipsilateral antenna. Animals also change their heading in direction of the stimulus, with the response magnitude depending significantly on stimulus azimuth. The spatial exploration pattern of both antennae is elliptical and slanted, with a bilateral overlap in front of the head. The horizontal beating field of both antennae follows the position of the stimulus with a significant linear dependency and tends to decrease in width towards more lateral stimulus positions for the ipsilateral antenna. Moreover, the vertical antennal beating field of the ipsilateral antenna becomes more elevated with increasingly lateral stimulus position. With the present study we establish and validate an experimental paradigm that allows us to analyze details of tactually induced changes in motor behavior of tethered walking insects.

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One appendage, two behaviors - pectoral motor control in the hatchet fish *Carnegiella strigata*

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Behavioral flexibility of pectoral appendages is a common feature throughout the animal kingdom. Therefore, studying neuronal pectoral appendage control in other species can provide us with novel insights that might foster the progress of the control of bio-inspired prostheses. One fish group that shows specialized pectoral behaviors are hatchet fish. Their hypertrophied abductor muscles enable them to explosively leave the water in response to a potential threat. We show that hatchet fish also display an additional, previously undocumented behavior, which we termed fin flickering, that likely serves a postural function. In contrast to the escape behavior, fin flickering is characterized by a smaller amplitude and more variable duration of pectoral fin motion.

How can such widely different behaviors be mediated by the same set of motoneurons? Retrograde tracing of the nerves innervating the pectoral fin muscles revealed a widespread spatial distribution and a set of differently sized motoneurons, the latter being associated with different behaviors in other systems. Gap junction passable tracers did not reveal trans-synaptic labeling, even though gap junctions are known to occur between moto- and pre-motoneurons in the hatchet fish pectoral system. While the pre-motoneuronal network of escape responses is associated with the Mauthner escape system, and has been detailed previously, this system is, however, not able to generate fin flickering behavior, thus suggesting additional neuronal populations involved in pectoral control. Our data thus suggests that the observed pectoral fin abductive behavior dualism is likely associated to differences in motoneuron size and pre-motoneuronal control, instead of altered pre-motoneuronal activity. As large motoneurons likely only contribute to escape behavior, we expect to find prominent differences in the action potential firing behavior and membrane properties of differently sized motoneurons.

Natural variability of walking behavior in *Drosophila melanogaster*

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The expression of behavior is always subject to variability, both intra- and inter-individually. What we consider to be normal behavior is therefore never the arithmetic mean, but rather a continuum of expressions. Detailed examination of this continuum and the underlying mechanisms is essential for assessing the effect of experimental interventions and also provides insights into the neuronal control of the studied behaviors.

The natural variability of walking behavior in *Drosophila* has not yet been the primary focus of a study, because the crucial prerequisite is an extensive data basis and acquisition of high-quality data of walking flies was very labor-intense until recently. In this study, we used a deep artificial neural network approach to drastically enhance the process of extracting information from videos of freely walking *Drosophila*. This approach delivered high-quality results comparable to manual annotations and enabled an almost 50-fold faster data analysis. The resulting data set contained the body and leg tip positions of over 100,000 steps taken during normal, straight walking in a total of 103 individual male wildtype flies. Leg tip positions of 30 steps per fly were normalized to body length and pooled into a single matrix; we then used principal component analysis (PCA) on this matrix to reveal correlations and covariations in the movement of leg tips throughout the step cycle. The resulting principal components (PCs) were shown to describe different facets of variability in the data set: PCs 1 and 4 relate to previously described inter-leg coordination patterns, while PCs 2 and 3 described systematic differences between individual flies in a condensed form. Hence, these PCs provide the opportunity to concisely describe idiosyncrasies in individual walking behavior by putting them in relation to the reference data set. We then tested the suitability of this approach for assessing the effect of experimental interventions with data from an optogenetics experiment. Here, an inhibitory channelrhodopsin was used to temporarily silence groups of sensory neurons in the legs of *Drosophila* during walking. The resulting differences between walking in the dark (no inhibition) and under exposure to green light (inhibition in targeted neurons) was described, quantified, and compared in terms of the aforementioned PCs.

The neural basis of spectral prosody in avian vocal duets

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Antiphonal duetting in birdsong, as conversation in human speech, is a form of vocal turn-taking. Turn-taking requires participants to control and adjust their vocal production in regards to conversation cues received from a partner. It has been suggested that birds pay attention to the prosodic structure to coordinate their song. We hypothesize that prosodic cues, such as changes in the “intonation pattern” of song elements, facilitate duet coordination by predicting the boundaries of the partners‘ turns.
In songbirds, vocal production is controlled by an interconnected network of brain nuclei, the so-called vocal control system. One of these nuclei, HVC, plays a crucial role in arranging individual vocal emissions into sequences. We hypothesize that if prosodic cues are used by songbirds in vocal turn-taking, the production of song elements that differ in prosodic pattern should be controlled differently by HVC.
To tackle these questions, we used duet singing White-browed Sparrow Weavers (Plocepasser mahali) as animal model. Pairs of P. mahali sing antiphonal duets by precisely alternating their vocalizations. We recorded the individual vocal and HVC activity in both partners of duetting pairs, and found that P. mahali duet songs are indeed organized into spectral prosodic patterns. The maximum frequency contour of male birds’ song syllables could be classified either as rising (R) or as falling (F) pattern. The pattern of the maximum frequency contour of female birds' song syllables could also be divided into two groups: low (LM) - and high (HM) – frequency modulated syllables. Based on these prosodic patterns, we constructed a markov chain for each individual song to determine if there are underlying duetting rules to guide the turn-taking in the duet song. Both male and female prosodic patterns were shown to alternate between each other. Female prosodic types LM and HM showed greater than expected transition probabilities to male prosodic types R and F, respectively.
In addition, we found that in male P. mahali, HVC activity was different depending on the prosodic pattern of produced syllables. Median spike time relative to syllable onset was significantly higher in F-type syllables than in R-type syllables. This indicates, that the right HVC is active later during the production of F-type syllables than during the production of R-type syllables. While in R-type syllables, maximum frequency is rather low at the beginning of the syllable, in F-type syllables, maximum frequency is rather low at the end of the syllable. Given that low frequencies are produced by the right syrinx, lateralization of vocal motor control might explain our observations.
Our study demonstrates the existence of spectral prosodic cues in birdsong and suggests song lateralization as the underlying neural mechanism for prosody generation.

Exploiting Low Dimensional Cortical Dynamics To Speed Up Brain-Machine Interface Calibration

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The neural activities recorded by electrode arrays during the planning and execution of a stereotypic behavior have been shown to vary strongly from day to day. Accordingly, a decoder trained on neural activities from one day, cannot be used at another day, but must be adapted or even trained from scratch. Adapting the decoder can be very time consuming, whereas retraining the decoder lead to a trade-off between number of examples acquired for training and the decoders accuracy and generalization capabilities To accelerate this retraining or adaptation, we exploit that the neuronal activity dynamics in cortex usually takes place within a low dimensional manifold which is preserved over days and weeks. By finding these manifolds for each day and comparing the neuronal trajectories , a mapping that aligns them with each other can be found (Gallego et al, Nature Neuroscience 2020). With this mapping decoders trained on one day can be used on another.
Here we compare this technique with the naive training of new decoders dependent on the number of samples available. For this, we use electrode array data from areas M1, PMd and PRR in freely behaving macaque monkeys performing a planning task. We find that especially for very few samples, manifold realignment outperforms naive training highlighting it potential applicability for brain-machine interfaces.

Respiratory cycle timing during vocal exchanges in the squirrel monkey (Saimiri sciureus sciureus)

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Introduction
Vocal exchanges of Saimiri sciureus (Sc) represents a good model for vocal communication in primates [1]. As precise respiratory control is regarded as a prerequisite for articulated speech in humans. [2], respiratory movements during vocal exchanges of Sc were analyzed.

Methods
Five adult Sc were trained to sit in a restraining chair inside a sound attenuated faraday chamber for sessions of about 1-2 h a day. At the end of the training period, animals were presented trill calls of familiar individuals by a computer during the last 30 minutes of the day's session (playback sound). Trills as well as isolation peeps were successfully elicited in this way. Respiratory movements were magnetometrically recorded [3].

Results
Fig A shows a histogram of all inspiration times during vocal exchanges from 1000 ms before and 2500 ms after the playback sound. As reference a similar histogram was calculated for the same number of trails without a response (Fig B). About 200 ms after the start of the playback sound the frequency of inspiration is elevated, while immediately after the playback sound inspiration frequency is down to zero. This distribution is significantly different from the time before the playback stimulus(p= 0.0065). This indicates that the respiration gets synchronized by the playback sound, such that inspiration timing is adopted to the needs of vocal communication.

The distribution of inspiration time preceding the response is focused around the onset of playback(a), and the time between inspiration and response(b) is ca. 500 ms.
But the most striking feature of both distributions is that their variance is larger than the variance observed in the distribution of the time between playback and response
This further demonstrates that the timing between the last inspiration and the start of vocalization is adopted for a fast vocal response.

This study has highlighted 3 points that describe the adaptation of the respiratory system to the needs of vocal communication.
1. During periods of vocal activity timing of respiration gets irregular.
2. Timing of inspiration preceding vocalizations is not random but differentially tuned for a short vocal response time.
3. Furthermore during vocal exchanges or responses, the inspiration preceding vocal activity is initiated early during listening to the playback call/ the partners call. An already started respiratory cycle is extended throughout the playback call, to allow the animal a fast vocal response after the end of the playback call.
Such a fast response is necessary specifically in larger free ranging groups of squirrel monkeys. As vocal exchanges establish the linkage between animals within a group [4] a fast response time clearly shorter than

the time between spontaneous calls is necessary for the calling animals to identify any other calls as a specific response to its own call

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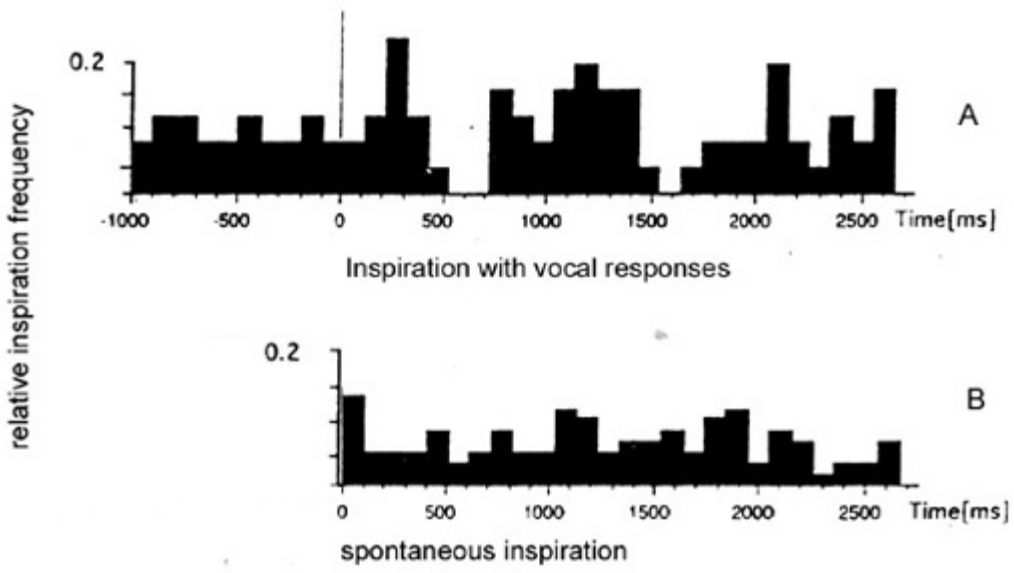

Poster Topic

T22: Homeostatic and Neuriendocrine Systems, Stress Response

T22-1 Differential gene expression in the hypothalamus of hibernating garden dormice (Eliomys quercinus)
Elena Haugg, Janus Borner, Sylvain Giroud, Annika Herwig

T22-2 When honey bee recruits search, they scout
Divya Ramesh, Arumoy Chatterjee, Deepika Bais, Axel Brockmann

T22-3 The interplay between LepR+ and Nts+ neurons of the lateral hypothalamus orchestrates foraging behaviour
Anne Petzold, Hanna van den Munkhof, Rebecca Figge, Tatiana Korotkova

T22-4 Gastric and lower oesophageal sphincter motility is affected by an ethanolic extract of *Alpinia officinarum*
Laura Menne, Kristin Elfers, Gemma Mazzuoli-Weber

Differential gene expression in the hypothalamus of hibernating garden dormice (Eliomys quercinus)

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Hibernation of garden dormice (Eliomy quercinus, Gliridae) is characterized by drastic reduction in metabolism associated with hypothermia, defined as a body temperature below 18 °C. Torpor bouts with thermoconforming body temperature of 5 °C are regularly interrupted after eight to ten days by few hours of interbout arousals with normothermic values of 37°C. There is evidence that the hypothalamus, maintaining chronobiological cycles as well as body temperature, plays an important role in hibernation. Many genes and proteins, including those with regulatory function in metabolism, thyroid system and circadian rhythm, have been suggested to orchestrate hibernation, yet molecular regulatory mechanisms on the transcriptomic level have largely remained unknown.

Twelve dormice (89.8 +/- 10.8 g body mass) were sacrificed during early torpor (one or two days torpid, n = 4), late torpor (nine or ten days torpid, n = 4) and interbout arousal (two hours normothermic, n = 4). Implanted transmitters revealed a core body temperature of 4.8 +/- 0.5 °C in torpid and 36.8 +/- 0.4 °C in normothermic animals.

After sacrifice, brains were quickly removed and shock frozen, followed by a dissection of the hypothalami. Total RNA was purified, from which mRNA was isolated and sequenced. Data analysis was performed after reference assembly and sequence annotation using the RefSeq of the well-known house mouse (Mus musculus, Muridae), a facultative heterotherm showing at least fasting induced torpor. After statistical normalization, the most up- and downregulated genes of pairwise compared physiological states (early torpor, late torpor, interbout arousal) are presented. Furthermore, indicator genes known to have potential regulatory function in metabolism, thyroid system and circadian rhythm (e.g. SRIF, VIP, GLUT, DIO, CRY, PER, QRFP) are screened for differential expression.

When honey bee recruits search, they scout

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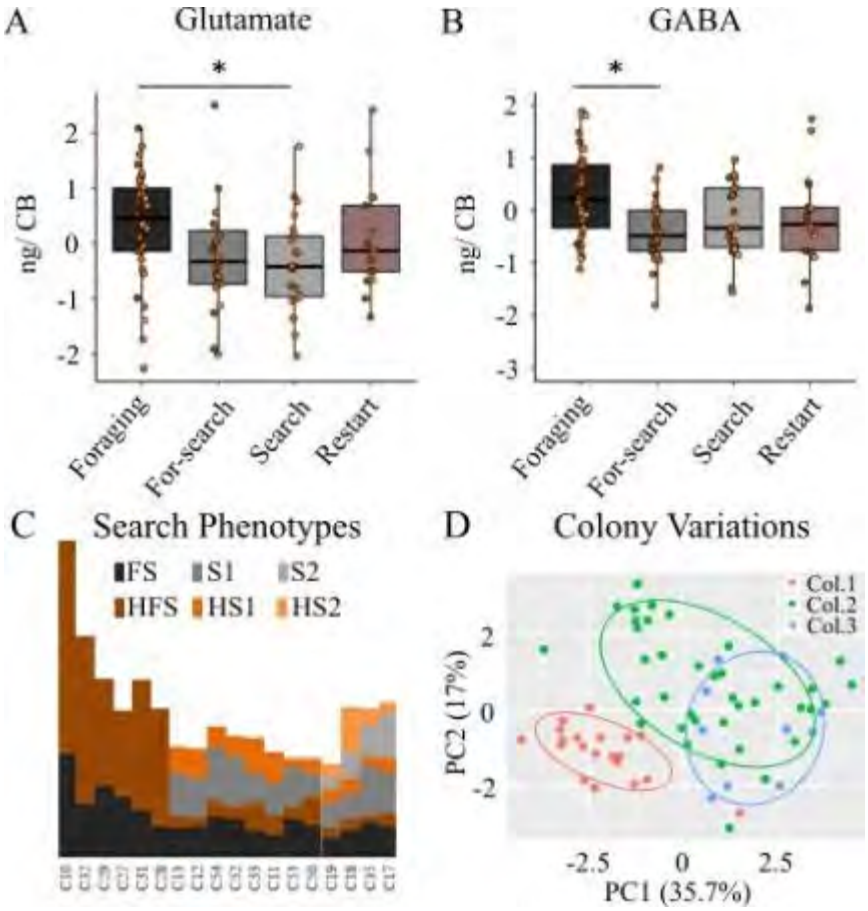
Division of labor among the non-reproductive worker caste is a hallmark of eusocial insect societies. In honey bees, for example, there are different forager types with respect to the food or resource they collect, or whether they search for novel food locations or exploit existing ones. These foraging related behavioral phenotypes differ in hormone and neuromodulator titers and brain gene expression patterns.

One of the first mechanistic hypotheses regarding the regulation of division of labor in social insects was that workers differ in their behavioral response thresholds. Following and more elaborate hypotheses proposed that behavioral specialization is based on a temporal persistence of a certain brain state associated with specific neuromodulator titers and specific expression levels of receptors and other genes involved in cellular information processing. Most recently, it was even hypothesized that these brain states might be associated with specific gene-regulatory networks that are synchronously expressed in all brain cells involved in regulating the specific behavior.

Given the idea that behavioral specialists may present a specific brain state which increases the probability of performing the corresponding behavior, we were interested whether common modulatory systems underlie scouting specialization and simple search behaviour of recruits. We used mass spectrometric measurements to test whether the search behavior of recruits that was induced by the removal of a visited feeder is associated with short-term changes in neuromodulators involved in social scouting. Behaviorally, individual bees differed in their search phenotypes, and colonies differed in the composition of these phenotypes. Similar to scouting behavior, GABA, glutamate and catecholaminergic systems were involved in recruit mediated food search. We found that the central brain regions including the mushroom bodies and the central complex, showed changes due to the initiation and maintenance of a search phenotype, while the optic lobes were involved only during reinitiating of foraging. Furthermore, differences within search phenotypes, were correlated with differences in aspartate and histamine titers, indicating that search is a complex behavior under the regulation of multiple modulatory systems.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Search
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C
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FS
S1
S2
HFS
HS1
HS2
D
Colony Variations
Col.1
Col.2
Col.3
PC2 (17%)
2
0
-2
-2.5
0
2.5
PC1 (35.7%)

The interplay between LepR+ and Nts+ neurons of the lateral hypothalamus orchestrates foraging behaviour

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Aim: The lateral hypothalamus (LH) has long been implicated in the regulation of consumptive behaviours through neurochemically and functionally diverse populations. Among those, a local circuit of LepR-LH and Nts-LH neurons emerged as a major modulator of consumptive behaviours. It is still elusive whether LepRLH and NtsLH ensembles are relevant for the processing of environmental cues and their interaction with internal cues.

Methods: We performed deep-brain single-cell Ca2+ imaging of LepR-LH and Nts-LH neurons using a microendoscope in the freely moving, spontaneously behaving mouse. Optogenetic and chemogenetic perturbation allowed us to evaluate the functional relevance of these populations.

Results: LepR-LH neurons preferentially responded to food cues in the hungry state, and to water cues in the thirsty state. The satiety hormone leptin reined in LepR-LH activity to limit over-consumption in response to hunger. Conversely, Nts-LH neurons preferentially responded to water. Thirst activated NtsLH neurons which support the water seeking drive. Both populations encoded social cues, and could be classified based on their social response profiles. Social coding was more prevalent among LepR-LH neurons than Nts-LH neurons, and supported food consumption in the face of food competition.

Conclusion: We confirm that both LepR-LH and Nts-LH neurons play a role in consumption. In addition, we demonstrate that these populations respond differently to homeostatic challenges: LepR-LH neurons limit consumption to rein in excessive consumption, while Nts-LH neurons support water consumption in the face of feeding pressure in response to hunger. Both populations are differently affected by social stimuli. We suggest that an interplay between both populations is crucial for the successful navigation of the foraging environment.

Gastric and lower oesophageal sphincter motility is affected by an ethanolic extract of *Alpinia officinarum*

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Neurons of the enteric nervous system (ENS) form a continuous network along the gastrointestinal tract (GIT). They are able to control all digestive functions independently from the central nervous system. Dysfunctions of those neuro-muscular circuits controlling gastric motility cause functional disorders of the upper GIT and imply various symptoms, which negatively affect patients' quality of life. Phytopharmaceutical substances are often used in those patients, since they appear to be better tolerated than common drugs. However, it is important to clarify their mechanisms of action. In this study, we investigated the effects of an ethanolic extract of lower galangal roots (*Alpinia officinarum*) on the motility of the stomach and the lower esophageal sphincter (LES) of the guinea pig. In our organ bath set up we recorded *in vitro* motility of muscle-myenteric plexus preparations from three gastric sub regions as well as from LES. Baseline pre-treatment muscle tension was compared to muscle tension after addition of the compound. In order to characterize the mechanisms underlying the effects of the ginger root and especially to understand if the effects were nerve mediated we performed experiments with tetrodotoxin (TTX, 1 µmol/l) and a transient receptor potential ankyrin (TRPA) 1 calcium channel blocker (HC030031, 10 µmol/l).

Alpinia officinarum ethanolic extract consistently led to a significant and reversible relaxation of both circular and longitudinal gastric fundus and corpus muscles with a more pronounced effect in the circular muscle layer. The relaxation effect was dose-dependent and mostly TTX insensitive. Induced relaxation of circular muscle tension was significantly inhibited by incubation with HC030031 prior to addition of the compound. LES preparations were less responsive indicating differential responses to *Alpinia officinarum* dependent on the gastric tissue location.

In conclusion, results of the present study show that an ethanolic extract from *Alpinia officinarum* causes a primary myogenic mediated relaxation in guinea pig gastric corpus and fundus muscle tone, which is at least in part based on inhibited influx of extracellular calcium via TRPA1.

The results obtained can serve as basis for further investigations with the aim to find an alternative and additional herbal treatment of functional gastrointestinal disorders based on *Alpinia officinarum*.

Poster Topic

T23: Neural Networks and Rhythm Generators

T23-1 A single neuromodulator is sufficient to reduce interindividual variability
Anna C. Schneider, Dirk Bucher, Farzan Nadim

T23-2 Long range glutamatergic and GABAergic inputs to the Nucleus of the Tractus Solitarius.
Orlando José Cortés Campo, Philip Tovote

T23-3 Parallel circuits underpin reproductive state-dependent behavior in *Drosophila* females
Anja Beatrice Friedrich, Ariane Boehm, Sydney Hunt, Sophie Aimon, Julia Claussen, Marie-Helen Link, K.P. Siju, Corinna Dawid, Ilona C. Grunwald Kadow

T23-4 Hippocampal CA1 pyramidal cells with dendritic axon origin receive specialized interhemispherical synaptic inputs

Nikolas Stevens, Christian Thome, Martin Both, Andreas Draguhn

T23-5 Functionally and morphologically distinct oscillations the in the fronto-temporal network linked to vocal production
Francisco Garcia Rosales, Julio C. Hechavarría

T23-6 Understanding the signatures of anesthesia.
Jesus J. Ballesteros Carrasco, Sourish Chakravarty, Indie Garwood, Emery N. Brown, Yumiko Ishizawa

T23-7 Pigment-dispersing-factor- and GABA-dependent circadian clock neurons modulate light responses of the compound eye and locomotor activity in the Madeira cockroach *Rhyparobia maderae*
Jenny A. Plath, Julia Y. Gestrich, Waliu Alaka, Marius Bartholmai, Ragna-Maja von Berlepsch, Pablo Rojas, Huleg Zolmon, Nali Hussein, Martin Garcia, Monika Stengl

T23-8 Compartmentalization of brain activity during sleep in zebra finches
Hamed Yeganegi, Janie M. Ondracek

T23-9 Does a weakened I_H abolish inhibitory postsynaptic potentials in the *reeler* somatosensory cortex?
Anouk Johanna Maria Meeuwissen, Martin Mock, Jochen F. Staiger, Julien Guy

T23-10 Chemoconnectomics and temporal activity of PTTH neurons in pharate *Drosophila melanogaster*
Emad Amini, Christian Wegener

T23-11 Maturation of information processing across cortical development
Soledad Dominguez, Liang Ma, Han Yu, Dion Khodagholy, Heiko J. Luhmann, Jennifer N. Gelinas

T23-12 Levels of chaos in neuronal transitions are altered by history-dependence of neuromodulators
Josselyn Gonzalez, Rosangela Follmann, Epaminondas Rosa, Wolfgang Stein

T23-13 Neuromodulator-induced rescue of temperature-elicited motor pattern breakdown: a comparative study with three decapod crustaceans.
Wolfgang Stein, Carola Städele

T23-14 Respiration paces prefrontal neuronal activity during intense threat
Shani Folschweiller, Jonas-Frederic Sauer

T23-15 The organisation of cricket rivalry and courtship behaviour indicated by lesions to the abdominal CNS
Chu-Cheng Lin, Berthold Hedwig

T23-16 Layer specific functional connectivity between RFA and CFA in lightly anesthetized mice in vivo
Svenja Kreis, Muthuraman Muthuraman, Sergiu Groppa, Heiko J. Luhmann

A single neuromodulator is sufficient to reduce interindividual variability

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Neural circuits produce similar output across individuals. This is surprising since the underlying ionic currents, which give rise to the activity of single cells within a circuit and of the circuit itself, vary largely across individuals in the same cell type. Neural circuits operate in the presence of multiple neuromodulators *in vivo*, yet ionic currents are often measured in isolation. We hypothesize that neuromodulation plays a key role in reducing interindividual variability.

We focused on the synaptically isolated LP neuron of the crab stomatogastric ganglion (STG). Only one copy of this neuron exists in each animal. LP produces regular bursting oscillations driven by periodic inhibition from a group of pacemaker neurons. When synaptically isolated, LP is quiescent. After removing endogenous modulatory input to the STG, we compared, across animals, the variability of endogenous ionic currents I_{HTK}, I_A and I_h in control saline and in the presence of the peptide modulator proctolin (10^{-6}M). We found high levels of variability of all three currents, as previously reported, but no change in variability due to proctolin modulation. Additionally, we found that two modulatory currents activated by proctolin (and other peptide modulators) were just as variable. We concluded that proctolin did not activate a consistent level of modulatory input to LP. There was no linear correlation in the levels of the proctolin-activated currents and any of the other currents we measured.

We measured excitability (f-I curves) and rebound properties (latency, spiking structure) of LP in control saline and in the presence of one (proctolin) or two (proctolin + CCAP) peptide modulators at a total concentration of 10^{-6}M. We then compared the variability measured as coefficient of variation or standard deviation of these responses across preparations under the different modulatory conditions. For the f-I curves, we applied 0-5nA in 0.5nA increments, and then reversed the steps to measure hysteresis as ratio of spike frequency during depolarizing to repolarizing current injection. We fitted the spike frequencies over injected current with a power function and compared variability of the fitting parameters, as well as hysteresis. For rebound spiking structure, we fitted the cumulative spike histogram with a sigmoid and compared the fitting parameters total spikes, time of midpoint, and steepness.

Our results demonstrated that neuromodulators significantly increased LP's excitability and decreased hysteresis. Changes in rebound spiking structure were consistent with the results of increased excitability, although there were no differences for midpoint and steepness. However, in all cases, even those where neuromodulation did not significantly change a parameter, variability was reduced when modulators were present. The addition of a second modulator did not additionally reduce variability. These results indicate that, at relatively high concentrations, a single neuropeptide modulator can reduce interindividual variability, leading to more consistent output at the single-cell level. The reduction in variability is not due to the modulator activating a consistent level of a modulatory ionic current, nor to activating an ionic current that is proportional to the intrinsic currents. Thus, the reduction in variability of the neuron's activity across animals is most probably due to the nonlinear interactions among intrinsic ionic currents in the presence of the

modulator.

Long range glutamatergic and GABAergic inputs to the Nucleus of the Tractus Solitarius.

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Challenged with threats, living organisms exhibit a plethora of adaptive survival responses. The biological machinery underlying the survival responses and their neuronal substrates that support them has been conserved throughout evolution. In order to react to innate or learned threatening stimuli the mammalian brain generates integrated affective states that encompass various defensive responses including behavioral changes, adaptations of the autonomic system and endocrine fluctuations. Brain areas such as central amygdala (CeA), periaqueductal gray (PAG) and nucleus of the solitary tract (NTS) have been demonstrated to play important roles in mediating behavioural and autonomic aspects of the defense reaction. However, while the integrated nature of defensive reactions has long been known, the underlying circuit mechanisms remain poorly understood. Novel technologies such as the genetic dissection of brain circuits enable us to gain a better understanding of the neurochemical and molecular identity and importantly, the specific connectivity of targeted neuronal populations. This allows for detailed characterization of the anatomical basis for defensive circuit functions.

In this study, we used genetic and viral approaches to intersectionally trace the connectivity of the NTS, a central regulator of cardiovascular functions, and the inputs that this medullary structure receives from vesicular glutamate transporter 2 positive neurons (Vglut2+) of the PAG and glutamate decarboxylase 2 enzyme positive cells (Gad+) within the CeA.

A retrogradely transported adeno-associated virus (AAV) injected into the NTS confirmed the inputs from PAG and CeA to the NTS. Likewise, a co-infection mediated by the injection of a double-conditional, anterogradely transported AAV injected into the PAG (Figure 1) and the CeA allowed to label the synaptic terminals from neurons located in these two brain regions innervating the NTS as well as the synaptic terminals from their collaterals distributed throughout the brain. The main results suggest that a population of glutamatergic neurons of the ventrolateral column of the PAG (vlPAG), innervates the NTS along its anterior-posterior extension and sends collaterals to other nuclei in the ventral hindbrain, including the magnocellular medulla (Mc), a brain region essential for freezing. Additionally, the obtained data revealed long range GABAergic projections from the CeA to the NTS, sending collaterals to the vlPAG which are major parts of the circuitry fundamental for both, behavioural and autonomic the defense responses. Our data builds the basis for functional studies aiming at shedding light on how these projection pathways coordinate integration of different components of the defense reaction.

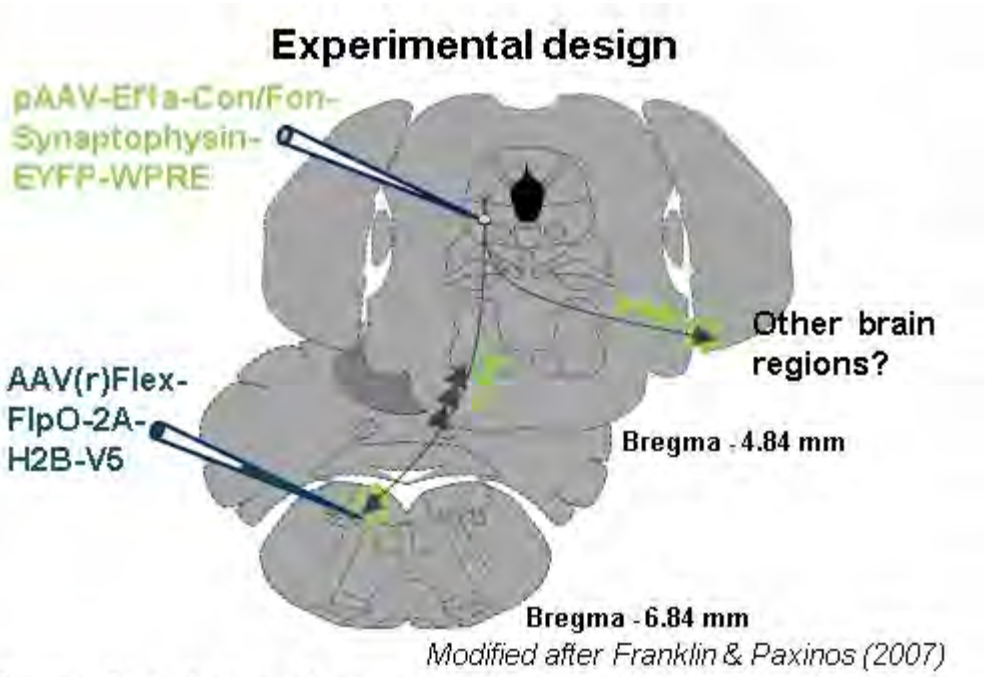

Figure 1. Scheme of an intersectional dissection mediated by retrograde and anterograde tracings combining the Cre/loxP and Fpl/FRT systems. In green, an injection of a double conditional AAV into the vlPAG, carrying genetic material whose translation depends on the presence of Cre and Flp recombinases.

Parallel circuits underpin reproductive state-dependent behavior in *Drosophila* females

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Internal states such as hunger or reproductive state strongly drive and alter innate and learned behaviors. Mating for example induces a drastic, sometimes long-lasting, change in internal state in most female animals. In Drosophila females it induces changes in food preferences and oviposition sites. We previously showed that mating induces a switch towards a change in preference to an important nutrient, polyamine, following a neuropeptidergic modulation in the olfactory sensory neurons (OSN). This behavioral change implies a flexible, more complex processing of these chemical cues in the female brain. Here, we show that this mating-induced flexibility in behavior of *Drosophila* females relies on parallel processing in two interconnected olfactory brain regions, the lateral horn (LH) and the mushroom body (MB). Using systematic behavioral screening, connectomic analysis, and in vivo imaging, we identify specific LH ouput neurons required for the attraction of females to polyamines. Moreover, we find that an enduring increase in mated female preference for polyamine requires Rutabaga, an adenyl cyclase with a highly conserved role in associative learning and memory, as well as neuronal output of the MB. We uncover two different MB pathways required for the reproductive state-dependent choice behavior, with a prominent role for the β'1 region. Specific dopaminergic neurons (DAN) and MB output neurons are sufficient to replace mating experience and induce the lasting behavioral switch in female preference. Our data in the fly provides insights into the neural circuits involved in a lasting modulation of female behavior.

Hippocampal CA1 pyramidal cells with dendritic axon origin receive specialized interhemispherical synaptic inputs

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Hippocampal principal neurons receive synaptic inputs from multiple brain regions at their dendrites which are then integrated at the axon initial segment. A large fraction of hippocampal cells has axons emerging from a basal dendrite rather than from the soma. We have previously shown that this axon-carrying dendrite (AcD) constitutes a privileged pathway for synaptic integration and action potential generation. We have therefore studied the prevalence and distribution of pyramidal cells with dendritic axon origin throughout the adult mouse hippocampus. Further, we identified the origin of functional synapses on the axon-carrying dendrites.

We found considerable variability of the AcD prevalence along the hippocampal sub-regions and the dorsal-ventral axis of the brain. The strongest concentration of such cells was found in area CA1 of the ventral hippocampus with up to 70% of neurons exhibiting a dendritic axon origin (N = 8 hippocampi from 10 animals).

To assess the functional role of cells with axon-carrying dendrites (AcDcells), we investigated the origin of inputs to cells with this peculiar morphology. We focused on CA1 pyramidal cells which receive strong innervation from ipsilateral and contralateral CA3 neurons and by ipsilateral CA2 neurons. In order to identify differences in the innervation of cells with and without dendritic axon origin, we patched individual neurons and used optogenetic stimulation to specifically activate fibres from different hippocampal input sites, measuring excitatory postsynapitic potentials (EPSPs) and -currents (EPSCs) at different stimulation strengths and locations.

We found that contralateral inputs(EPSCs) from CA3 on the basal dendrite were approximately twice as strong in cells with dendritic axon origin compared to other CA1 neurons (amplitude AcDcells =-286.3 pA ±44, nonAcDcells=-127.4 pA ±33.65 (mean ±sem); n(AcDcells)=28, n(nonAcDcells)=14 from 6 animals; unpaired t-test $p<0.05$;). Stimulation of inputs to apical dendrites showed no differences between the two cell types. No difference in innervation was seen in inputs from ipsilateral CA3 neurons (amplitude AcDcells =-292.6 pA ±39.85, nonAcDcells=-302.9 pA ±75.7 (mean ±sem); n(AcDcells)=25 n(nonAcDcells)=10 from 6 animals; unpaired t-test $p=0.89$). Consistent with the literature, the input to basal dendrites compared to apical dendrites was stronger with contralateral stimulation and weaker with ipsilateral stimulation. These data show a specific asymmetry in synaptic activation of cells with or without dendritic axon origin. Further studies are underway to correlate these functional data with morphological innervation patterns from different presynaptic regions. Input from CA2 is currently under investigation. Preliminary data show similar input strengths from CA3 ipsilateral, contralateral and CA2 ipsilateral. In summary, our work provides additional insight into the functional role of cells with dendritic axon origin in the hippocampal network.

Functionally and morphologically distinct oscillations the in the fronto-temporal network linked to vocal production

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The ability to vocalize is essential for the success of many animal species. However, the precise neural mechanisms that underly vocalization control, and the nature of large-scale interactions in brain networks linked to vocal production, remain poorly understood. In mammals, the frontal cortex seems to be involved in the planning and execution of vocal production. Frontal areas are also connected with the auditory cortex (AC), an important hub for audition that participates in the feedback control of vocal outputs. Although a fronto-auditory circuit appears crucial for coordinating vocal production, little is known regarding the manner in which these regions interact during vocalization utterance.

This study addressed the fronto-auditory network during vocalization production in Carollia perspicillata bats. Bats constitute an excellent model to study vocal production because of their dependence on vocalizations for communication and navigation. In the frontal cortex, we focused on the frontal auditory field (FAF), a structure that appears involved in vocal coordination. Given the FAF's functional connection with the AC, it is possible to speculate that the FAF-AC network plays a crucial role for vocalization production. Whether and how this happens is still unknown.

We performed electrophysiological recordings in FAF and AC while animals vocalized freely. Simultaneous, pre-vocal neural activity in each region was contrasted between the production of echolocation pulses (sonar) or non-specific communication calls (non-sonar). We observed that the power of ongoing oscillations (local-field potentials, LFP) in both frontal and auditory cortices allowed to predict ensuing vocal type in a frequency specific manner. In the FAF, low- (1-12 Hz) and high-frequency (70-120 Hz) pre-vocal LFP power increased during vocalization, with stronger increase when animals uttered sonar calls. In the AC, differences in power increase occurred in the low-beta band (12-20 Hz), with an opposite effect. Such functional divergence was also reflected in the underlying neuronal mechanisms, as indicated by oscillation morphology in each region. These results suggest that ongoing oscillations in FAF and AC have different and complementary origins, signaling complex oscillatory interactions in the network.

Oscillations in the FAF-AC network were also causally linked based on a transfer entropy framework. Top-down information flow (FAF->AC) occurred mostly in low- (delta, 1-4 Hz) and high-frequency (gamma, 70-120 Hz) bands. In low frequencies, pre-vocal top-down influences were weakest during sonar call production, and strongest in the absence of vocal outputs. For gamma frequencies, pre-vocal top-down influences were strongest when animals produced sonar calls. The directionality patterns changed from pre-vocal to post-vocal times. In low frequencies, top-down influences made way to bottom-up information flow (AC->FAF), but only in the case of sonar vocalizations. Thus, pre-vocal activity in the FAF-AC network appears dominated by top-down influences in a frequency and vocalization dependent manner, which changes in post-vocal periods. This dynamic reorganization of information flow might contribute to the feedback correction of uttered vocalizations. Altogether, our data expose large-scale mechanisms in sensory and association cortices that could be essential for vocal production and control in the mammalian brain.

Understanding the signatures of anesthesia.

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General anesthetics are drugs that induce reversible unconsciousness. These altered states of consciousness are associated with highly structured oscillations and neural dynamics. While most common anesthetics potentiate GABAergic transmission, others interact with NMDA or α2 adrenergic receptors. We therefore used three different anesthetics: propofol, ketamine and dexmedetomidine. We studied the induction of and recovery from unconsciousness with each of these drugs. We related the non-human primate behavioral changes during these processes to electrophysiological recordings of intracortical local field potentials and multiunit activity obtained from a sensory-motor cortical system. The GABA agonist propofol leaded, in a fast transition, towards a state of slow oscillations across the cortex where the neural activity was depressed and phase-locked, generating the so called “burst suppression” state. The NMDA antagonist ketamine gradually built-up a slow frequency-modulated increase in gamma activity. Under ketamine anesthesia, the average neural activity remained unchanged but was disorganized. The sensory processing was preserved for longer time than the multisensory processing in higher cortical areas. The α2-adrenergic agonist dexmedetomidine induced a state of delta-alpha oscillations and kept the interregional coherence intact, showing sleep-like neural signatures. The recovery from dexmedetomidine involved a time of neural dynamics drift between two main states, until it was stabilized on the conscious state. The use of an α2-adrenergic antagonist to reverse the effects of dexmedetomidine bypassed the intermediate states completely and immediately, stabilizing the neural dynamics on the conscious state within minutes. Together with research on close-loop control and precise signal characterization, this knowledge allowed us to control the dosage of propofol based on individuals’ EEG, in real time, achieving high accuracy maintaining a stable level of anesthesia with only machine control of the infusion. Understanding how unconsciousness can be induced and reversed and how neural signals are related to these changes would reveal basic mechanisms of brain function.

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Pigment-dispersing-factor- and GABA-dependent circadian clock neurons modulate light responses of the compound eye and locomotor activity in the Madeira cockroach *Rhyparobia maderae*

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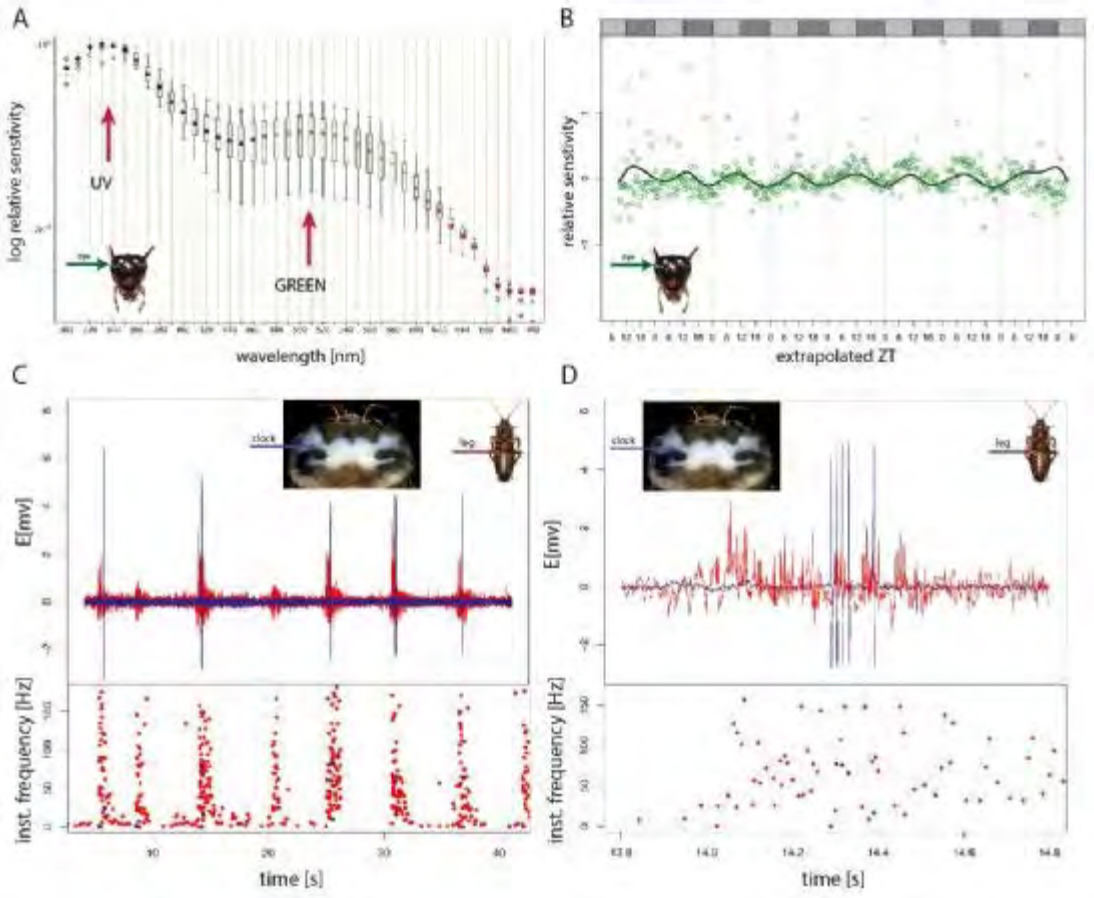
Circadian clocks of mammals and insects that orchestrate sleep-wake cycles entrain to the daily light-dark rhythm and appear to be composed of morning cells locked to dawn and evening cells locked to dusk. In the night-active Madeira cockroach we investigate how circadian clock neurons regulate sleep-wake cycles entrained by the compound eye. Transplantation experiments identified the accessory medulla (AME), a small neuropil in the brain's optic lobes, as the circadian clock of the cockroach. The AME consists of a glomerular, GABAergic core which receives indirect compound eye light input and a shell which sends outputs to midbrain neuropils regulating sleep-wake rhythms. The AME clock is innervated by twelve pigment-dispersing factor (PDF) expressing neurons that regulate sleep-wake cycles. While clock neurons that project to the contralateral clock control locomotor activity rhythms, it was suggested that ipsilateral clock neurons regulate sleep. Backfills from the contralateral AME combined with Ca^{2+}-imaging experiments found that PDF activated ipsilateral (iPDFMEs) and inhibited contralateral projecting PDF-expressing clock neurons (cPDFMEs). Thus, PDF release from iPDFMEs as morning cells appears to activate sleep promoting circuits during the day while inhibiting the cPDFMEs as evening cells that promote activity. Since UV exposure promotes inactivity while green light exposure activates cockroaches, we hypothesized that UV-light activates iPDFMEs inducing sleep GABA-dependently, while green-light activates cPDFMEs inducing activity. Different electrophysiological recordings of clock cells were performed to characterize PDF-, GABA, and light responses of ipsi- and contralateral AME neurons, combined with simultaneous electromyograms (EMGs) of the leg muscle. We observed correlations between changes in locomotor activity and changes of activity in non-light responsive AME clock cells. Also, with electroretinograms (ERGs) circadian rhythms in light sensitivity of the compound eye were examined in combination with simultaneous EMGs, to search for phase-coupled clock output rhythms. To determine whether EMG rhythms reflect locomotor activity rhythms, subsequently, cockroaches were transferred to running wheels. Indeed, EMG rhythms approximated daily locomotor activity rhythms. Since immunocytochemical stainings suggested that PDF and GABA as clock outputs affect the compound eye as well as clock circuits of morning and evening cells that control locomotor activity rhythms, we tested this with different experimental approaches. Preliminary GABA-injections reduced ERG responses to green and UV light in the morning (Zeitgebertime 2) in comparison to control injections. Furthermore, GABA and PDF affected neurons arborizing in the lamina, where short compound eye photoreceptors terminate. Currently, we investigate the effects of GABA and PDF-injections on light responses at different Zeitgebertimes. Additionally, ERGs are measured in RNAi-knockdown animals for PDF-precursor and PDF receptor. In summary, PDF release during the day keeps sleep- and activity promoting neuronal circuits in antiphase. Furthermore, preliminary results suggest that PDF modulates light input to the clock as well as modulating compound eye light

responsiveness via clock outputs apparently also via affecting GABAergic pathways. [Supported by DFG grants STE531/18-3 and STE531/26-1: SPP 2041 to MS and by Förderlinie Graduiertenkollegs: Biological clocks, Univ. Kassel]



Compartmentalization of brain activity during sleep in zebra finches

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Sleep in humans is characterized by the switching between two distinct brain states: slow wave sleep (SWS) and rapid eye movement (REM) sleep. During SWS in humans, the effective connectivity between cortical regions breaks down and is accompanied by a loss of consciousness [1]. Electroencephalography (EEG) recordings in birds also reveal distinct stages of SWS and REM, despite their different brain structure [2, 3]. However, the network connections that exist during different sleep stages remain unexplored in these animals. We recorded EEG during sleep in 7 zebra finches (3 adults, 4 juveniles) and monitored body movement with infrared video recording. We used graph theory to explore highly correlated EEG channels. We found that during sleep, small clusters of highly correlated networks exist, and that these clusters are spatially organized into frontal and caudal networks in each hemisphere. Furthermore, in some birds, we observed a decrease in correlation that occurred within minutes after the onset of behavioral sleep. This decorrelation was more pronounced between hemispheres, but also occurred between sites within hemispheres. These results highlight the similar compartmentalization of brain activity that occurs in both birds and mammals during sleep, despite the different neural architectures in these animals.

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Does a weakened I_H abolish inhibitory postsynaptic potentials in the *reeler* somatosensory cortex?

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In the severely disorganized *reeler* somatosensory cortex, thalamocortical axons manage to target misplaced layer 4-fated neurons. Although the *reeler* somatosensory cortex upholds proper activation in response to sensory input, thalamocortical input onto layer 4-fated spiny stellates was found to be weakened compared to wild-type, raising the possibility that specific intracortical mechanisms rescue the weakened thalamic input to the *reeler* cortex. Here, we hypothesized that such a compensation mechanism may involve weakening of feedforward or feedback inhibition provided by fast-spiking interneurons.

In order to study layer 4-fated neurons in the disorganized *reeler* cortex, we used the Scnn1a-tdTomato-Reeler mouse line, where layer 4-excitatory neurons express cre-dependent tdTomato. In vitro paired whole-cell voltage clamp recordings between fast-spiking interneurons and spiny stellates allowed for assessing connection probability and strength in *reeler* versus wild-type.

In *reeler*, a drastic decrease in connection probability and strength was found between fast-spiking interneurons and spiny stellates when using standard potassium-based intracellular solution. Remarkably, repeating these recordings with cesium-based intracellular solution in *reeler* restored the connection probability and strength to wild-type levels. Immunohistological staining for synaptotagmin-2, marking presynaptic boutons of fast-spiking, parvalbumin-expressing interneurons, showed that inhibitory synapses in both *reeler* and wild-type are likely to have a similar somatodendritic distribution.

The connection probability, connection strength and synapse location between fast-spiking interneurons and excitatory spiny stellates therefore seem unaffected in *reeler*. This suggests that dendritic integration of inhibitory postsynaptic potentials (IPSPs) is impaired in *reeler*. Dendritic excitability is controlled by the hyperpolarization-activated H current (I_H), an inward current mediated by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels. I_H depolarizes the membrane potential with respect to the reversal potential of $GABA_A$ mediated currents, which maintains the driving force underlying IPSPs. We hypothesize that in *reeler*, a weakened I_H reduces the driving force responsible for IPSP formation and hampers their propagation to the soma, causing an overall decrease in somatic inhibition. If true, enhancing HCN channel activity should restore the dendritic integrations of IPSPs in *reeler*. This poster shows promising preliminary experiments testing this hypothesis using paired intracellular recordings and pharmacology in thalamocortical slices.

Chemoconnectomics and temporal activity of PTTH neurons in pharate *Drosophila melanogaster*

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Eclosion, the emergence of the adult insect from the pupa, is a critical behavior and developmental step in holometabolic insects. When observed on the population level, eclosion is a rhythmic event. In *Drosophila*, eclosion is gated to the early morning by the interplay between the central circadian clock in the brain and a peripheral clock in the steroidogenic prothoracic gland (PG). We earlier showed that two neurons in each brain hemisphere producing the peptide prothoracicotropic hormone (PTTH) couple the central clock with the PG clock in pharate flies. Impairment of PTTH signaling leads to a loss of circadian gating and results in arrhythmic eclosion.
In this study, we focus on the timing of PTTH signaling. Using genetic silencing during different of pupal metamorphosis, we found evidence that PTTH signaling is important at the final pupal stage. We also characterized synaptic inputs to PTTH neurons from central clock neurons by syb-GRASP and a novel technique, BAc Trace. Our findings confirm the previous assumption that the sLNvs are important neurons for relaying clock information to the PTTH neurons, but other clock neuron groups seem to be involved too. We also characterized the putative chemoconnectome of the PTTH neurons, which confirmed sNPF as an important peptidergic input to the PTTH neurons and suggests that the PTTH neurons are exclusively peptidergic.

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Maturation of information processing across cortical development

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Neural networks underlying cognitive processes are characterized by complex communication within and between numerous brain regions. Here, we examine the maturation of information processing in primary somatosensory cortex. For this purpose, we use outbred mice from postnatal day 5-14 to perform in vivo electrophysiological recordings and wide-field calcium imaging. We acquire neural signals with a novel advanced neural interface device, the NeuroGrid, which permits minimally invasive, high spatiotemporal resolution recording, in combination with an immunohistochemical analysis to anatomically localize the recording electrodes. Furthermore, we have analyzed brain transcranial optical imaging at high spatial and temporal resolution . We found that there is an inflection point in oscillatory activity occurring specifically at P8-P9 that is reflected in the macrostructure of local field potential (LFP) organization and its state dependence, expression of characteristic oscillations, and spatial properties of these oscillations. These changes are associated by a transition in patterns of neural firing and can be transiently reversed by GABAergic receptor blockade. Based on these results, we propose that oscillatory activity transitions from a local synchronization to an information processing mode around P8-9, a time point that coincides with emergence of supragranular connectivity, mature GABAergic inhibition and gradual onset of exploratory behavior.

Levels of chaos in neuronal transitions are altered by history-dependence of neuromodulators

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While transitions between neuronal states are essential to cognitive and motor functions, they are less understood than the states themselves. Transitions often consist of irregular firing activity that computational models predict is chaotic, meaning that it is deterministic and sensitive to initial conditions. In fact, prior research shows that neuronal systems can exhibit levels of chaotic activity. Furthermore, these levels of chaos can be reduced through network interactions to achieve stable network activity. This indicates that minimizing the levels of chaos is desirable for the function of neuronal networks. Therefore, we hypothesize that biological neurons possess mechanisms to reduce chaos during transitions.

To characterize the levels of chaos during transitions, we use a combined experimental and computational approach. In our experimental approach, we induce transitions in the well-characterized pyloric central pattern generator of the crustacean stomatogastric nervous system using the neuropeptide proctolin. In our model approach, we use the Huber-Braun single neuron model and implement the excitatory, depolarizing current that proctolin activates, I_{MI}.

In agreement with previous studies, I_{MI} in the model was sufficient to elicit transitions between stable activity states with chaos occurring between states. However, chaos was only observable with sufficiently long transition durations, i.e. the time interval during which the transition takes place, or when discrete models with distinct I_{MI} values were compared. When we implemented a time-dependent I_{MI} in our model, we found that the time course of I_{MI} application and, thus, the transition duration played a significant role in the dynamics of the transition. Specifically, longer transitions showed chaos while shorter transitions did not.

To test whether this was the case in the biological system, we synaptically isolated the lateral pyloric neuron (LP) and bath-applied proctolin. This increased firing rates and elicited rapid transitions from silent or arrhythmic spiking to bursting. We quantified the levels of chaos using Lyapunov exponents which measure how quickly a system becomes unpredictable. Although the system exhibited chaos throughout the transition, the levels of chaos did not change significantly during the transition itself. Taken together with the model results, this suggests that rapid neuronal transitions suppress increases in chaos.

To further explore how the history-dependence of neuromodulators affect chaotic transitions, we used dynamic clamp to inject discrete levels of I_{MI} into LP, omitting the time-dependence of proctolin. Increasing I_{MI} induced transitions from silent or arrhythmic to tonic. We found that the levels of chaos did not change significantly throughout this transition, possibly due to LP being unable to burst with I_{MI} alone. To test this,

we performed similar proctolin and dynamic clamp experiments with the inherently bursting pyloric dilator neuron (PD) of the pyloric circuit. We are currently analyzing the results of these experiments.

Neuromodulator-induced rescue of temperature-elicited motor pattern breakdown: a comparative study with three decapod crustaceans.

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Temperature changes are a particular problem for the nervous system, as they can disrupt the well-balanced ionic conductances necessary for maintaining neuronal function. Poikilothermic animals are especially prone to the effects of environmental temperature changes, as they do not actively maintain their body temperature. Yet, many vital behaviors in poikilotherms function across a wide temperature range, making them ideal to study temperature effects. Using the well-studied gastric mill pattern generator of the Jonah crab *Cancer borealis*, we have previously shown that membrane leak conductance increases with temperature, ultimately shunting neuronal function and eliminating spike activity. Yet, neuropeptide modulation from descending projection neurons enables thermal protection and allows the animals to maintain normal neuronal function at elevated temperatures. Specifically, the tachykinin-related peptide CabTRP Ia rescues neuronal oscillations in gastric mill rhythm-generating neurons. CabTRP Ia is released from a pair of descending projection neurons (MCN1) and activates a voltage-dependent inward current (IMI) that counterbalances the increase in leak conductance at elevated temperatures. Neuromodulator release from descending projection neurons is a universal mechanism to adjust neuronal activity in many taxa, suggesting that temperature compensation via neuropeptide modulation is a widespread phenomenon and evolutionarily conserved.

We are testing this hypothesis by comparing temperature compensation mechanisms in the lateral gastric (LG) motor neuron of several closely and distantly related crustaceans with various temperature tolerances. In the two closely related species, *Cancer borealis* (Atlantic Ocean) and *Cancer magister* (Pacific), rhythmic LG activity terminated when the temperature was raised from 10 to 13°C (N=10). Both of these species experience similar temperature fluctuations in their natural habitat. Our analysis shows that in both species membrane leak increased with temperature, which eliminated spike activity and disrupted neuronal function. CabTRP Ia effectively acted as a negative leak to restore spiking. Thus, the same temperature compensation mechanism was present in both *Cancer* species.

To test whether the same mechanisms are also present in a more distantly related crustacean species, we are currently using the European green crab, *Carcinus maenas*. This species tolerates large and rapid changes in ambient temperature and is an aggressive invader of new habitats. Our initial experiments (N=4) indicate that the higher temperature tolerance of these crabs is not reflected in their gastric mill rhythm-generating neurons. Similar to the two *Cancer* species, a temperature change from 10 to 13°C disrupted rhythmic spiking in LG. We are currently testing the contribution of neuromodulation to the high temperature tolerance of *Carcinus maenas*.

Respiration paces prefrontal neuronal activity during intense threat

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Respiration, subdivided in inspiration and expiration, has been shown to affect human emotion recognition via nasal airflow (Zelano et al., 2016. *J. Neurosci.* **36**, 12448–12467). Furthermore, recent studies performed on rodents revealed that respiration plays a key role in higher order cognitive processes, namely by entraining the medial prefrontal cortex (mPFC) neuronal activity during freezing induced by Pavlovian auditory conditioning (Moberly et al., 2018. *Nat. Commun.* **9**). We recently found that the mPFC activity is reliably entrained by respiration during despair-like behavior in a tail suspension test (TST) (Biskamp et al., 2017. *Sci. Rep.* **7**), suggesting that respiration-related rhythms (RR) might aid prefrontal processing during threat. To address this hypothesis, we performed local field potential and single unit recordings in the mPFC of mice while monitoring the respiration during different behavioral states. We found that respiration paces the activity of a majority of mPFC neurons during immobility, whether this immobility was emotionally neutral, as in the home cage, or linked to threat during TST. Nonetheless, we observed that neurons fire preferentially during inspiration when the mice where in their home cage, and switched toward firing more during the transition from expiration to inspiration when the mice were subjected to TST stress. Furthermore, immobility during the TST induced a robust increase in the percentage of cells coupled to the respiration, but solely in the superficial layers 2/3, when compared to neutral immobility. This localized change in RR entrainment suggests that a different macro-circuit of the mPFC is recruited by the respiration during intense threat. The respiration frequency and amplitude is strongly modulated by cognitive states, and unlike other sensorial afferences, projections from the olfactory bulb by-pass the thalamus to directly project to brain regions involved in emotions and cognition. These characteristics, along with increasing electrophysiological evidences, indicate that the RR role in the brain extends beyond olfactory processing.

The organisation of cricket rivalry and courtship behaviour indicated by lesions to the abdominal CNS

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The organisation of cricket singing behaviour has long been dominated by the assumption, that the mesothoracic ganglion houses the CPG for motor pattern generation underlying singing (Huber 1962). For the generation of the calling song, we recently demonstrated in *Gryllus bimaculatus* that the network for motor pattern generation is organised along the abdominal ganglion chain (Schöneich and Hedwig 2011, 2012, Jacob and Hedwig 2016). Here we look into the organisation of the rivalry and courtship singing by comparing the behaviour in normal males with their behaviour 1-7 days after lesions to the different connectives along the abdominal ganglion chain.

Rivalry songs did not occur after the connectives between T3 and A3 or between A3 and A4 were cut. Songs were still generated in the normal way when any connective posterior to A4 were lesioned. These results indicate that an intact connection between the A3 and A4 ganglia is crucial for the control of the rivalry song, akin to the organisation of the calling song.

For the control of courtship singing the more posterior ganglia are crucial. Courtship singing fails if any connective between T3 and A5 is lesioned, whereas the connection to the terminal ganglion is not required. As an intact connection between A5 and A6 was crucial for courtship song generation, the relevance of these posterior abdominal ganglia is implicated.

These data support the importance and the very specific roles of the different abdominal ganglia for cricket singing behaviour.

Layer specific functional connectivity between RFA and CFA in lightly anesthetized mice in vivo

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Background: The function and connectivity between supplementary motor area (SMA) and primary motor cortex (M1) is very well known in humans and primates. In rodents, however, the function and connectivity between the different motor areas is still under discussion. The motor cortex of rodents can be divided into at least two functionally distinct areas, the rostral forelimb area (RFA) and the caudal forelimb area (CFA). The CFA is proposed to be the equivalent of the M1 in the humane brain; the RFA could be a homolog to the premotor cortex (PM) or the SMA.

Methods: In this study, we performed multi-site extracellular recordings of spontaneous neuronal activity of all RFA- and CFA-layers to gain inside into the connectivity between these areas in lightly anesthetized mice in vivo. Therefore 8-14 weeks old C57Bl6-N mice were anesthetized with 1 mg/g urethane (i.p.). A craniotomy was performed over RFA and CFA. Silicone MEA electrodes were inserted into CFA and RFA. A vertical inter-electrode distance of 100 µm allowed to cover all cortex layers simultaneously. We analyzed LFP-signal in different frequency bands from 0-100 Hz as well as single-unit activity. Correct electrode placement was verified using histology. The oscillatory proxies were analyzed between the two regions using LFP coherence and the causality with time-resolved partial directed coherence and finally validated with weighted phase lag index.

Results: We showed that the spectral power is significantly higher in CFA compared to RFA, especially in the β and γ frequency band. Additionally, we could show frequency bands specific functional connectivity between RFA and CFA. The strongest LFP-coherence was found in the theta and beta frequency band. Using SU-correlation and tPDC we found layer specific, bidirectional, effective connections between these two motor cortex areas. We showed that the flow of information is higher for the direction of RFA towards CFA. We showed a significant difference in the direction of information transmission between RFA layer 5 and CFA layer 2/3 especially in the beta and high gamma band.

Discussion: Using a combination of LFP power, SU-correlation, LFP coherence and tPDC we were able to clearly show that RFA and CFA are two distinct areas. Our results provide insights into functional and directed connectivity in the motor network of adult wild-type mice, which would be helpful to further elucidate the pathophysiological changes of this network in movement disorders and to develop target-specific therapeutic interventions.

Poster Topic

T24: Attention, Motivation, Emotion and Cognition

T24-1 The caloric value of food influences the connectivity of mushroom body-related modulatory dopaminergic neurons in the *Drosophila* brain
Büsra Coban, Haiko Poppinga, Francisco J. Rodriguez Jimenez, Thomas Riemensperger, Ilona, C. Grunwald Kadow , André Fiala

T24-2 The Medial Orbitofrontal Cortex Mediates Effort-Related Responding through Interactions with the Ventral Tegmental Area
Wolfgang Hauber, Angeline Votteler, Susanne Sommer, Alexandra Münster

T24-3 Dopamine's function in the algorithmic basis of foraging decisions
Sarah Starosta, Sergey Shuvaev, Duda Kvitsiani, Aubrey Siebels, Alexei Koulakov, Adam Kepecs

T24-4 Attentional modulations overcome large activity differences between competing neuronal populations in area V1/V2
Lukas-Paul Rausch, Maik Schuenemann, Eric Drebitz, Daniel Harnack, Udo Ernst, Andreas K. Kreiter

T24-5 Inhibitory control of the nucleus reuniens by the medial prefrontal cortex
Felix Kohlhaas, Gilda Baccini, Johanne Gertrude de Mooij-van Malsen, Peer Wulff, Alina Mogk

T24-6 Information processing in monkey`s visual cortex is causally dependent on precise γ-synchronization
Eric Drebitz, Lukas-Paul Rausch, Andreas K. Kreiter

T24-7 Characterizing integrated defense state dynamics
Nina Schukraft, Jérémy Signoret-Genest, Dennis Segebarth, Philip Tovote

T24-8 The roles of higher visual areas in surround suppression in the primary visual cortex
Kai Lun Teh, Emerson Harkin, George Stuyt, Jerome Lecoq, Matt Valley, Sarah Naylor, Linzy Casal, Yazan Billeh, Gabe Ocker, Daniel Millman, Saskia E.J. de Vries, Richard Naud, Lucy Palmer, Jens Kremkow

T24-9 Effects of prolonged starvation on fly locomotion and place preference
Deepthi Mahishi, Tilman Triphan, Ricarda Hesse, Wolf Huetteroth

T24-10 The role of social housing: Genotype of cage mates and prior experience influences social play behavior in juvenile *Cacna1c* haploinsufficient rats

Theresa Marie Kisko, Rukhshona Kayumova, Rebecca Bogdan, Rainer K.W. Schwarting, Markus Wöhr

T24-11 Attentional shift of individual neurons' activity relative to the neighboring population dynamics explains attentional improvement of behavior
Ehsan Aboutorabi, Sonia Baloni Ray, Daniel Kaping, Farhad Shahbazi, Stefan Treue, Moein Esghaei

T24-12 A novel paradigm for studying interactions between rats and mice: development of an apparatus to assess innate fear
Marti Ritter, Lara Chirich, Ronny Bergmann, Lara Sach, Panayiota Poirazi, Robert Sachdev, Matthew Larkum

T24-13 Songbirds can learn flexible contextual control over syllable sequencing
Lena Veit, Lucas Y Tian, Christian JM Hernandez, Michael S Brainard

T24-14 Approach or avoid? Pre-adult social experience forges life-long behavioural differences in individual crickets
Julia Sophie Balsam, Paul Anthony Stevenson

T24-15 Strength of attention-dependent modulation of monkey V1 epidural field potentials correlates with behavioral performance.
Lara Syrek, Detlef Wegener

The caloric value of food influences the connectivity of mushroom body-related modulatory dopaminergic neurons in the *Drosophila* brain

Büsra Coban[1], Haiko Poppinga[2], Francisco J. Rodriguez Jimenez[3], Thomas Riemensperger[1,4], Ilona, C. Grunwald Kadow [3], André Fiala[2]

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The experience of getting food with particularly high or low nutritional value can influence an animal's behavior. Since animal behavior is typically determined and orchestrated by neuronal circuits, we have asked whether brain connectivity is influenced by the caloric value of food. To this end, we have made use of the model organism *Drosophila melanogaster*, and we focused on dopamine-releasing neurons targeting the mushroom body of the central brain. This brain circuit integrates sensory information with positive or negative valences, thereby mediating associative learning, and it integrates information about internal states such as metabolic conditions. We exposed adult fruit flies to hypo-caloric, iso-caloric or hyper-caloric food conditions for a relatively extended period of time (7 days). Not surprisingly, calorie restriction resulted in an increased food uptake increase and an appetitive memory formation. Interestingly, we found alterations in the connectivity between dopaminergic neurons and intrinsic mushroom body neurons (Kenyon cells) as a result of calorie restriction. As a measure for connectivity, we have utilized reconstituted split GFP (rsGFP) across putative synaptic contacts. The connectivity of very specific dopaminergic neurons innervating specific axonal compartments of the mushroom body undergoes structural remodeling in dependence of the nutritional value of food such that connections become weakened. This decrease of synaptic contacts relies on Allatostatin A-signaling, and can be optogenetically mimicked by elevating the cAMP level. Additionally, we observed an increase in the overall calcium activity of these dopaminergic neurons, which could perhaps lead to this structural remodeling. We discuss this finding in the light of maintaining homeostatic synaptic balances.

The Medial Orbitofrontal Cortex Mediates Effort-Related Responding through Interactions with the Ventral Tegmental Area

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The orbitofrontal cortex (OFC) supports learning and decision making in multiple ways, for instance, by providing predictions about outcomes associated with actions [1]. This brain region is highly heterogeneous and can be subdivided on the basis of cytoarchitecture, connectivity and behavioral function. The medial subregion of the OFC has been specifically linked to motivational states and control of goal-directed action [2]. Our recent findings further suggest that the posterior subdivision of the medial OFC (mOFC-p) mediates functions related to response effort. For instance, pharmacological stimulation and inhibition of the mOFC-p bidirectionally altered instrumental responding of rats in a progressive ratio (PR) task that demands increasingly more effort for a fixed outcome [3]. Yet, the neural circuitry through which the mOFC-p modulates effort-related responding is still unknown. Anatomical studies demonstrate that the mOFC-p projects prominently to the posterior ventral tegmental area (pVTA). Given these findings, we investigated the role of the mOFC-p and interactions with the pVTA in effort-related responding using a combination of behavioral, pharmacological, optogenetic and neural circuit analysis methods in rats.

Using a novel PR task, we showed that pharmacological inhibition of the mOFC-p increased lever pressing for food under a PR schedule of reinforcement, while optogenetic stimulation of the mOFC had opposite effects. These findings provide further support for a bi-directional modulation of effort-related function by the mOFC-p. Then, we investigated effects of disconnecting the mOFC-p and pVTA on PR responding using unilateral pharmacological inhibition of both areas. Results show that this asymmetric intervention enhanced PR responding suggesting that the mOFC-p controls effort-related function through interactions with the pVTA. VTA dopamine (DA) neurons play a key role in PR responding and are, therefore, one putative major target of the mOFC-p to support effort-related motivational function. Given the antagonistic GABA/DA interactions in the pVTA, it is conceivable that a unilateral stimulation of mOFC-p GABAergic transmission in combination with a contralateral stimulation of pVTA GABAergic transmission enhanced DA neuron activity in both hemispheres thereby invigorating PR responding. In ongoing studies, we test the hypothesis that a reduced excitatory mOFC-p drive on pVTA GABAergic relays disinhibits VTA DA neurons and enhances PR responding.

Collectively, our findings suggest that the mOFC-p and pVTA are key components of a neural circuit mediating the willingness to expend effort to reach a goal. Rodent studies of effort-related processes not only can provide insights into the neural circuitry and neurochemistry of motivation but could also provide a further understanding of the neural basis of effort-related dysfunction in psychiatric disorders associated with OFC dysfunction such as schizophrenia or major depression [4].

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Dopamine's function in the algorithmic basis of foraging decisions

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In everyday life, we are continually confronted with decisions about whether to stay engaged with the current option or to switch to a new one. These stay-or-leave decisions include e.g. employment, house purchases or partner selection and have been studied intensively in behavioral ecology as foraging decisions. However, little is known about their neural basis. Thus, we set out to study the behavioral algorithm and neural mechanisms underlying stay-or-leave decisions in a behavioral paradigm inspired by foraging theory from neuroethology. In this paradigm, mice were facing the decision when to leave depleting reward sources. To explore the trial-by-trial choice strategy and its neural correlates, we implemented several reward manipulations and performed optical recordings (fiber photometry; Gcamp6f) of dopamine neuron activity in the Ventral Tegmental Area (VTA) – a brain area involved in reward-guided behaviors (Schultz et al, 2015). We compared the observed mouse behavior to a range of computational models and identified a novel decision rule that accounts for animals' behavior in a range of conditions, including different inter-trial intervals, reward depletion rates, and average reward rate of the environment. Specifically, we found that the animals compare the next expected reward to the exponential average of the previous rewards – a decision rule we named the leaky MVT, for some similarity with the conclusions of the Marginal Value Theorem (MVT; Charnov, 1976; Constantino & Daw, 2015). We further show that the leaky MTV decision rule may be learned via a reinforcement learning (RL) paradigm called R-learning (Schwartz, 1993), but is not consistent with classical V-, or Q-learning paradigms as these make qualitatively different predictions for the reward manipulations. Finally, we show that dopaminergic signaling in the VTA best correlates with the Reward Prediction Error (RPE) of R-learning, pointing to the potential learning mechanism that optimizes stay-or-leave choices. Overall, our work offers an algorithmic decision rule and neuronal implementation for an ethologically relevant behavior based on qualitative different model predictions.

Attentional modulations overcome large activity differences between competing neuronal populations in area V1/V2

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Selective attention is a fundamental cognitive function for goal-oriented behavior: By focusing on individual stimuli while ignoring irrelevant stimuli, it allows for detailed processing of relevant information, which is not possible for the excessive amount of sensory information provided by complex environments.
In the visual system, it is well documented that shifts in spatial attention are associated with modulations of neuronal responses and that attentional mechanisms resolve the competition between efferent signals of upstream neurons for processing by receiving neurons in downstream areas. When spatial selective attention is devoted to one of multiple stimuli within receptive fields of neurons in visual areas V4 or MT, these cells respond as if only the attended stimulus was present. The underlying neural mechanisms are still debated, but computational studies predict that (e.g., for routing-by-synchronization mechanisms), a small rate advantage for neural populations in V1/V2 passing the attended signal to V4 suffices to establish such selective processing [1]. But what if the attended stimulus is less salient than a closely spaced highly salient distractor stimulus, causing much weaker activation of the presynaptic V1/V2 population, thus challenging any neural mechanism by which selective attention routes visual information?
We tested this prediction in areas V1/V2 of macaque monkeys performing a demanding shape-tracking task that required sustained attention for one of several stimuli. We investigated to which extent attention-dependent rate modulations compensated for large contrast-dependent response differences. Our results show that attentional rate modulations consistently and effectively counteract large stimulus-induced activity deficits of neuronal populations encoding the target compared to those responding to the distracter stimulus. The rate advantage for the low contrast target was established mainly by facilitating neuronal activity evoked by the target stimulus and, to a lesser extent, by suppression of distractor responses. The magnitude of the target facilitation correlated strongly with the difference between the low and high contrast stimulus' firing rates whereas distractor suppression correlated well with the remaining rate difference after target facilitation. We observed an attention-dependent average rate gain of 70% for the low contrast stimulus for an average rate difference of 80%. Simultaneously, firing rates in response to the high contrast distractor were attenuated by 15% on average. Such a rate advantage for V1/V2 populations representing the target was not achieved in error trials. The attention-dependent firing rate gain observed for the challenging stimulus configurations of non-matching contrast by far exceeded those observed for stimulus configurations of equal contrast (25% target facilitation and 14% distractor suppression on average).
Our findings reveal a surprisingly strong capacity for attention-dependent rate modulations in areas V1/V2. They support models of a control mechanism where spatial attention moderately elevates the population rate for the attended stimulus above that for the unattended stimuli to enable subsequent mechanisms for selective processing of signals encoding the behaviorally relevant object.

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Inhibitory control of the nucleus reuniens by the medial prefrontal cortex

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The integration of executive and mnemonic functions is required for adaptive behaviour and mental health. In rodents as well as in humans such integration is thought to depend on a circuit connecting the medial prefrontal cortex (mPFC) and the hippocampus (HC). The connections between mPFC and HC are bidirectional and strongly dependent on the nucleus reuniens (RE), a midline-thalamic region that - far from being a simple relay structure - works as a bridge between prefrontal and hippocampal functions. RE dysfunction is implicated in clinical disorders that impair behavioural flexibility and temporal organization of memory such as dementia or schizophrenia. The excitatory connections within this higher-order cortico-thalamic-cortical circuit have been extensively described and have received an increasing amount of attention in the past few years. However, little is known about the inhibitory control of this system. The RE is devoid of local GABAergic control, and its only inhibitory input described so far originates in the nucleus reticularis.

We have here used a combination of retrograde and anterograde viral tracing methods to explore the existence of inhibitory cortical control of the RE. We report GABAergic projection neurons which are distributed throughout the different layers of the frontal cortex. We collected information on their histochemical identity, anatomical pathway and synaptic target fields within the RE. Our work describes a novel class of GABAergic projection neurons which may present an important element in prefrontal-thalamic-hippocampal circuitry and communication.

Information processing in monkey`s visual cortex is causally dependent on precise γ-synchronization

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Successful behavior in our complex environment imposes high demands on the brains' ability to integrate and process the behaviorally relevant information. The integration of information across extended parts of the visual field allows identifying objects and differentiating them from the background and other objects. This ability arises from the convergent anatomical connections along the visual processing pathways. Neurons with small receptive fields (RFs) located in upstream cortical areas deliver their output convergently to the same downstream neurons. These downstream neurons can, in turn, process and integrate information originating from more extensive parts of visual space. However, such convergent inputs often implicate a need for selection when conveying information on multiple independent objects, which cannot be processed in parallel. Selective attention is well-known to resolve this conflict in favor of the behaviorally relevant information. However, the neuronal mechanism behind the attention-dependent selection is under intense debate. One hypothesis states that up-and downstream neurons that encode the attended object synchronize their γ-band activity—implicating that the relevant signals arrive at the receiving neurons' most sensitive phase within their γ-cycle, close to the spiking threshold. In contrast, upstream neurons representing non-attended objects do not synchronize their activity and often fail to deliver their spikes at the receiving neurons' sensitive phase. This difference in synchronizing with the common downstream receiver neurons is thought to result in differences in signal routing effectiveness, thus constituting an effective selection mechanism. While several studies confirm important predictions of such an attention-dependent routing-by-synchronization mechanism, it is still an open question whether γ-synchronization between sender and receiver neurons is causally responsible for selective signal routing or rather epiphenomenal. To investigate this question, we recorded neuronal activity in macaque monkeys' (*Macaca mulatta*) visual area V4 while the animals performed a demanding attention task. Simultaneously, single biphasic electric current pulses with low amplitude were applied to upstream neurons located in area V2, causing several neurons to spike. The rationale was to investigate whether the effect of such an artificially evoked volley of spikes depends on the ongoing γ-activity of the receiving neurons in V4. Indeed, we found that the electrically evoked spikes had a significant and γ-phase-dependent impact on spiking-activity of V4 neurons and animals' behavior. Both monkeys showed a significant increase in response times (median: 25 and 47 ms) if the electrically evoked spikes arrived during the sensitive γ-phase of the V4 neurons, whereas there was no significant effect on response times during other phases. The same pattern was observed for the spiking-activity of V4 neurons. We found a significant increase in spiking-activity 7 – 15 ms following electrical stimulation, but only if the V2 spikes arrived at the sensitive phase of the V4 γ-cycle. At other γ-phases, no significant effect on V4 spiking was observed. These results demonstrate that selective signal routing and information processing within the visual cortex causally depend on γ-phase synchronization between afferent input and receiving neurons.

Characterizing integrated defense state dynamics

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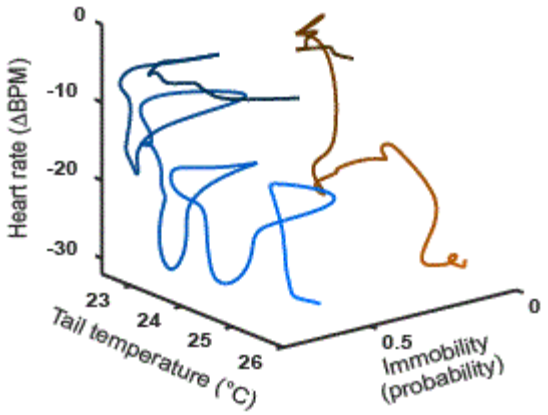
The appropriate reaction to a threat is essential for survival. Depending on the stimulus nature and its imminence, rodents exhibit specific defensive reactions characterized by diverse behavioural strategies and concomitant autonomic responses. However, while the behavioural component appears well described, cardiovascular changes have proven to be much more elusive, with sometimes conflicting results. Those contradictory findings most likely stem from the fact that although the terminology used is similar (e.g. “fear-induced”), the actual threat stimuli are heterogeneous (conditioned vs. innate responses) and that the exact conditions under which the measurements were performed are not directly comparable (homecage, baseline or not, etc). Importantly, common experimental approaches treat various aspects of the defense reaction as separate entities and analyses of output parameters are performed at different timescales. This is why, in order to capture the integrated nature of defensive reactions, we devised a strategy that does not rely on a preconception about the animal’s state. Instead, we sought to infer states dynamically from changes in several behavioural and cardiovascular readouts. We hypothesized that integrating multiple measures at different levels and over a wide temporal range would allow us to describe defensive states more accurately, find patterns in apparent heterogeneity, and expose underlying mechanisms.

To this end, we performed electrocardiogram recordings in freely behaving mice placed in different anxiety and fear paradigms. In addition to cardiovascular and behavioural data, we also extracted tail temperature from thermal movies acquired simultaneously, to assess peripheral vasodilatory processes. We performed appropriate pre-processing on each type of data to obtain biologically relevant readouts, and subsequently analyzed dynamics of these parameters.

We first focused on a conditioned fear paradigm in which mice display a relatively limited array of behaviours, making it an ideal entry point to tackle complexity. We were able to identify several key characteristics of the states displayed in that particular context. Notably, while there was an overall strong correlation between some behavioural and heart rate responses, the amplitude of the latter wasn’t constant, and seemed to reflect a slower reactivity of the cardiovascular system. We identified stereotypical, fast integrated changes reflected by both, autonomous and behavioural measurements, that we term microstates, as well as more sluggish changes in the different readouts, which we call macrostates. To extend these concepts to the other conditions, we implemented an unbiased approach to find stereotypical associations to define hierarchically organized, discrete defensive states.

The current results underline the relevance of an integrated approach to define defensive states, which allows to capture more complexity from the data and discriminate with more accuracy than single readout-based definitions, while preventing a priori semantic biases. The various states that can be described at different timescales (microstates vs. macrostates) and identifying potential switch-points also provide a valuable tool to now look for neuronal correlates and mechanisms in brain regions involved in the defensive reaction.


0
-10
-20
-30
Heart rate (ΔBPM)
23
24
25
26
Tail temperature (°C)
0
0.5
Immobility
(probability)

The roles of higher visual areas in surround suppression in the primary visual cortex

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The neuronal activities in the primary visual cortex (V1) can be modulated by visual stimuli that are not within their classical receptive field (RF), namely the extra-classical RF. Specifically, the visual stimuli that extend from the classical RF to the extra-classical RF have been shown to suppress the responses of V1 neurons, with iso-oriented surrounds having stronger suppression than ortho-oriented surrounds (Henry et al., 2013; Self et al., 2014). The local somatostatin (SST) interneurons in V1 and cortico-cortical feedback from higher visual areas (HVAs) have both been suggested to play a role in this surround suppression of the neuronal responses (Adesnik et al., 2012; Nienborg et al., 2013; Nassi et al., 2013). However, how exactly the information is integrated in the circuits to give rise to this modulation remains unclear. In particular, little is known about the role of HVA feedback in surround suppression in the V1.

Here we test the hypothesis that HVAs encode extra-classical RF information which is integrated with the feedforward sensory information of the classical RF via apical dendrites of pyramidal neurons and SST neurons in V1. To test this, we used two-photon calcium imaging in awake mice to measure the activity of both soma and dendrites of V1 neurons, as well as soma of neurons in HVAs that have feedback projections into V1. Two HVAs were imaged, which are the anterolateral (AL) and lateromedial (LM) areas. Feedback neurons in these HVAs were identified by injecting rAAV2-retro-CAG-mRuby3 in V1. All experiments were conducted in the OpenScope observatory at the Allen Institute for Brain Science. OpenScope is the first shared observatory for neuroscience and allows making use of the high quality and standardized experiments of the Allen Brain Observatory. All experiments were run by the optical imaging experts at the Allen Institute.

To investigate surround suppression, we measured classical RFs using the locally-sparse-noise stimuli, size-tuning using windowed gratings, and extra-classical RF effects using center-surround gratings with different combinations of center and surround orientations. We are currently analyzing the large dataset to compare the somatic, proximal dendritic, and apical dendritic activities of the V1 pyramidal neurons to the activities of HVA neurons and SST interneurons in order to determine how the information from different cell types and areas are being integrated to induce the surround suppression effects in V1.

Here, we will present the concept behind the OpenScope observatory and our preliminary analysis on the role of HVAs in surround suppression in V1.

Effects of prolonged starvation on fly locomotion and place preference

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Animal behaviours are demonstrably governed by sensory stimulation, previous experience and internal states like hunger. With increasing hunger, priorities shift towards foraging and feeding. During foraging, flies are known to employ efficient path integration strategies. However, general long-term activity patterns for both hungry and satiated flies in conditions of foraging remain to be better understood. Similarly, little is known about how chronic contact chemosensory stimulation affects locomotion. To address these questions, we have developed a novel, simplistic fly activity tracking setup – the Panopticon. Using a 3D-printed Petri dish inset, our assay allows recording of walking behaviour, of several flies in parallel, with all arena surfaces covered by a uniform substrate layer. We tested two constellations of providing food: i) in single patches, and ii) omnipresent within the substrate layer. Fly tracking is done with FIJI, further assessment, analysis and presentation is done with a custom-built MATLAB analysis framework. We find that starvation history leads to a long-lasting reduction in locomotion, as well as a delayed place preference for food patches not driven by immediate hunger motivation.

The role of social housing: Genotype of cage mates and prior experience influences social play behavior in juvenile *Cacna1c* haploinsufficient rats

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Similar to humans, healthy development of social behavior and communication in rats is highly dependent on early social practices and in particular early social play experience. The risk gene known as *CACNA1C* has been repeatedly implicated in numerous neuropsychiatric disorders in which key characteristics include deficits in social functioning and communication. Recently, we reported sex-dependent impairments in social play behavior and ultrasonic vocalizations in juvenile heterozygous *Cacna1c* (+/-) rats tested as same sex pairs. Male *Cacna1c*$^{+/-}$ rats showed no behavioral alterations, however, there was a prominent genotype effect in female pairs. *Cacna1c*$^{+/-}$ female pairs showed an increase in the time spent playing and in the time spent pinning. In fact, the increased pinning exceeded the known sex difference between juvenile male and female rat pairs, speaking for a hyper-masculinized playful repertoire in *Cacna1c*$^{+/-}$ females. Interestingly, wildtype female *Cacna1c* (+/+) pairs also showed increased rates of social play suggesting that play behavior in *Cacna1c*$^{+/+}$ females may be influenced by their *Cacna1c*$^{+/-}$ female cage mates. Consequently, we designed an experiment to test the influence of cage mates on social play behavior. Here, we housed juvenile females together in either mixed or same genotype cages and then tested them in a social play paradigm with either a same or mixed genotype partner from either a same or mixed genotype cage. We hypothesized that *Cacna1c*$^{+/+}$ rats display female-typical rates of play when housed in same genotype cages but will show previously seen increased play behavior when housed in mixed genotype cages. The results indicate that housing conditions and genotype both influence play behavior with a surprisingly opposite result to those previously seen. Here, pairs of female *Cacna1c*$^{+/+}$ rats overall spent more time playing than do pairs of *Cacna1c*$^{+/-}$ rats in both housing conditions. More importantly, we found that the sequence of play partner across testing days contributes strongly to the time spent playing and influences how playful the female rats are with subsequent partners. In this regard, playing with a female *Cacna1c*$^{+/+}$ partner first is more likely to result in higher duration of subsequent playful interactions when *Cacna1c*$^{+/-}$ females are paired with a *Cacna1c*$^{+/-}$ partner. Whereas, playing with a *Cacna1c*$^{+/-}$ partner first results in low frequencies of playful interactions. Taken together, our findings indicate that the social housing environment and prior play experience are essential to modulate social play behavior in juvenile female *Cacna1c* haploinsufficient rats.

Attentional shift of individual neurons' activity relative to the neighboring population dynamics explains attentional improvement of behavior

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There is strong evidence for a correlation between the spiking activity of individual neurons relative to the average activity of the neighboring neural network in which they are embedded (reflected in local field potentials (LFPs)). To improve the representation of attended stimulus and consequently behavioral performance selective attention modulates 1) this correlation of sensory neurons' with their neighboring population activity as well as 2) the neurons' stimulus response. Here we show evidence for an encoding strategy in the visual cortex, that enables a selective routing of the behaviorally relevant (but not the irrelevant) information to downstream areas by aligning the underlying oscillatory population activity to a specific phase.

We simultaneously recorded spiking activity and LFP for 90 well-isolated motion pattern-selective neurons in the medial superior temporal (MST) cortex of monkeys performing an attention (spatial and feature-based) task.

Our data show that MST neurons tend to fire spikes at a specific phase of ongoing population beta oscillations (19-24 Hz). Interestingly, this preferred spiking phase shifts significantly depending on the behavioral relevance. Our data show that when the animal attends towards the receptive field (where the preferred stimulus is presented), the neuron fires spikes at a later phase compared to when attention is shifted away from the receptive field (where the anti-preferred stimulus is presented) (0.77 rad for neurons with at least 10 percent of attentional modulation in their spike rate; paired Watson-Williams test, FDR adjusted p-value < 0.05). This attentional shift in preferred spiking phase is mainly caused by the shift of the local focus of attention, rather than the attended visual feature (here, the specific motion direction). Importantly, the attentional shift of preferred spiking phase is significantly correlated with the animal's behavioral performance in detecting the visual change. We propose that this systematic change in preferred spiking phase may lead to better readout of relevant visual information by making neurons which represent the attended/unattended locations relay their information at distinct phases linked to periods of good/poor perceptual sensitivity for the higher-level cortical area. This strategy limits perception selectively to relevant visual sensory inputs and allows sensory events lead to better-matching behavioral outcomes.

A novel paradigm for studying interactions between rats and mice: development of an apparatus to assess innate fear

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Fear is a central emotion guiding the behavior of human beings and other animals. Fight or flight, a standard behavioral outcome, is a reaction to threatening situations. To understand how the brain functions to perceive fear and reacts to elements of the environment that can potentially be threatening, we designed a behavioral apparatus to assess the animal's behavior during the perception of innate but ambiguous threat.
The movement and position of a treadmill were tethered to 1) a visual virtual reality; 2) a reward delivery lick-port and 3) a tube that held an innate threat – a rat. As mice moved forward, the lick-port positioned under the tube containing the threat moved toward them. When mice moved backward, the lick-port and tube moved away.
To introduce ambiguity in the innate fear condition we used five conditions: First, the acclimation condition, when there was no rat; second, the rat was present but not visible; third the rat, was present and could be seen by the mouse; fourth, the rat could stick its head out, and hover over the mouse as it licked the reward; and finally, an optional looming stimulus, which generates a shadow from above and has been shown to cause innate fear in mice.
Surprisingly, in these conditions some mice show no evidence of innate fear – they do not run away, they do not freeze, but their approach to the reward slows down. Currently, we are quantifying the changes in facial expression, vocalization, pupil dilation and adding a comparison to the looming stimulus. These social interactive behaviors are being prepared for use with calcium imaging, recording and optogenetics.

Songbirds can learn flexible contextual control over syllable sequencing

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The flexible control of sequential behavior is a fundamental aspect of speech, enabling endless reordering of a limited set of learned vocal elements (i.e. syllables or words). Songbirds are phylogenetically distant from humans, but share the capacity for vocal learning as well as neural circuitry for vocal control that includes direct cortical-brainstem projections. Based on these similarities, we hypothesized that songbirds might likewise be able to learn flexible, moment-by-moment control over vocal production. Here, we demonstrate that Bengalese finches, which sing variable syllable sequences, can learn to rapidly modify the probability of specific sequences (e.g. ‘ab-c’ versus ‘ab-d’) in response to arbitrary visual cues. Moreover, once learned, this modulation of sequencing occurs immediately following changes in contextual cues and persists in the absence of external reinforcement. Our findings reveal that songbirds, like humans, can learn flexible, contextual control over syllable sequencing and establish birdsong as a model for investigating the neural mechanisms that underlie this capacity

Approach or avoid? Pre-adult social experience forges life-long behavioural differences in individual crickets

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One of the most influential recent findings in the field of animal behaviour is that individuals of the same species, including many invertebrates, exhibit consistent between individual differences in specific behavioural traits (“animal personality”). However, there is still no consensus regarding the proximate mechanisms that generate behavioural phenotypes. We investigate how aggressive social experience influences the acquisition of behavioural individuality in adult male Mediterranean field crickets (*Gryllus bimaculatus*). Using automated high-speed video analysis (EthoVision XT), we measured the motor responses to a single brief touch of one antenna with a freshly severed donor male antenna, which also occurs naturally when conspecifics meet. After staging a single fight between adults that were previously socially isolated for 48 h (short term isolates), we found that the winners tended to turn towards the direction of the stimulus (positive thigmotaxis), whereas the losers turned away (negative thigmotaxis). Surprisingly, however, the same was also evident for prospective winners and losers before fighting, suggesting that the difference must be established earlier in development. Supporting this, adults isolated since their last nymphal stage that only had prior contact to adults during nymphal life, all showed negative thigmotaxis in response to antennal stimulation. Furthermore, individuals that had no prior contact to adults at any time in their life showed no preferential response direction to the stimulus. In the latter animals we found that after a single fight, the winners mostly approached the stimulus, whereas the losers turned away. However, this effect was transient and no longer evident after 24 h. We next tested the effect of multiple agonistic experiences and found that individuals that won, respectively lost, 6 consecutive fights subsequently showed positive, respectively negative thigmotaxis for almost the entirety of remaining adult life (> 6 days). We conclude that aggressive experience, even during pre-adult development, has a life-long influence on an individual cricket’s decision to approach or avoid a conspecific in response to antennal contact.

We are now analysing how turning responses are influenced by octopaminergic and nitridergic drugs, which promote and respectively suppress aggression in crickets (cf. Stevenson and Rillich, Neuroforum 25(1), 2019). Firstly, we found that short term isolates that are predisposed to avoid the touch stimulus, change to approach it after treatment with either the octopamine agonist chlordimeform (CDM), or after blocking NO synthesis with L-NAME. Conversely, individuals initially predisposed to approach the stimulus showed avoidance responses after treatment with the octopamine receptor blocker epinastine. These initial data support our hypothesis that different behavioural phenotypes, e.g. for turning, are established by neuromodulators that are released in response to social experience.

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Strength of attention-dependent modulation of monkey V1 epidural field potentials correlates with behavioral performance.

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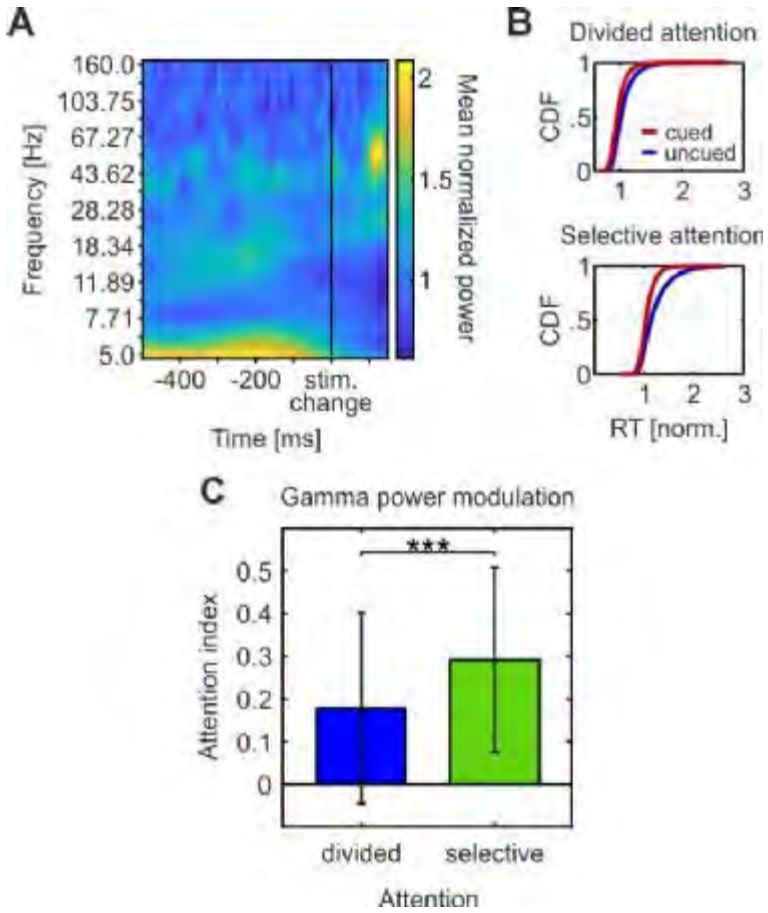
Epidural field potentials (EFPs) constitute a promising technique in both clinical applications and basic science research due to their reduced invasiveness and high coverage density over large cortical areas. To investigate neural signatures of selective visual attention in EFPs and their correlation to signatures of attention in behavioral data, we recorded EFPs with a chronically implanted multielectrode array from monkey primary visual cortex (V1). The monkey was engaged in a covert-attention task and was required to detect a small rotation at one of two objects triangular objects located in the visual periphery, with a spatial cue indicating the target location.
Responses were allowed to both the cued and the uncued object. We used a non-binary rewarding scheme (Fischer & Wegener, J. Neurophysiol. 2019) to associate detection of cued and uncued stimulus changes with different amounts of reward and applied two different reward regimes, supporting either more distributed or more selective attention. We hypothesized that attentional modulation of the EFP should follow the strength of behavioral effects in the two reward regimes.

We compared reaction times (RT) of the two regimes and found a decrease in absolute numbers of responses to uncued changes and a significant increase in RT differences between responses to cued and uncued changes for the regime supporting more selective attention.
Gamma-band power of EFPs recorded within the two regimes showed a significant attentional modulation with selective attention but only weak attentional modulation with distributed attention. This modulation was strongest in trials with fast responses as compared to trials with medium or slow responses. The strength of attentional modulation significantly decreased with increasing distance from the attended object, consistent with a zoom-lens structure of selective attention in V1.

Together, the results show that EFP gamma-band attentional modulation in monkey V1 correlates strongly with behavioral measures of attention, including a close link to reaction time performance and the spatial structure of the attentional focus.

Supported by DFG WE 5469/3-1.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43.62
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18.34
11.89
7.71
5.0
Frequency [Hz]
-400
-200
stim. change
Time [ms]
2
1.5
1
Mean normalized power
B
Divided attention
CDF
cued
uncued
Selective attention
RT [norm.]
C
Gamma power modulation
***
Attention index
divided
selective
Attention

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The effects of systemic noradrenergic blockade on cued fear extinction and its retention

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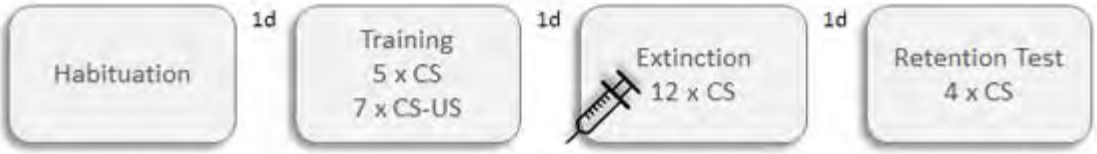
Considerable research efforts have been devoted to the pharmacological enhancement of fear extinction, in part because of its potential to improve exposure therapy for patients suffering from anxiety-related disorders. Here, we focus on the effects of propranolol, a beta-adrenergic receptor antagonist that can be safely used in rodents and humans. Prior research suggests that the administration of propranolol before extinction training may be useful when stress levels are high (e.g., in case of immediate extinction), but can be detrimental to extinction learning under more typical extinction conditions (e.g., one day after acquisition of the fear memory), although findings in the literature are somewhat mixed.

In a series of six preregistered experiments (118 adult male rats of which 32 non-naïve Wistar and 86 naïve Sprague-Dawley), we examined the effects of systemic propranolol (10 mg/kg) administered 20 minutes prior to the start of extinction training. The standard procedure (see figure) started with habituation to the context, 24 hours later followed by an auditory fear conditioning session (5 unreinforced tone CSs followed by 7 CS-US pairings). One day later, animals received injections of propranolol or saline and were given (partial) extinction training (12 CSs). Yet another 24 hours later, rats underwent an extinction retention test (4 CSs). In some experiments, the standard procedure was extended with a spontaneous recovery test one week later. Additionally, one experiment included a third group that received the benzodiazepine midazolam (3 mg/kg) instead of propranolol or saline before extinction training.

In 3 out of 6 experiments, we found lower freezing during extinction training in the propranolol versus saline group ($p = .02$, $p = .02$, $p < .001$). In 2 more experiments, we found a (trend toward a) faster decline in freezing during extinction training in propranolol rats ($p = .02$, $p = .09$). Furthermore, we found less freezing, i.e., better retention of extinction learning, in one experiment ($p = .049$) and a trend toward the same effect in another experiment ($p = .05$), both of which had also shown significant acute effects of propranolol on the day before. In the 4 other experiments, freezing during the extinction retention test was statistically indistinguishable between both groups. In contrast, rats that had received midazolam showed a trend toward more freezing during extinction retention ($p = .09$) and at the spontaneous recovery test ($p = .14$) compared to the saline control group (effects were statistically significant after exclusion of one saline animal with outlying freezing values).

The findings with midazolam are in line with the prior literature which indicates that benzodiazepines can decrease the effectiveness of fear extinction and, likewise, of exposure therapy. Our observation that propranolol has acute fear-reducing effects during extinction training also aligns with the existing literature. However, the finding in two of our experiments that propranolol may improve retention of extinction learning is in contrast with the majority of prior research, which typically finds either no effect of noradrenergic blockade or worse retention, i.e., more fear at test.

Overall, our results suggest that midazolam may impair retention of extinction learning, whereas propranolol can enhance it, although the latter effect appears to be subtle.


Habituation
1d
Training
5 x CS
7 x CS-US
1d
Extinction
12 x CS
1d
Retention Test
4 x CS

Neuropeptides as potential modulators of the behavioral-stage transitions in the ant *Cataglyphis nodus*

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Ants are widely distributed all around the globe. Their enormous ecological success is based on a highly dynamic and successful interplay of the colony members. In most ant species, the workers follow an age-related, innate sequence of different behavioral stages. Ants of the genus *Cataglyphis* undergo several distinct internal stages before they finally start to forage outside of the nest. Although the behavior and ecology of *Cataglyphis* ants have been extensively studied, the intrinsic mechanisms underlying the behavioral changes during behavioral maturation are largely unknown. Recent studies suggested neuropeptides as potential neuromodulators, initiating the behavioral transitions in social insects. Since neuropeptidomic studies are missing in *Cataglyphis*, we combined transcriptome analysis with Q-Exactive Orbitrap mass spectrometry (MS) and direct tissue profiling by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS to provide a most comprehensive neuropeptidomic data set of the ant *Cataglyphis nodus*. We further localized 35 peptides in 14 µm thin brain sections by using MALDI-MS imaging. As many of the neuropeptides were found in the central adjoining neuropils, an area which lacks clear boundaries between the individual neuropils, we further reconstructed a three-dimensional brain model. This neuronal map of the *Cataglyphis* brain allows to address the precise location of the neuropeptides within 33 distinct brain neuropils which are connected by 30 fiber tracts. Based on recent studies and the spatial distribution of the neuropeptides in the *Cataglyphis* brain, we propose that the neuropeptides allatostatin-A (Ast-A), corazonin (Crz) and tachykinin (TK) are suitable modulators of the behavioral-stage transitions. To reveal stage-related changes of the spatial distribution and the expression level of the neuropeptides, we employed immunohistochemistry and quantitative PCR. Our data provides evidence for a behavior-related expression of Ast-A and Crz in the *Cataglyphis* brain. Given the presence of Ast-A and Crz in important control centers of the brain, our results indicate a neuromodulatory role of the neuropeptides during the behavioral maturation of *Cataglyphis* ants.

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cFos does not simply reflect an increase in neuronal activity.

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The activity-dependent expression of immediate early genes, such as cFos, is widely used as an indicator of which neurons participated in a behavioral task. It is generally believed, that if a neuron was strongly activated or underwent an event leading to synaptic potentiation, then cFos will be expressed. However, surprisingly little is known about how cFos expression is related to the neuronal firing pattern and which molecular cascades are important. We therefore investigated the relationship between neuronal activity and cFos expression and used pharmacological manipulation to investigate the pathways leading to cFos. When high potassium was used to depolarize all cells in organotypic rat hippocampal slice cultures, we found that cFos expression is not uniform but depends on neuronal type and is especially low in the CA2 region. cFos expression is abolished by blocking either CREB, MEK or calcineurin signaling pathways (AND logic) or by preventing neurons from firing action potentials with tetrodotoxin (TTX) during high potassium stimulation. We next used the channelrhodopsin ChrimsonR to precisely drive action potentials with red light flashes throughout the slices in the presence of blockers of fast synaptic transmission. At a high frequency (50 Hz), 30 action potentials are sufficient to induce cFos in DG, CA3 and CA1 hippocampal regions and 300 action potentials maximally drove cFos. cFos expression was also frequency dependent. There was a clear U-shaped dependence of cFos expression on firing frequency with high cFos driven at high and low frequencies but not by intermediate frequencies. In contrast to high potassium stimulation, cFos expression after both low and high frequency firing was not blocked by inhibiting either CREB, MEK or calcineurin alone. Action potentials were required as cFos expression after low frequency stimulation was abolished, when sodium channels were blocked with TTX. We conclude, that increasing cFos expression does not simply indicate increasingly active neurons. Rather, cFos expression is highly dependent on neuronal identity and increases after both short high intensity bursts of activity or after prolonged low frequency firing. In addition, the molecular pathways leading to cFos expression after stimulation-induced firing differ from those seen using high potassium to globally depolarize not only neurons but other cells in the slices.

Spatio-temporal dynamics of cFos ensembles in the dentate gyrus: Ensemble overlap is influenced by learning and temporal proximity but not spatial search consistency.

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Neuronal activity that occurs during memory acquisition can be identified and tagged by cFos-dependent tagging. Using this technique to manipulate neuronal ensembles (also known as engram cells or memory traces) has shown that memories can be either suppressed or reinstated. These studies, however, used the conditioned freezing response to a foot shock as a readout for memory retrieval and not a goal-oriented active behavior. We therefore, questioned how a spatial memory task (Morris water maze (WM)), that requires several training days to master, would be reflected at the overlap between cFos ensembles of two given training days. Moreover, we questioned how optogenetic silencing of early training cFos ensembles (day 1) affected memory performance on subsequent days. Here, we use a cFos reporter mouse line (TetTag) combined with adeno-associated viruses to deliver a chloride-conducting channelrhodopsin (iChloC) to selectively tag and silence a given cFos ensemble during a defined time window. Based on contextual fear conditioning experiments, we expected that silencing cFos+ ensembles from early memory encoding (day 1) would impair memory retrieval on subsequent training days, however, mice performed better during optogenetic inhibition. Moreover, mice learned a new platform location faster (reversal training, day 6) when day 1 cFos ensemble was silenced. These unexpected results were explained by the low degree of overlap (similar to chance) between day 1 & 6 cFos ensembles. To assess if the low overlap was due to the temporal distance between cFos tagging events (5 days apart) or because of the spatial configuration of the WM (original platform position vs. opposite quadrant) we studied the temporal and spatial dimensions independently. We found that cFos ensembles strongly segregate over time even when the animals were kept in their home cage. Conversely, when a fixed time window for cFos tagging events was set to 24 h, we found that animals with the highest spatial consistency between events had similar to chance overlap. Interestingly, learning either at the beginning (original platform) or relearning (reversal, opposite) slightly increased overlap above chance between subsequent tagging days 1 & 2 or 5 & 6 respectively. Our results suggest that even in a constant environment, cFos+ ensembles in the dorsal DG segregate as a function of time, but become partially reactivated when animals try to access memories of past events.

Postsynaptic plasticity of cholinergic synapses underlies the induction and expression of appetitive memories in Drosophila

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In vertebrates, memory-relevant synaptic plasticity involves postsynaptic rearrangements of glutamate receptors. In contrast, previous work indicates that Drosophila and other invertebrates store memories using presynaptic plasticity of cholinergic synapses. Here, we provide evidence for postsynaptic plasticity at cholinergic output synapses from the Drosophila mushroom bodies (MBs). We find that the nicotinic acetylcholine receptor (nAChR) subunit α5 is required within specific MB output neurons (MBONs) for appetitive memory induction, but is dispensable for aversive memories. In addition, nAChR α subunits mediate memory expression downstream of α5 and the postsynaptic scaffold protein Dlg. We show that postsynaptic plasticity traces can be induced independently of the presynapse, and that in vivo dynamics of α2 nAChR subunits are changed both in the context of associative and non-associative memory formation. Therefore, regardless of neurotransmitter identity, key principles of postsynaptic plasticity support memory storage across phyla.

A set of elementary operations captures recombination of neuronal ensembles during basal conditions and learning.

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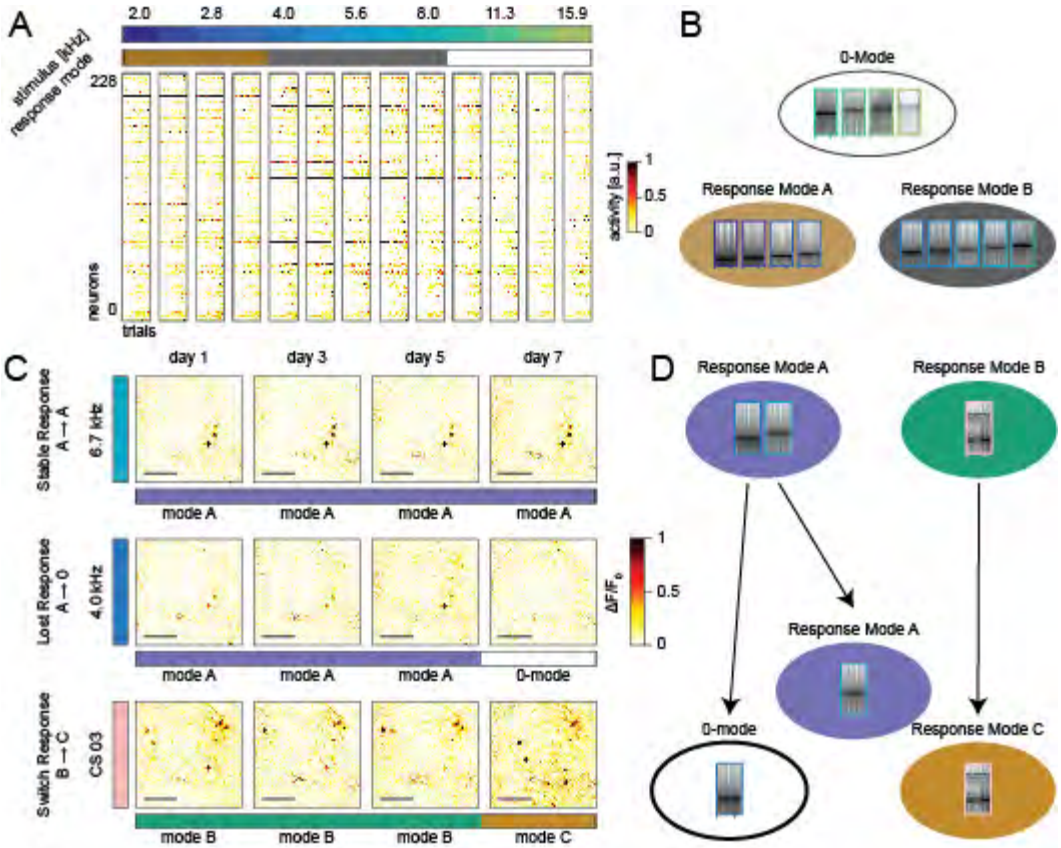
Neuronal ensembles are believed to form essential functional units within neural networks. How structurally and functionally stable are such ensembles? This question is of particular interest in light of evidence that synaptic connections show substantial remodeling on the time scale of days. At the same time, the perception of external stimuli has to be highly reliable, while allowing for flexible integration of novel experiences to form associations to already known stimuli.
Using chronic imaging of local population activity in mouse auditory cortex, we show that neuronal responses to short sounds typically cluster into a near discrete set of response modes. Moreover, we find that response modes show significant remodeling over several days. We identify a set of elementary operations capturing the dynamics of sensory representations, which involve changes in the set of stimuli driving particular response modes as well as their formation and elimination.
We demonstrate that stable encoding of external stimuli is maintained in activity patterns of local populations despite ongoing remodeling of sensory representations. Training a decoder based on logistic regression using information on a single time point either from single cell activity or from response mode patterns, respectively, is sufficient to decode stimulus identity over long time scales. Auditory fear conditioning introduces biases in the frequency of specific operations mediating increased associations of sensory stimuli and improved sound encoding. The ongoing recombination of neuronal ensembles provides a mechanism for the integration of novel stimulus information by associative learning paradigms.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5.6
8.0
11.3
15.9
stimulus [kHz]
response mode
228
neurons
0
trials
activity [a.u.]
1
0.5
0
B
0-Mode
Response Mode A
Response Mode B
C
day 1
day 3
day 5
day 7
Stable Response
A → A
6.7 kHz
mode A
mode A
mode A
mode A
Lost Response
A → 0
4.0 kHz
mode A
mode A
mode A
0-mode
Switch Response
B → C
CS 03
mode B
mode B
mode B
mode C
ΔF/F0
1
0.5
0
D
Response Mode A
Response Mode B
Response Mode A
0-mode
Response Mode C

A novel automated system for training common marmosets on auditory discrimination

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The common marmoset (Callithrix jacchus) has become an invaluable non-human primate (NHP) model for biomedical research in general and the neurosciences in particular. Factors such as the relative ease of breeding, early sexual maturation and short life span allow for detailed longitudinal investigations into neurodevelopment or neurodegeneration, among others. Their large vocal repertoire paired with strong similarities in hearing perception to humans opens the possibility to assess different aspects of the auditory system in a NHP. The family based lifestyle including alloparenting, food sharing, and imitation learning further makes marmosets an ideal model for social neuroscience. Additionally, with the generation of genetic models of human mental diseases, the marmoset model is rapidly bridging experimental and theoretical gaps between mouse and primate research lines, producing critical advancements.

As such, it is crucial to develop techniques which aid to further evaluate cognitive capabilities of this species in general and cognitive aspects of hearing in particular. In this study, we describe a novel, touchscreen-based, home-cage mounted, automated operant conditioning device which has been successfully employed to train and test common marmosets on sound discrimination. The system mostly consisted of off-the-shelf or 3D-printed components, was entirely programmed in Python, and based on the Raspberry Pi platform, for maximum flexibility of use. Further, this approach allows for simple adaptation by others.

Across 14 animals tested, we found that marmosets required on average a low number of sessions (mean: 3 sessions) to learn how to interact with the touch screen. Second, marmosets performed roughly 120 self-initiated trials within a session (median: 117 trials, SD: 40 trials). Marmosets reached 90% of the total trials per session after 117 minutes.

Additionally, we successfully trained six out of eight marmosets in a sound discrimination two-alternative choice task. Here, an infant marmoset call paired with an infant marmoset face had to be discriminated from a pure tone train paired with a geometric figure.

Our novel touchscreen based, automated and unsupervised training procedure opens the possibility for testing new and more complex protocols for assessing cognitive hearing in the common marmoset in large

scale settings.

Context matters: revealing hidden sensorimotor memories in mice with AD-relevant pathology

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Memories have to be accessible for them to be useful. Alzheimer's disease (AD) is thought to be a progressive form of dementia in which cognitive capacities slowly deteriorate due to underlying neurodegeneration. Interestingly, anecdotal observations have demonstrated that Alzheimer's patients can exhibit cognitive fluctuations during all stages of the disease. This 'paradoxical lucidity' suggests that memories may exist in the AD brain but they are somehow inaccessible. In particular, it is thought that contextual factors are critical for unlocking these hidden memories. To date, however, exploration of the neural basis of paradoxical lucidity has been hampered due to the lack of a behavioral approach in mouse models to dissociate memories from contextual performance.

In previous work, we demonstrated how interleaving 'reinforced' trials with trials without reinforcement ('probe' trials) allowed us to distinguish between acquisition of sensorimotor memories (in 'probe' trials) versus contextual expression of these same memories (in 'reinforced' trials). Here, we test whether this same manipulation can be used in fully trained AD-related mice (mice with cortical Aβ accumulation, APP/PS1^{+}) to determine whether amyloid accumulation impacts underlying sensorimotor memories (measured in 'probe' trials) and/or contextual-performance (measured in 'reinforced' trials) in an age dependent manner. In young adult APP/PS1^{+} mice, contextual-performance is significantly impaired compared to age-matched controls. Surprisingly, these same animals show only minor impairments in underlying sensorimotor memories. However, middle aged APP/PS1^{+} mice show deficits in both contextual-performance and sensorimotor memories. These effects were recapitulated by using a reinforcement learning model that accounts for changes in contextual signals. The main network model parameters affected between the control and the APP/PS1^{+} mice were those governing contextual scaling and behavioral inhibition. These results suggest that Aβ deposition impacts circuits involved in contextual computations before those involved in acquiring knowledge. Additionally, we performed two-photon calcium imaging in the auditory cortex of behaving animals and found a reduction in stimulus selectivity in APP/PS1^{+} mice particularly apparent in reinforced trials, suggesting aberrant inhibitory network integration of contextual signals.

The function of the *radish* gene in *Drosophila* larval learning and memory

Andreas Stephan Thum[1], Deepthi Mahishi[1], Alexandra Großjohann[1], Elsa Kolbe[1], Nadine Ehmann[1], Robert Kittel[1], Astrid Rohwedder[1], Ines Köhler[1], Dennis Pauls[1]

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The *Drosophila* larva is an attractive model system to study fundamental questions in the field of neuroscience. Like the adult fly, the larva offers a seemingly unlimited genetic toolbox, which allows one to visualize, silence or activate neurons down to the single cell level. This, combined with its simplicity to modify specific gene function in nearly every possible way, offers a useful system to study the molecular correlates of complex processes including associative odor-taste learning and memory formation. Here, we analyse the function of the *radish* gene.

The *radish* gene is still one of the great mysteries in *Drosophila* learning and memory research. Although it was described more than 25 years ago, its function is still unknown. *Radish* is central to larval and adult anaesthesia-resistant memory. Using new genetic techniques, we reveal here for the first time details of the protein's molecular structure and anatomical localization. Supported by functional imaging and neurogenetic behavioural approaches, our results provide the basis to describe how *radish* regulates learning and memory in the *Drosophila* larva.

Dendritic integration of dopamine signals in dopaminergic neurons of the fly

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Dopaminergic signaling is thought to be at the basis of goal directed behavior and instrumental for specific forms of learning. Amongst these is associative learning which is found in vertebrates but also invertebrates. In the Drosophila brain dopaminergic neurons innervate a higher-order processing centre called the mushroom bodies. Here dopaminergic signaling alters the synaptic weights between intrinsic Kenyon cells that represent sensory information and efferent cells called mushroom body output neurons. Individual dopaminergic neurons can signal specific stimuli and encode valence. However, it is less clear how competing inputs to the dopaminergic neuron network are integrated. Here we present evidence for an inhibitory signaling pathway at the level of dopaminergic neurons innervating the fly mushroom bodies. Moreover, we provide first evidence that this signaling pathway is located at the dendritic sites of these neurons and resembles mammalian dopamine receptor 2 (D2) auto receptor signaling.

Exploratory attentional resource allocation in a probabilistic foraging paradigm in the Mongolian gerbil

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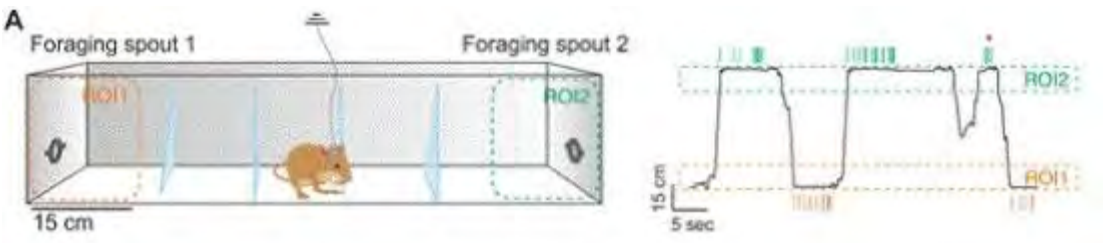
In our constantly changing world, it is necessary to continuously adapt choice options to current needs and-if necessary-to change our current behavioural strategy and explore the environment for the adaptive reallocation of resources. For example, imagine a Mongolian gerbil that forages a desert habitat for distributed food patches. When these patches become exhausted, the gerbil is in an exploitation/exploration dilemma: Should it exploit the current patch further or should it explore an alternative patch, suffering travel costs but enjoying potentially higher food density? Such a foraging example shows a fundamental resource allocation problem. The patch-leaving decision needs to be made based on probabilistic information (how much food is usually available in the alternative patch?) and in a potentially changing environment.

Here, we present a new behavioural paradigm in the Mongolian gerbil based on a probabilistic foraging paradigm (adapted from Lottem et al., 2018, *Nat Comm*). Patch-leaving behaviour is typically guided by the marginal value theorem (MVT): the current patch is abandoned if the food capture rate (i.e. the time or effort needed to obtain a food item) drops to the average capture rate in a patch. Thereby, in a random probability schedule, at a given food patch rewards decay exponentially per foraging attempt to zero. In contrast to a deterministic task, rule change cannot be inferred from a single reward omission, but is based on accumulating, gradual evidence. We developed a food-restriction schedule that allows us to train Mongolian gerbils with on average >40 switches between food patches per daily session (>120 food pellets). The stochastic nature of action-outcome associations inevitably leads animals to sample both sides, and hence, animals perform the task at hand without extensive pre-training sessions. We further influence exploration/exploitation trade-offs by varying the path length (i.e. the travel costs) in the foraging arena. Gerbils infer from the statistics of food delivery when to leave a ‘depleted site’ and explore another site despite the costs of traveling and uncertainty of future reward at another food source. Together, we present here a probabilistic foraging paradigm in the Mongolian gerbil as an ideal model system to investigate exploratory resource allocation.



Circadian modulation of associative conditioning in *Drosophila*

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Circadian modulation of associative conditioning in *Drosophila*

Felix Frantzmann, Robert J. Kittel, Dennis Pauls

Learning and memory is one of the most important cognitive tasks achieved by animals. Memory expression helps securing resources that change availability under temporal and spatial contingencies like food and mating partners or memorizing the location of nests and hives which are crucial for survival.
One of the factors influencing learning and memory has been shown to be time delivered by the circadian clock. Time keeping plays a decisive role since many environmental characteristics show rhythmic variations, for example the availability of food sources. Time-specific memories are present at various scales, since different times can be embedded into memories. Time information can vary from the time of day, temporal sequence of events, the elapsed time before or after an event, or even the seasonal time. While the circadian clock and its rhythm have been extensively studied concerning their influence on optimizing the physiological state and behavior of animals throughout the day, the question arises how animals associate the “when” with “what” and “where” to remember a biologically significant event precisely in time and space? This is a crucial knowledge gap for our understanding of learning and memory.
In this study we focus on different neurons of the fly´s circadian clock to untangle how a timestamp is set on mushroom body neurons. In detail, in a first set of experiments we focus on how odor-shock associations are modulated by the circadian clock. In line with previous studies we found that flies can learn odor-shock associations throughout the day, however, learning scores show circadian fluctuations. Further, our data suggests that dorsal clock neurons of the DN1p cluster might be essential to time-stamp memory processes within the mushroom bodies through PDF-dependent DH31 signaling.

Anesthetics uniquely decorrelate hippocampal network activity, alter spine dynamics and affect memory consolidation

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General anesthesia is characterized by reversible loss of consciousness accompanied by transient amnesia. Yet, long-term memory impairment is an undesirable side-effect. How different types of general anesthetics (GAs) affect the hippocampus, a brain region central to memory formation and consolidation, is poorly understood. Using extracellular recordings, chronic 2-photon imaging and behavioral analysis, we monitor the effects of isoflurane (Iso), medetomidine/ midazolam/ fentanyl (MMF), and ketamine/xylazine (Keta/Xyl) on network activity and structural spine dynamics in the hippocampal CA1 area of adult mice. GAs robustly reduced spiking activity, decorrelated cellular ensembles, albeit with distinct activity signatures, and altered spine dynamics. Network activity under all three anesthetics was distinct from natural sleep. Iso anesthesia most closely resembled unperturbed activity during wakefulness and sleep, and network alterations recovered more readily than with Keta/Xyl and MMF. Correspondingly, memory consolidation was impaired after exposure to Keta/Xyl and MMF, but not Iso. Thus, different anesthetics distinctly alter hippocampal network dynamics, synaptic connectivity, and memory consolidation, with implications for GA strategy appraisal in animal research and clinical settings.

Appetitive and aversive learning of amino acids in larval *Drosophila*

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Amino acids are important nutrients for all animals because they are necessary for protein synthesis in particular during growth, as well as for neurotransmission. However, little is known how animals use past experience to guide their search behaviour towards amino-acid-rich food. Larval *Drosophila* continuously require ingesting protein source for growth, which makes them a suitable model to investigate the mechanism of amino acid searching behaviour. The larval brain consists of a relatively low number of neurons, about ten times smaller than the brain of the adult fly; nevertheless, larvae are intelligent enough to form and express associative memory of odours and taste stimuli to search for a food source or to escape from toxic substances. Schleyer et al. (2015) showed that sugar and amino acid induce independent appetitive memories. We performed learning experiments for 20 individual amino acids, and found that larvae learn all individual amino acids as reward (Kudow et al. 2017). However, it is unlikely that animals ever meet a food containing only single amino acids under natural conditions. As a next step, we therefore used a mixture of 20 amino acids as the reinforcer. Surprisingly, we found that the amino-acid mixture is not only rewarding but also punishing to larvae. Currently, we work on identifying the neuronal circuits underlying the rewarding and punishing functions of amino acids.

Dendritic signal integration in a *Drosophila* Mushroom Body Output Neuron (MBON) essential for learning and memory

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The ability to associate neutral stimuli with either positive or negative valence forms the basis for most forms of decision making. Long-term memory formation then enables manifestation of these associations to guide behavioral responses over prolonged periods of time. Despite recent advances in the understanding of the neuronal circuits and cellular mechanisms controlling memory formation, the computational principles at the level of individual information processing modules remain largely unknown. Here we use the *Drosophila* mushroom body (MB), the learning and memory center of the fly, as a model system to elucidate the cellular basis of memory computation. Recent studies resolved the precise synaptic connectome of the MB and identified the synaptic connections between Kenyon cells (KCs) and mushroom body output neurons (MBONs) as the sites of sensory association. We build a realistic computational model of the MBON-α3 neuron including precise synaptic connectivity to the 948 upstream KCs innervating the αβ MB lobes. To model membrane properties reflecting *in vivo* parameters we performed patch-clamp recordings of MBON-α3. Based on the *in vivo* data we model synaptic input of individual cholinergic KC-MBON synapses by local conductance changes at the dendritic sections defined by the electron microscopic reconstruction. Modelling of activation of all individual synapses confirms prior results demonstrating that MBON-α3 is electrotonically compact. As a likely consequence of this compactness, activation pattern of individual KCs with identical numbers of synaptic connection but innervating different sections of the MBON-α3 dendritic tree result in highly similar depolarization voltages. Furthermore, we show that KC input patterns reflecting physiological activation by individual odors *in vivo* are sufficient to robustly drive MBON spiking. Our data suggest that the sparse innervation by KCs can control or modulate MBON activity in an efficient manner, with minimal requirements on the specificity of synaptic localization. This KC-MBON architecture therefore provides a suitable module to incorporate different olfactory associative memories based on stochastically encoded odor-specificity of KCs.

Precise predictions lead to increased efficiency of processing in stimulus-specific visual cortex

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Prior knowledge and predictability facilitates perception. As a stimulus is repeatedly presented, the brain learns to predict it with higher certainty. Incoming sensory input is matched to the prediction, and the mismatch is signalled as a prediction error (PE). Thus with every repetition, a lower PE is generated. This is thought to aid in efficient processing of information. Further, predictions are often made taking into account contextual information and at different levels of precision. A highly precise prediction could be at the level of identity of a person- ie. expecting your friend's face when you knock at their door. Whereas, a prediction at the level of a category- ie. expecting human faces at a train-station would entail forming predictions with a low precision. Here, we address the question: How does the brain efficiently process stimuli in conditions when the predictions are made at different levels of precision- ie. identity and category-level to guide perception ?

We approach this question using invasive electrophysiology in human epilepsy patients. The participants perform a task where they are shown sets of repeating visual face stimuli which follow a reference image/ prior providing contextual information. This repetition of stimuli elicits reduction of PEs- also known as repetition suppression. It also elicits repetition enhancement effects, which is taken as strengthening of predictions. The prior gives the context, ie. whether the predictions are to be made at the level of specific facial identity or more imprecise predictions at the level of category.

We study these repetition effects by focusing on the high-gamma frequency band (70-200Hz). We observe 38% of all visually responsive electrodes show repetition suppression, exhibiting PEs and 18% show repetition enhancement, ie. strengthening predictions.
Predictive-coding frameworks suggest that predictions and PEs are passed as information exchange between higher and lower hierarchical areas in the brain and suggests the role of higher regions in sending predictions. We hypothesised that electrodes holding predictions would have higher activity when predictions are very precise. We observe this specifically in higher regions of the processing hierarchy, ie. in the medial Temporal lobe, the inferior frontal gyrus, as well as the fusiform gyrus.
Further, we observe a global reduction of PE throughout the visual hierarchy, for both identity and category-level predictions. This indicates a general efficient coding regime being implemented in the perception of repeated and predictable stimuli. To address how precision of prediction affects the magnitude of PEs, we hypothesized that higher PE would be computed when the precision of predictions is lower. This is built on the concept of precision weighting, which suggests that the computation of PEs depends not only on the content of predictions and input to that region, but is also weighed to the reliability ascribed in them. We observed this effect in the stimulus specific area, ie. the fusiform gyrus. This indicates that in the

computation of PEs, the effect of precision of predictions exists only when received by neurons that are highly selective for the stimulus category.
Thus, this suggests that predictions facilitate an efficient processing of stimuli throughout the hierarchy and making a precise prediction for identity of faces leads to a further increased efficiency only in the stimulus specific area.

Serotonergic Memory Suppression Balances the Consolidation of Conditioned Avoidance or Approach Behavior

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The brain selects only relevant information for long-term memory (LTM) storage. Memory suppression mechanisms are thought to coordinate the competition between appropriate and inappropriate information during memory consolidation by largely unknown mechanisms. We have recently identified a molecular and cellular correlate of a memory suppression mechansim in the Drosophila brain. In detail, the serotonergic projection neuron (SPN) exhibits a default inhibition on the formation of aversive LTM via the modulation of dopaminergic input to the flies' memory center, the mushroom body (MB). This serotonergic "memory checkpoint" sustains a default inhibition of memory consolidation for aversive associations controlled by phosphodiesterase (PDE)-mediated suppression of neuronal activity in the SPN. Only in relevant contexts, memory suppression by PDE is released and activation of the SPN allows LTM consolidation in the MB. Intriguingly, while blocking SPN activity during consolidation suppresses aversive LTM, the expression of reward-driven, appetitive LTM is strongly increased. Excitatory and inhibitory serotonin receptors in the downstream dopamine circuit mediate this diametrical effect on the conditioned avoidance or approach behavior in a state-dependent manner. Together, this work proposes a mechanism of memory suppression by serotonin, which realizes context-dependent plasticity to bias the storage of positive or negative associations.

Opponent types of learning through occurrence vs. termination of reinforcement

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Obtaining reward and avoiding punishment are important behavioural goals in humans and animals. To attain these goals it is crucial to learn not only about the occurrence of reward or punishment, but about their termination as well. These two aspects of experience with reward or punishment entail opposite affect: Just as it feels bad to receive punishment and good to receive reward, the relief upon punishment termination feels good and the frustration upon reward termination feels bad. This effect is called timing-dependent valence reversal and has been studied in flies, rodents and humans – mostly, however, regarding punishments. Using Drosophila larvae, we identified two different dopaminergic mushroom body input neurons (DAN) whose optogenetic activation can likewise confer timing-dependent valence reversal, in either the appetitive and the aversive domain, respectively. We study the temporal dynamics of timing-dependent valence reversal induced by these two DANs and its dependency on dopamine signaling.

Chronic social stress impairs learning and memory in crickets

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Early life adversity and stress have numerous deleterious effects on health, behaviour and cognition in humans and other mammals. Using a chronic social defeat-stress paradigm, similar to that used in rodents, we have shown that pre-adult social subjugation in insects (crickets) leads to life-long changes in adult behaviour, seemingly via effects of specific neurotransmitters (Poster Balsam & Stevenson). We now report that chronic social defeat-stress in crickets impairs learning in an appetitive, differential olfactory learning paradigm.

Since the response to stress depends on an individual's susceptibility and resilience to adversity, we devised a novel method to score the learning capacity of individual crickets. Briefly, we used automated video tracking (EthoVision XT) to evaluate responses to an unconditioned and a conditioned odour (N=50 each). From this data we constructed a mathematical model which discriminated between the two groups with 93% accuracy, giving a probability (0-1) for each animal that it exhibited a conditioned response ($P_{resp.}$). The model was tested with new crickets receiving either the unconditioned stimulus (US, sugar water), conditioned stimulus (CS, odour) or both (CS+US; N=25 each) and gave a $P_{resp.}$ median(IQR) for CS+US: 0.997(0.82/1.0); US: 0.09(0/0.29); CS:0.02(0/0.18). Given this high efficacy, we used the model to calculate the effect of social stress on learning.

For this, mature adult males were isolated from a breeding stock 48 h previously and deprived of water for 24 h (N=64). In a training regime, individuals were alternately presented with 2 different odours (amyl acetate and 1-octanol, 3 x each, 5 min intervals), one was rewarded with sugar water (CS^+), the other not (CS^0). After 30 min, each odour was presented once and the response probabilities were calculated ($P_{resp.}^{+}$ and $P_{resp.}^{0}$; note: presentation sequences and the odour rewarded were changed systematically in 8 different regimes, but there were no significant differences and hence the data pooled). As expected, the rewarded odour response was significantly greater than for the non-rewarded odour: median(IQR) $P_{resp.}^{+}$ 0.98(0.65/1); $P_{resp.}^{0}$ 0.20(0/0.71); Wilcoxon signed-rank test, $p<0.0001$. Individual learning indices were calculated from $L_{in} = P_{resp.}^{+} - P_{resp.}^{0}$ and gave a group median(IQR) of L_{in} 0.39(0/0.89), N=64.

Other individuals were subjected to chronic-defeat stress by confronting them multiple times with an aggressive dominant male (6x at 1 h intervals + 3x next day). At the first confrontation the focal animals typically fought the dominant, but retreated first to become the losers. In later confrontations the focal animals retreated immediately. Surprisingly, compared to non-stressed crickets, defeat-stressed crickets responded less to the rewarded odour ($P_{resp.}^{+}$ 0.22) and more to the non-rewarded odour ($P_{resp.}^{0}$ 0.65) resulting in significantly lower, often negative learning scores: median(IQR), defeat-stressed: L_{in} -0.01(-0.43/0.02), non-stressed control: L_{in} 0.25(0/0.63); Mann-Whitney-U-test, $p=0.0002$, N=20 each. A duplicate experiment with crickets raised without any contact to adults revealed the same effect of defeat-stress on learning (L_{in} defeat-stressed: -0.23; L_{in} non-stressed control: 0.27). Current experiments are revealing neurotransmitters that implement the effects of defeat-stress on learning and change in valence reported here. Supported by the DFG (STE 714/5-1).

Subtle effects of a history of avoidance on later extinction of an auditory fear memory in rats

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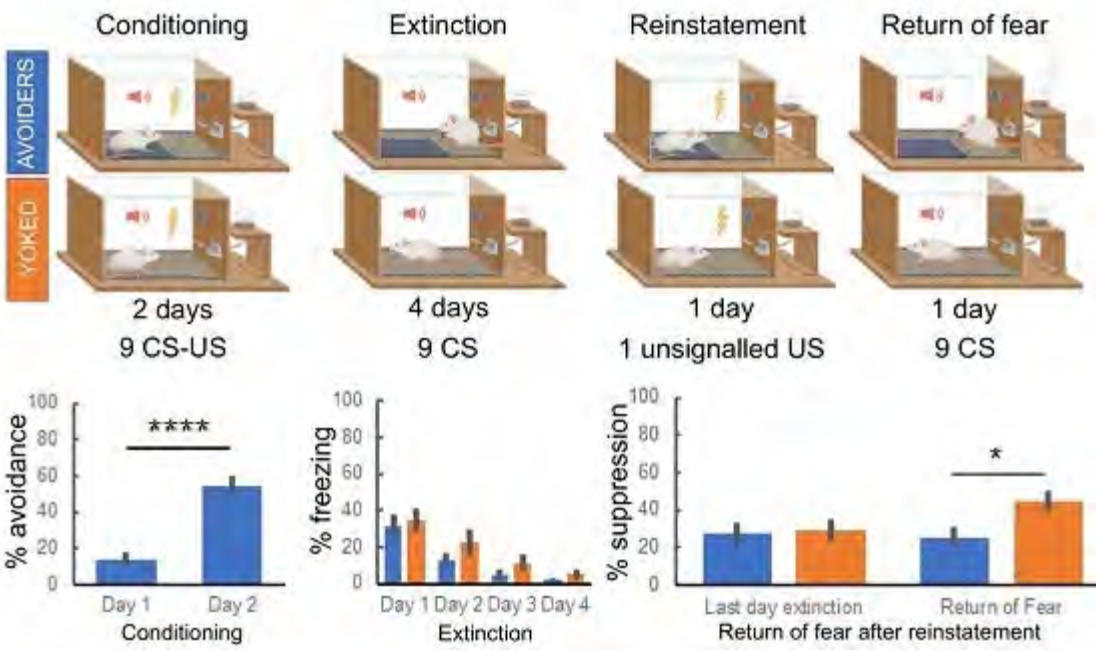
A promising avenue for understanding the basis of anxiety disorders is the identification of learning mechanisms that are involved in the transition from adaptive to maladaptive fear. Some of the processes that appear to be important in this transition are (deficits in) extinction and (excessive) avoidance. Previous studies suggest that a history of controllability over stressors may enhance Pavlovian extinction in humans and rats. However, it is presently unknown how a history of avoidance influences extinction learning. Using a platform-mediated avoidance procedure, we performed two experiments where we studied the effect of avoidance acquisition on later Pavlovian extinction of an auditory fear memory in rats. Male Sprague-Dawley rats (N = 24 per experiment) were food-deprived and trained to press a lever for food. They were then divided into two groups: avoiders and yoked animals. Avoiders had the possibility to avoid the CS-signalled US by stepping onto a platform (thus giving them some control), while yoked animals (without a platform) received the same CS-US contingency as the avoider they were yoked to. In the first experiment, the avoidance platform was absent during extinction training, while in the second experiment, the platform was present. We scored time spent on the platform, freezing, rearing and suppression of bar pressing to evaluate whether animals learned to avoid the US and to examine how the possibility to avoid affected subsequent extinction. We found that two days of conditioning are sufficient for avoidance learning using the platform-mediated avoidance procedure. Surprisingly, regardless of the possibility to perform an avoidance response during extinction training, we showed that a history of avoidance does not impact the capacity to acquire extinction. Finally, in a series of exploratory follow-up tests in experiment 2, we observed that a history of avoidance did partially reduce reinstatement. These results suggest that, even though avoidance learning may not affect the subsequent extinction of an auditory fear memory, it could have an impact on reinstatement. This result merits further investigation, given that reinstatement is one of the mechanisms for relapse in patients suffering from anxiety disorders.


Conditioning
Extinction
Reinstatement
Return of fear
AVOIDERS
YOKED
2 days
9 CS-US
4 days
9 CS
1 day
1 unsignalled US
1 day
9 CS
% avoidance
****
Day 1
Day 2
Conditioning
% freezing
Day 1 Day 2 Day 3 Day 4
Extinction
% suppression
*
Last day extinction
Return of Fear
Return of fear after reinstatement

The effects of nasally-applied neuropeptide S on T-maze learning and reversal learning

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Neuropeptide S (NPS) is a 20 amino acid long neuropeptide that is expressed in neurons of a specific area between the locus coeruleus and Barrington's nucleus. Its receptor NPSR1 is distributed throughout the brain. Studies showed that NPS plays role in anxiety, stress, arousal, and learning. While the majority of experiments was performed with NPS injection into the cerebral ventricle, some studies used nasal application of NPS and observed behavioral effects including anxiolysis. However, it was not known whether nasal NPS application also affects cognitive functions. The aim of our study was to investigate the effects of nasal NPS administration on learning and reversal learning. Therefore, C57Bl/6 mice received nasal application of either saline or NPS and were subjected a simple T-maze task, i.e., they had to learn that one of the two T-maze arms were baited with a food reward. After five sessions with 10 trials each, reversal learning was performed with five further sessions. The results showed that NPS does not affect the learning performance within the first five sessions, however, reversal learning was improved with nasal NPS administration.

Since reversal learning is an important experimental paradigm to study cognitive flexibility in both humans and animals, this data indicates facilitating effects of nasal NPS administration on cognitive flexibility. Cognitive flexibility is an executive function that allows switching from one concept to another concept and is impaired in several neuropsychiatric disorders. Our data suggest that NPS as a target to improve deficits in cognitive flexibility.

Highly correlated network dynamics underlie sharp wave-ripple activity in sleeping zebra finches

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Sharp wave ripples (SWR) represent one of the most synchronous population patterns in the mammalian brain. Although SWRs are highly conserved throughout mammalian evolution, the existence of SWRs in non-mammalian species remains controversial. We reexamined the existence of avian SWRs by recording the brain activity during sleep and under anesthesia in the zebra finch, an oscine vocal learner. Electrophysiological recordings using silicon probes implanted in the avian telencephalon revealed highly dynamic switching between high and low delta phases during sleep. High delta phases were composed of large-amplitude, negative deflections (sharp waves) that coincided with a high frequency oscillation (ripple). Correlation analysis revealed that these events were highly synchronous and spanned a large anatomical range of the avian telencephalon. Finally, detailed spike analysis revealed that an increase in the population spiking activity coincided with the occurrence of SWRs, that this spiking activity occurred in specific sequences of spike patterns locked to the SWRs, and that the mean population spiking activity peaked prior to the trough of the negative deflection. These results provide the first evidence of avian SWRs during natural sleep, and highlight the role that large-scale network activity may have in the evolution of learning and memory.

The role of specific dopaminergic neurons for gating memory retrieval

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Integrating past experiences, the present situation and future behavioural options is one of the most essential functions of brains – in any animal. This integration is particularly obvious at the moment of memory retrieval: a hungry animal exploring the environment may use its past experience with food-associated odors to guide its search for food. Once a food source is found, however, it is adaptive to stop that learned search and to rather exploit the food source. In other words, the absence or presence of food gates the retrieval of the memory and its transition to behaviour. We study the neuronal circuitry that underlies this gating process in larval *Drosophila melanogaster*. We find that optogenetic activation of the dopaminergic neuron DAN-i1 resembles natural food rewards in that it conveys both a rewarding signal to establish memory for associated odors that can inform future learned search, and a gating signal that can terminate that search but leaves innate olfactory behavior unaffected. Using high-resolution behavioral tracking, we further find that the DAN-i1 reward signal induces the same locomotor 'footprint' for learned olfactory search as food rewards, and that the DAN-i1 gating signal can tune down these very behavioral modulations. EM data-based analyses of all the synaptic connections between DAN-i1 and its two main targets, the Kenyon cells and the mushroom body output neuron MBON-i1, suggest that the reward and the gating signal can be locally read by the Kenyon cells and MBON-i1, respectively. This provides an elegant circuit motif to gate memory retrieval and to terminate a search mission once it is accomplished. Given the similarities in dopamine neuron function across the animal kingdom, we wonder whether such a circuit motif may reflect a general principle. Currently, we extend our analysis to the aversive domain and explore which individual punishment-signaling dopaminergic neurons can also gate memory retrieval.

Feed-forward inhibition in Dentate Gyrus-CA3 drives time-dependent re-organization of memory ensembles in prefrontal cortex

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Hippocampal-cortical communication is thought to determine the extent to which memories generalize over time, but the underlying circuit mechanisms are poorly understood. By identifying Ablim3, a cytoskeletal F-actin binding protein that is exclusively localized to dentate granule cell (DGC) mossy fiber terminals, as a molecular brake of DGC connectivity with parvalbumin inhibitory neurons (PV-INs), we demonstrated a critical role for DGC recruitment of feed-forward inhibition
(FFI) onto CA3 in time-dependent memory generalization. Viral downregulation of Ablim3 in DGCs enhanced excitatory drive onto stratum lucidum PV INs, elaborated PV IN synaptic contacts with CA3 neurons and increased FFI in DG-CA3. Mice with increased FFI in DG-CA3 exhibited reduced remote memory generalization. These observations motivated investigation of how FFI in DG-CA3 affects neuronal ensemble dynamics in hippocampal-prefrontal cortical networks over time. Towards this goal, we performed single-photon calcium imaging in hippocampal CA1 and the anterior cingulate cortex (ACC) of awake, behaving mice - in which we increased FFI in DG-CA3 - to longitudinally track neuronal ensembles during encoding and recall of contextual fear memory in the fear conditioned and neutral contexts at recent and remote timepoints. We first developed a Matlab-based automated workflow to standardize the extraction and sorting of temporal and spatial information from both CA1 and ACC datasets to allow for comparison between these two brain regions. Consistent with the notion that memory traces re-organize during consolidation in hippocampal-cortical networks, we directly observed time-dependent refinement of context-associated ensembles in ACC over time. Importantly, increasing FFI in DG-CA3 potentiated this refinement and we observed a decrease in the overlap between ensembles of the fear-conditioned and neutral contexts in CA1 and ACC at remote timepoints. Analysis of active neurons revealed that FFI reduced numbers of correlated pairs of active neurons in ACC, but not CA1, following context exposure. Together, these findings provide direct evidence for time-dependent evolution of memory ensembles and identify FFI in DG-CA3 as a neural substrate for memory consolidation in hippocampal-cortical networks. Targeting Ablim3 may represent a strategy to promote hippocampal inhibition, dampen hippocampal hyperactivity and promote memory consolidation in aging and Alzheimer’s disease

Rewarding properties of the APL neuron in larval *Drosophila*

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Inhibitory systems are important controllers of sensory systems and behaviour, allowing the processing of relevant information against environmental noise, and the selection of adaptive motor actions from a pool of competing behavioural options. Several studies have shown the critical role of GABAergic synaptic inhibition in odour processing, olfactory learning and behavior in invertebrates, including *Drosophila melanogaster*. In this project, we focus on a single, GABAergic anterior paired lateral (APL) neuron, identified in both adult and larval *Drosophila*. Although the role of APL in memory acquisition and retrieval has been investigated in adult, the reduced complexity of the larval olfactory system, which is well characterized at synaptic resolution and without cellular redundancy, allows the interpretation of behavioural and physiological data with ease, precision and completeness.

Using a combination of behavioural analysis, optogenetics and connectomics, we aim to understand how APL functions in the larval brain and how it modulates associative olfactory memory formation and retrieval. We discovered, surprisingly, that activating APL optogenetically is sufficient to establish a reward memory. Systemic inhibition of dopamine signaling impairs this memory, suggesting an implication of downstream dopaminergic neurons. We follow up on this asking (i) which dopaminergic neurons are involved, (ii) whether this rewarding effect depends on GABA-synthesis in APL, and (iii) working in collaboration with colleagues from Leipzig University contributing to physiological expertise.

Poster Topic

T26: Computational Neuroscience

T26-11 Dissecting dynamic gain reveals differential contributions of subthreshold impedance and spike generation dynamics
Elinor Lazarov, Ricardo M Merino, Michael J Gutnick, Fred Wolf, Andreas Neef

T26-12 Neural models for the (cross-species) recognition of dynamic facial expressions
Michael Stettler, Nick Taubert, Ramona Siebert, Silvia Spadacenta, Peter W. Dicke, Peter Thier, Martin A. Giese

T26-13 Genetic Basis of Phase Amplitude Coupling Entrained by Working Memory
Anahita Nazari, Jacob Khezri, Stefan Treue, Moein Esghaei

T26-14 Biophysically grounded mean-field models of neural populations under electrical stimulation
Caglar Cakan, Klaus Obermayer

T26-15 Modulation of Fronto-Striatal Connectivity by using intermittant Theta Burst Stimulation (iTBS). A 18 Flourine-Desmethoxy Fallypride (DMFP) Positron Emission Tomography (PET) study.
Usman Jawed Shaikh, Antonello Pellicano, Andre Schüppen, Oliver Winz, Alexander Heinzel, Felix Mottaghy, Ferdinand Binkofski

T26-16 Dimensionality of neural circuit manifolds associated with a salt-and-pepper organization of cortical stimulus preferences

Michael Sternbach, Fred Wolf

T26-17 Interneuron Inhibition Stabilizes Pyramidal Neurons against Cortical Spreading Depression
Allison Harris, Wolfgang Stein

Hue dependence of contextual influences on color vision predicted by a non-uniform population code

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The perceived color of a stimulus is influenced by colors in the visual context around the stimulus. A putative neural correlate of such contextual interactions is the response modulation of neurons in the visual system by stimuli outside of their classical receptive field. For orientation perception, contextual response modulation is thought to underlie perceptual phenomena like the tilt illusion [1]. Computational models assuming a population code and surround suppression in the early visual cortex can account for the corresponding psychophysical findings [2], [3], providing a functional explanation in line with the notions of efficient coding and visual saliency. The same kinds of models likewise predict the contextual influences in color vision qualitatively. However, quantitatively, contextual effects in color vision show a dependency on the hue of the context [4]. We present a population coding model with non-uniform model parameters that predicts the hue-dependence of color shifts induced by chromatic surrounds. In the model, stimulus hue was encoded by a population of neurons with von Mises tuning and color preferences distributed in color space. The modulatory influence of the contextual surround was implemented via divisive inhibition. Tuning widths and the strength of surround suppression were not uniform but varied as a function of hue angle, motivated by experimental findings of non-uniformities in the chromatic tuning properties of V1 neurons [5]. To determine the readout of the stimulus value encoded in the population, we considered the Population Vector (PV) and alternatively Maximum Likelihood (ML) decoding [6]. In contrast to the uniform model, the non-uniform model was able to produce color tilt curves of different magnitudes for different surround hue conditions, improving the fits to the experimental data by 15%. While for the uniform model both decoders showed comparable performances, for the non-uniform model the ML decoder performed better than the PV decoder in fitting the experimental data. This was mainly due to the ML decoder capturing better the context-induced bias with surround hue. In conclusion, the results are consistent with a non-uniform population code underlying the hue-dependence of contextual influences on color vision.

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Encoding beats beyond Nyquist frequency by a smooth threshold non-linearity

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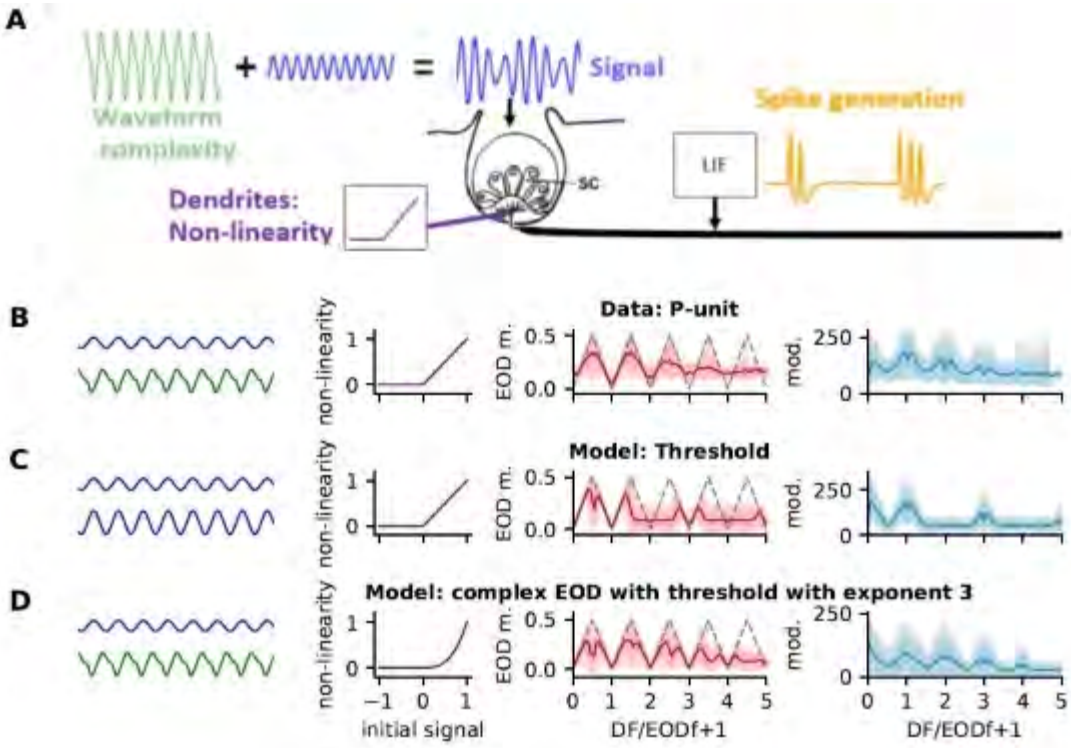
Beats, periodic amplitude modulations resulting from the superposition of two signals of different frequencies are common sensory stimuli in auditory systems as well as in electrosensory systems (Joris et al., 2004). Usually the second signal is assumed to be close in frequency to the carrier signal. The beat frequency is then given by the difference frequency between the two stimulus frequencies. Here we study the temporal encoding of difference frequencies far beyond the Nyquist frequency (half of the frequency) of the carrier signal in the electric fish Aperonotus leptorynchus. These fish generate sinusoidal electric organ discharges (EOD) with a fish specific frequency, EODf , in the range of 500 to 1100 Hz. The beat carries behavioral relevant information about species, sex, size, distance and dominance status of the sender and is encoded by electrosensory afferents, called P-units, by modulations of their firing rate following the beating amplitude modulation (Walz et al., 2014). We recorded spiking activity of P-units in response to beats at difference frequencies ranging from -800 to 2700 Hz relative to the fish's own EODf. The P-units responded to these beats in similar ways at every integer multiple of the EOD frequency. The frequency of the firing rate modulation followed a Toblerone shaped curve with minima at integer multiples of EODf (red line, Fig. 1 B). The modulation depth of the firing rate response also followed this repetitive pattern (blue line, Fig. 1 B). This repetitive response pattern cannot be explained by the difference frequency as the frequency of the amplitude modulation of the carrier signal that one gets mathematically from the analytical signal using the Hilbert transformation. How do the P-units retrieve this repetitive Toblerone curve from the superposition of the two input signals? Extending the concepts introduced by (Sinz et al., 2020) in the context of spike-time locking to multiple frequencies, we demonstrate that this requires a threshold operation applied to the stimulus and higher harmonics of the carrier signal at every integer multiple of the carrier frequency. The threshold operation has been suggested to correspond to the synaptic transmission of the electroreceptor cells onto the primary afferents (Chacron et al., 2001) but is not sufficient to explain the Toblerone curve (red line, Fig. 1 C). Higher harmonics of the carrier can be obtained by smoothing out the threshold operation by raising the thresholded signal for example to a power of three or five (Fig. 1 D). In addition, the natural EOD waveform already provides the necessary harmonics (Fig. 1 D). After thresholding, exponentiation and low-pass filtering the resulting signal already shows the repetitive beat frequency. Feeding this into a leaky integrate-and-fire model predicts the P-unit responses well. The power spectrum of the carrier waveform determines how strong the P-units respond to difference frequencies beyond Nyquist frequency and thus this could potentially be a selective force on the shape of the EOD waveform. The simple and general mechanism we propose (smooth threshold operation) allows for P-units to respond to beat frequencies far beyond the Nyquist frequency of the carrier. This is a stimulus regime that so far has been neglected in electrophysiological experiments but has been shown to be of behavioral relevance in courtship (Henninger et al., 2018) and potentially also in inter-species interactions (Henninger et al., 2020). The same mechanisms might also allow other sensory systems, in particular the auditory system, to respond to high difference frequencies.


A
Waveform complexity
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Spike generation
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A synthetic biology approach to the evolutionary transformation of visual cortex architecture

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(Introduction) The arrangement of orientation selective neurons in the visual cortex into functional domains most likely evolved in parallel in primates and in carnivores [1]. It presumably emerged from a prior rodent-like salt-and-pepper layout. In order to investigate transition scenarios between the two types of functional architecture we developed a synthetic biology approach [2].

(Methods) We used neuronal interface technology to connect a computational model of the retino-thalamic pathway to an in-vitro model of cortical input layer 4 (L4). The latter contained channelrhodopsin expressing principal neurons, either as a primary culture of cortical neurons or a horizontal brain slice of visual cortex L4. The two stages were connected via optogenetic holographic stimulation emulating thalamo-cortical synaptic input to L4 [3, 4]. We recorded neural activity either with a multielectrode array or by calcium imaging.
In the Hubel&Wiesel feed-forward model orientation selection in L4 is a result of convergent thalamic input [5]. We implemented such a feed-forward scheme in our system with variable size of orientation domains. We then explored the consequences of scaling the size of orientation domains down to the size of single neurons.

(Results) We measure the amount of significantly tuned neurons and the strength of orientation selectivity for different domain sizes. Intriguingly, the fraction of orientation selective neurons only weakly decreased with shrinking domain size. Surprisingly, even in the absence of orientation tuned input a considerable level of orientation selectivity was retained. In this limit of infinitesimally small domains, the emergent arrangement of orientation selective cells resembled a sparse salt-and-pepper layout.

(Conclusions) We successfully implemented a synthetic biology system to probe different realizations of thalamo-cortical connections. Our results indicate that evolutionary scaling of orientation domain sizes can induce a self-organized transition to and from a salt-and-pepper layout.

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Proprioceptive Encoding In Spiking Neural Networks

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The ability to walk requires complex coordination between the sensory and motor systems. As insects traverse their environment they are faced with complex substrates that requires initiating a climbing behavior. For climbing, body coordination must use information about body-posture, leading some insects to possess dedicated organ or neural circuits to hold a representation of body-pitch. Some insects are hypothesized to derive this information directly from their proprioceptive system. The neural circuit of proprioception involves several types of proprioceptors and an array of descending interneurons which encode the joint angle and velocity.

The current thesis examines whether such whole-body high-order para-meter can be inferred from the distributed activity of the proprioceptive system in the Indian stick insect Carausius morosus. A feed-forward spiking neural network was created, containing a model of the hair-field sensilla proprioceptor fitted using experimental data, a layer of inteneurons for encoding joint position and velocity and a layer of neurons to encode basic common spatio-temporal phases of the leg, known as movement primitives. The network was fed with experimental data of climbing stick insects, and the resulting spike trains were used as input for a linear regression model to estimate the body-pitch.

The results showed that the proprioceptor's model exhibit similar spiking dynamics of a phasic-tonic combination as those recorded from sensillum afferent. Moreover, when placing several sensilla in a row structure with direction sensitivity, two interneurons were sufficient to encode the joint angle time course with high precision. These neurons have similar dynamics to similar neurons that were recorded in the stick insect antenna. From the hair-row the joint movement velocity could be inferred, with linear relationship between spike rate and velocity. The movement primitives neurons encoded successfully different phases in the leg trajectory during walking. Furthermore, some of these neurons exhibited exceptional swing/stance encoding, which suggests that information about the gait movement is reflected in the proprioceptive level. Finally, the linear regression resulted in a maximum R-squared of ~0:85, suggesting that body-pitch representation can be extracted from proprioceptive activity, and that this is computationally inexpensive. Further developments, such as introducing noise to the system, and inferring the body-pitch from the proprioceptive layer itself, can reinforce the feasibility of this process occurring in the nervous system of the insect.

The impact of noise correlation on the stability of signal correlation

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Neuronal correlations are ubiquitous in networks of neurons and are believed to be an essential part of the neural code. Different types of correlations capture different aspects of the neural code: Signal correlations describe the similarity between the tuning properties of neurons, while noise correlations measure the similarity in trial-to-trial variability.
While studies in the past have mainly focused on the interaction between signal- and noise correlations concerning the coding performance of neural systems, little is known about how signal- and noise correlations change and affect each other over a period of days in a mature sensory cortex.
Here we investigate the evolution of signal- and noise correlations in chronic two-photon calcium imaging data of mature, awake mouse primary auditory cortex under baseline conditions.
We found that the structure of signal correlations is undergoing substantial reorganization, while at the same time keeping a steady-state distribution over time. Noise correlations, on the other hand, remained more stable, although they were on average lower than signal correlations.
Furthermore, we found that noise correlations had a stabilizing effect on signal correlations, i.e. that neuron pairs which exhibited both, a high signal- and noise correlation, maintained on average a higher signal correlation two days later, compared with neuron pairs with high signal correlation but low noise correlation. Interestingly, the reverse was not the case, i.e. signal correlations did not predict the stability of noise correlations.
Our findings underline the importance of noise correlations in sensory coding and hint towards a previously unknown role of noise correlations in stabilizing cortical circuits.

Simulation-Based Predictions of the Effects of Ion Channel Mutations on Neuronal Firing Behaviour and Neurological Severity

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Mutations in ion channels may alter the biophysical properties of the corresponding ionic currents and potentially cause neurological conditions like epilepsy, autism or intellectual disability. Much investigation into the impacts of ion channel mutations has used electrophysiological characterizations of changes in biophysical properties of ionic currents in expression systems with no endogenous ionic currents. Whereas the impact of a mutation on the affected current can be readily assessed, predicting its consequences on the level of neuronal firing in terms of gain or loss of function (increased or decreased firing, respectively) or the strength of this impact is difficult. Particularly when a mutation affects an ionic current in multiple ways that each provide contradictory indications as to whether a gain or loss of function will be realized, predicting the consequences on neuronal firing are impossible. Experimental data recorded in neurons expressing the mutated channels are available for a limited number of mutations only and require large amounts of effort. This makes a large-scale experimental investigation into the effects of mutations at the neuronal level impractical. In addition, potential dependencies of effects of mutations on the properties of other channels and currents present in a specific neuron are difficult to infer from such experiments. Modelling approaches can overcome these limitations and enable flexible analysis of the impacts of alterations of ionic currents on neuronal firing, thus bridging the gap between the characterization of biophysical properties and neuronal level effects including losses or gains of function. Here we used a single compartment conductance-based neuronal model to evaluate the impacts of alterations to activation and inactivation dynamics for all currents in a five-current model. The effects on neuronal firing were simulated for different types of single modifications and subsequently quantified. A correlation analysis between the magnitude of the modification and the changes in firing behaviour was performed. As a result, a summary lookup table was generated that enables qualitative insights into the potential effects of a wide range of modifications at the level of ionic currents. The lookup table bridges the span from electrophysiologically observable changes in biophysical properties of ionic currents to functional classifications in terms of gain or loss of function and their relative strength. This allows classification of mutations with contradictory effects on ionic currents based on their relative contributions. As a proof of concept, we used the look up table to predict changes in the firing rate of several described variants of the SCN8A gene (voltage dependent sodium channel Nav1.6) that are known to cause epilepsy. The resulting predictions explain 35% of the variance in epileptic severity caused by these variants, that is our rough predictions of the changes in firing rate carry over to some extent to the resulting neurological disorder. This encourages us to further pursue linking biophysical properties with firing behaviors and ultimately neurological conditions based on conceptual simulation studies.

Dual-task modulation of neural activity related to perception of time

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Time interval perception is an important cognitive ability of the human brain. Very often in daily life, time estimation is done concurrently with other tasks. The dual task paradigm was used in duration reproduction experiments to show that time perception is dependent on attention and memory (Fortin & Rousseau 1998; Polti et al. 2018). Dual tasks require to share cognitive resources and, accordingly, dual-task durations have been shown to be judged shorter than without a secondary task. Studying task-related EEG responses could help to reveal the underlying neural mechanisms of time estimation. We therefore conducted a duration reproduction experiment investigating EEG responses under simple (STRT) and dual task (DTRT) conditions. Participants (48, 23 female) had to reproduce 180 randomly presented audio-visual time intervals (1, 1.5, and 2 s) per condition by pressing a button (Fig. 1A). In DTRT participants had to remember whether one of 5 random letters had been presented during the production phase. EEG signals were recorded using the 32 channel LiveAmp amplifier with actiCap wet electrodes (Brain Products) in 3 sessions for each task (20 trials per duration per session). Central and frontal electrodes clusters were used for the analysis of Contingent Negative Variation (CNV; e.g., Kononowicz & van Rijn, 2014) and P300 response respectively.

Behavioral results showed no consistent contraction of perceived stimulus duration for DTRT, but a significantly increased regression effect as shown by the slope of reproduced over stimulus duration (t-test: $p>0.001$) being smaller for DTRT (0.49, SD 0.14) than for STRT (0.70, SD 0.09). Extracted ERP amplitude was compared using a three-way ANOVA (task, duration, channel). The CNV amplitude of STRT (-1.214, SD 0.172) was significantly larger ($F(1,47)=12.6$, $p=0.001$, $[eta]_p^2=0.21$) compared to DTRT (-0.518, SD 0.192). P300 amplitude due to offset of production phase was enhanced significantly ($F(1,47)=29.4$, $p<0.001$, , $[eta]_p^2=0.38$) in DTRT (1.46, SD 0.12) compared to STRT (0.88, SD 0.19).

Our behavioral result suggests that DTRT mainly added uncertainty resulting in increased reliance on prior information (Petzschner et al. 2015). This increase in uncertainty corresponded to a decrease in CNV amplitude, which is regarded as index of temporal accumulation, suggesting that both are linked to sharing the capacity for processing temporal and non-temporal information. The enhancement of P300 amplitude in DTRT compared to STRT was likely caused by increased working memory activation and attentional demand in the frontal lobe due to memorization as a second task.

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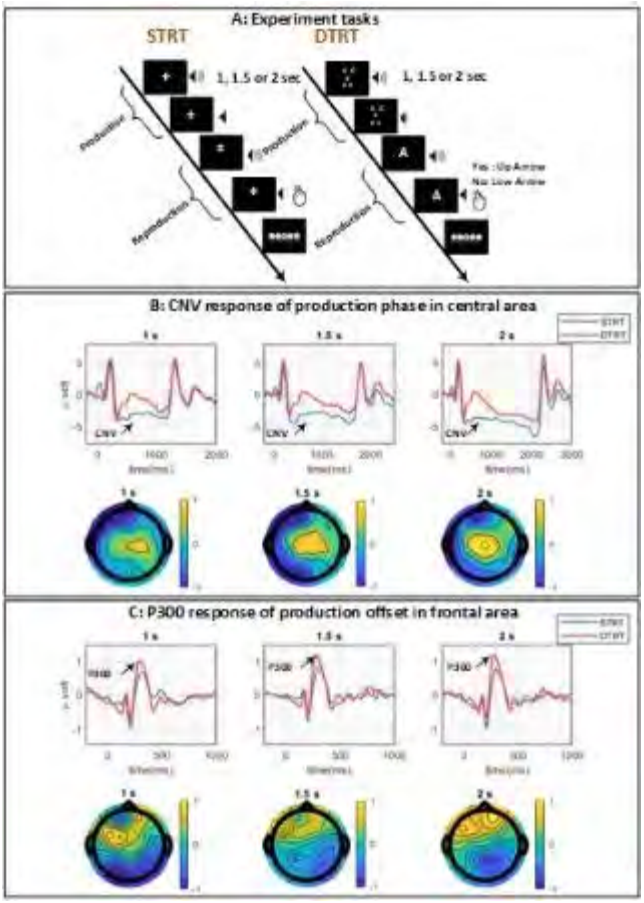
A: Experiment tasks
STRT
DTRT
1, 1.5 or 2 sec
1, 1.5 or 2 sec
Production
Production
Reproduction
Reproduction
Yes : Up Arrow
No: Low Arrow
B: CNV response of production phase in central area
1 s
1.5 s
2 s
CNV
C: P300 response of production offset in frontal area
P300

Modeling effects of Hebbian plasticity protocols in recurrent neural networks with homeostatic structural plasticity

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Plasticity is a remarkable feature of neural tissue that enables it to respond to specific stimuli with structural and functional adaptations. Such changes at synaptic contact sites, i.e., synaptic plasticity, are considered to play a pivotal role in complex brain functions. Activity-dependent plasticity has been extensively examined in past decades, with Hebbian and homeostatic plasticity being cornerstone concepts. The mechanisms by which these two forms of plasticity, which are based on positive or negative feedback, respectively, can co-exist in the same networks, neurons, and even synapses remains unclear [1]. However, the effects of classic Hebbian plasticity protocols have not yet been systematically observed within the context of homeostatic synaptic plasticity induction. In the present study, we adopt a computational approach to explore the effects of classic LTP/LTD protocols in a recurrent neural network with leaky integrate-and-fire point neurons, which follows a structural plasticity rule based on firing-rate homeostasis [2]. The network is inhibition-dominated with sparse connectivity between the nodes. The stability of neurons in this network is defined by a set-point of intracellular calcium concentration, reflecting its firing rate. Changes to the level of activity imposed by external input are compensated by counteracting changes in structural connectivity, thereby bringing the neuron back to its set-point of activity. Here we apply classic Hebbian plasticity protocols to a subset of excitatory neurons, using trains of very short but strong DC pulses (e.g., 100 pulses at 100 Hz; 900 pulses at 1 Hz), and record the resulting changes in firing rate and network connectivity. Results previously obtained with a small network (500 neurons) suggest reliable network remodelling following these protocols, similar to what was previously observed in a model of transcranial DC stimulation (tDCS) protocols [3]. Interestingly, we noted differential effects of structural plasticity among stimulated and unstimulated neurons, and between these groups. Extending the simulations to large-scale networks that more closely resemble the cortical network also shows network remodelling following stimulation. However, we observe that the magnitude and direction of remodelling among and between neuron groups is influenced by the size of the subset of neurons that we stimulate, drawing parallels with the role of focality of stimulation in brain stimulation protocols. Our simulations may thus provide a perspective in developing novel plasticity protocols for (non-)invasive brain stimulation, such as repetitive transcranial magnetic stimulation, in clinical practice that are optimised for the induction of homeostatic structural plasticity.

Abrupt transitions of response patterns emerging from gradual changes of network connectivity

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Recent experimental studies show substantial ongoing remodeling - even in the absence of an explicit learning paradigm - both on the level of synaptic connections and on the level of neuronal population activity. It is, however, unclear, how the changes in neuronal activity can be linked to changes in the underlying synaptic connectivity.
To shed light on these phenomena we study a circuit model of randomly connected excitatory and inhibitory neurons.
For strong recurrent connectivity and a high ratio of inhibition to excitation, the model reproduces key characteristics of neuronal activity patterns of mouse auditory cortex (Bathellier et al., 2012), including sparse activity, a broad distribution of firing rates, and a clustering of stimuli into a set of response modes. In this regime we find that gradual changes of synaptic strength can result in periods of stable responses which are interrupted by abrupt transitions towards new response patterns. To understand the mechanism underlying these transitions, we analyze the fixed points of this network model, employing a method similar to Sussilo and Barak, 2013. The regime, where we find this clustering into response modes, is characterized by multiple fixed points per stimulus, i.e. one stimulus can evoke different responses depending on the initial conditions of the system. Analyzing how the fixed points of a network change during ongoing, random synaptic drift reveals that abrupt transitions of response patterns coincide with topological changes in the structure of fixed points and not just a rerouting of response trajectories due to the displacement of unstable fixed points.
We conclude, that even slow ongoing synaptic drift can lead to abrupt transitions in stimulus responses, which can be understood by monitoring the fixed point structure of the underlying network.

Bathellier et al., Neuron 2012; 76, 435-449
Sussilo and Barak, Neural Computation 2013; 25, 626–649

Physiologically-inspired neural model for social interactions recognition from abstract and naturalistic stimuli.

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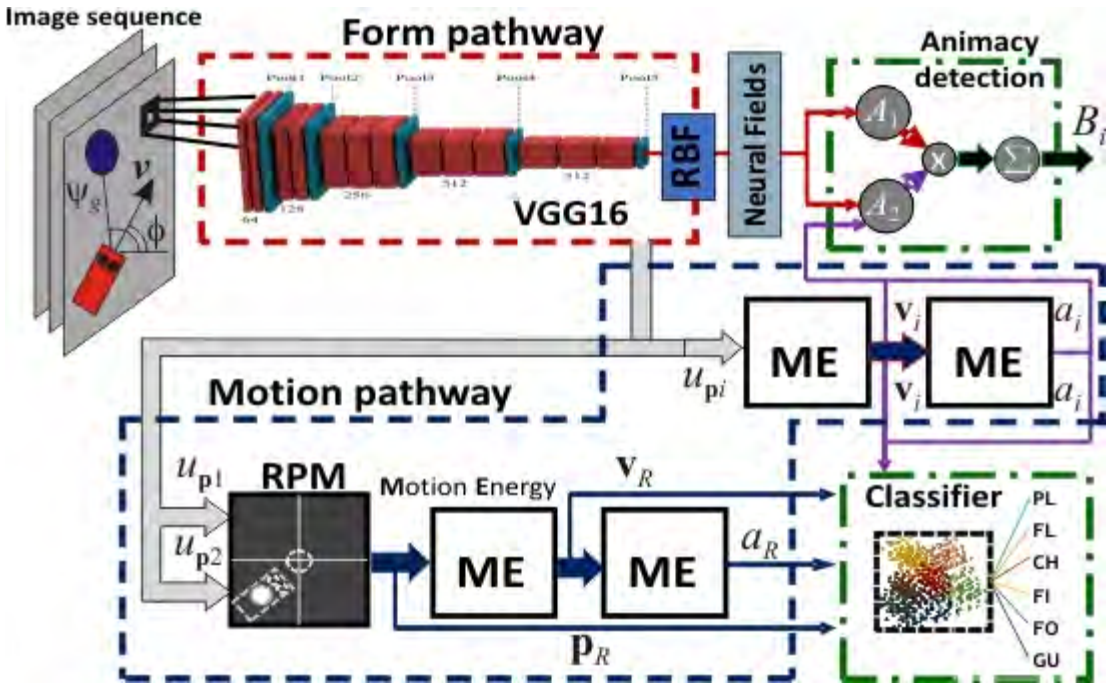
INTRODUCTION: Humans can perceive social interactions from natural as well as from schematic stimuli, as shown by the classical experiments by Heider and Simmel (1944). The neural circuits underlying this visual function remain completely unknown, and it has been suggested that the recognition of such stimuli is based on high level probabilistic inference. We present a simple neural model that is consistent with the basic facts known about neurons in the visual pathway that recognizes social interaction from naturalistic as well as from abstract stimuli. In addition, we present an algorithm for the generation of highly-controlled stimulus classes of naturalistic and abstract social interactions. Such stimuli are critical for electrophysiological experiments that clarify the underlying mechanisms.
METHODS: The model consists of a hierarchical shape-recognition pathway with partial position invariance that is modeled using a deep neural network (VGG16), followed by an estimation of the relative instantaneous positions and orientations of moving agents, which are then robustly tracked and encoded by a population code in a Dynamic Neural Field, see Fig.1. The relative positions, velocities and accelerations of moving agents are computed in a top level module, employing gain-field mechanism which is followed by the classifier of the interactive behaviors. The stimulus synthesis algorithm is derived from dynamic models of human navigation (Fajen & Warren, 2003, Warren, 2006) which are combined with methods for computer animation of quadrupedal animals.
RESULTS: The model successfully reproduces results of Tremoulet and Feldman (2000) on the dependence of perceived animacy of moving agents on their motion parameters and the body axis. Classifying abstract stimuli consisting of moving geometrical figures generated by the algorithm, we found highly reliable classification of 12 action categories. The model reproduces this classification and in addition can recognize interactions from real movies showing interacting animals. The most distinctive three behavioral classes scored better than 71% in terms of the true positive rate. A score for the different classifiers for 6 types of interactions is presented in Table 1. The model makes predictions about the behavior of a variety of different neuron classes, which can guide the analysis in ongoing physiological experiments. CONCLUSION: Simple neural circuits combined with learning are sufficient to account for simple forms of social interaction perception in real and artificial stimuli.
Acknowledgements: ERC 2019-SyG-RELEVANCE-856495, HFSP RGP0036/2016, BMBF FKZ 01GQ1704, NVIDIA Corporation.

Classifier	Accuracy
Linear SVM	99.0%
Gaussian kernel SVM	96.3%
LDA	94.7%
KNN	94.7%
Nonlinear LDA	94.3%
Neural Network	94.0%



Dissecting dynamic gain reveals differential contributions of subthreshold impedance and spike generation dynamics

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The dynamic gain function (DGF) quantifies the input-output-function of neurons driven by background fluctuations to an in-vivo-like operating point. Recent studies suggest that a wide bandwidth of the DGF, which reflects precise spike-timing, has functional importance, might be under evolutionary pressure (Lazarov et al. 2018) and may even be related to cognitive performance. The DGF shape is influenced by the nano-physiology of the axon initial segment, dendrite size (Eyal et al. 2014) and axonal resistance (Brette et al. 2013). Relative contributions of these factors are difficult to disentangle, as DGF entails signal transformations from input current to membrane voltage to firing probability.

Here, we decompose the DGF into its spike-generator component and a subthreshold impedance in neurons with various morphologies and firing patterns at different developmental stages and with perturbed axonal ion channel density. We find that the transformation from input current to membrane voltage is shaped by neuronal morphology and insensitive to correlations in the background fluctuations. Transformation from somatic voltage to firing probability has strong high-pass characteristics with a sharp cut-off, whose position is relatively insensitive to morphology, but affected by axonal perturbations. DGFs of all neurons showed improved encoding of high frequencies when background fluctuations was dominated by low frequencies. This ‘Brunel-effect’ (Brunel et al. 2001) manifests mainly in the supra-threshold spike-generation gain. The subthreshold impedance contributes by attenuating low frequencies.
Our results suggest that the active conductances controlling spike initiation play a crucial role for the DGF bandwidth and boosting of high frequencies.

Neural models for the (cross-species) recognition of dynamic facial expressions

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Dynamic facial expression recognition is an essential skill of primate communication. While the neural mechanisms of the recognition of static pictures of faces has been extensively investigated, the neural circuits of the recognition of dynamic facial expressions remain largely unclear. We studied possible neural encoding mechanisms by neural modelling, exploiting highly controlled and realistic stimulus sets that were generated by computer graphics, and which are also used in electrophysiological experiments.

METHODS: Combining previous physiologically plausible neural models for the recognition of dynamic bodies (Giese & Poggio, 2003) and of static faces (Giese & Leopold, 2005) with architectures from computer vision (Simonyan, 2014), we devised two models for the recognition of dynamic facial expressions. The first model is example-based and encodes dynamic faces as temporal sequences of snapshots, which are recognized by a sequence-selective recurrent neural network. The second architecture is based on a norm-referenced encoding mechanism. Individual face pictures are neutrally encoded by face-space neurons that are tuned to the differences between the actual stimulus frame and a reference face showing a neutral facial expression. Dynamic expressions can be recognized by a simple circuit that differentiates the responses of these face-space neurons. Both models were tested using movies of highly realistic human and monkey face avatars that were animated using motion capture data from humans and monkeys. Expression strength and style was precisely controlled using motion morphing techniques (Taubert et al. 2020).

RESULTS: Both models recognize reliably dynamic facial expressions of humans and monkeys from movies. They make quite different predictions for the behaviour of face-selective neurons, especially for stimuli that interpolate between different expressions. The norm-referenced model shows a highly gradual, almost linear dependence of the neuron activity with the expressivity of the stimuli, which is not the case for the example-based model. In addition, we also explored in how far the models account for the experimental observation (Taubert et al. 2020) that humans recognize human expressions on monkey faces spontaneously without any prior training on such stimuli.

CONCLUSIONS: Both models are physiologically plausible and accomplish the recognition of dynamic faces from movies. The models make very different predictions about the behaviour of face-tuned single cells. Norm-referenced encoding might support the generalization of expressions across different basic face shapes, and even across faces from different species.

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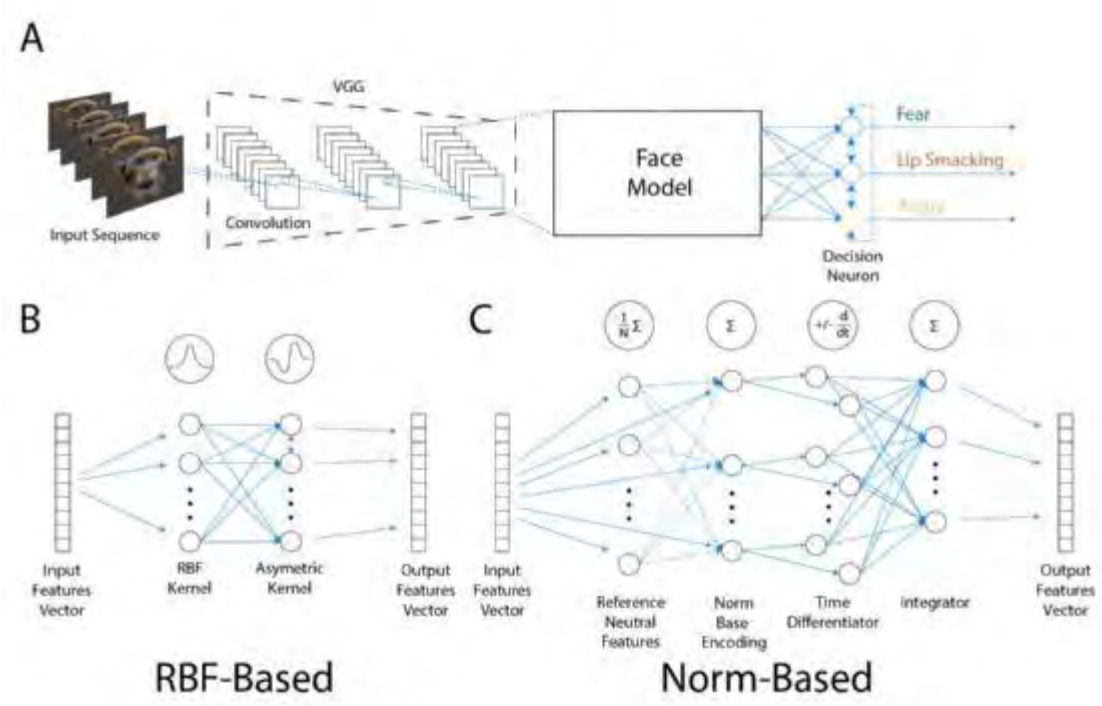

Genetic Basis of Phase Amplitude Coupling Entrained by Working Memory

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The coupling between low and high frequency oscillatory activities is critical for cognitive functions, such as working memory (WM) and is linked to genetically-caused disorders (e.g. Schizophrenia). The inheritable factors of this coupling however are not known.
We analyzed MEG data from the human connectome project dataset (HCP 1200 Release), recorded from 82 subjects while performing a WM (zero-back or two-back) task. We focused on data from twins with different levels of genetic similarity (monozygotic [MZ] & dizygotic [DZ]), and unrelated subject pairs (UR). The blank period where the subjects maintained a previously presented picture (of either a tool or a face) in memory was analyzed. We observed a peak in the power spectral density between 6-10 Hz, which we considered as the phase-providing frequency. To find out which higher frequencies are driven by the phase of this Alpha band, we extracted 6-10Hz oscillations within the 100 ms interval before and after the peak of Gamma envelope during the blank period for each trial and averaged across trials for each recording site. The resultant signal, named the Gamma triggered Alpha (GtA), was calculated for 9 different Gamma bands from 40 to 190Hz (with 30Hz band width and an overlap of 15Hz). The resulting GtA was averaged for each electrode across subjects, the peak-to-peak magnitude difference of which was used as a measure of across-subject PAC similarity. To assess the statistical significance of this similarity, we randomly shifted the GtA's circularly for each subject 10,000 times. In each repetition, we calculated the average GtA across subjects and computed the z-score of the original peak-to-peak relative to the random distribution. This PAC similarity's spatial topography was observed to be similar across gamma bands ($R=0.36$, Pearson; $p<0.01$ for all frequency pairs). We next calculated the correlation between GtA's of the MZ pairs (n=15) and found it significantly stronger than the correlation in UR pairs (z-score>2, permutation test; n=10,000) in 5 of the 9 bands. To investigate if the stronger PAC similarity is due to genetic similarity (and not age, gender, …), we further analyzed same-sex DZ pairs. Mean correlation of the DZ twins (n=9) was compared to all possible selections of 9 out of 15 MZ pairs (5005). Results revealed that DZ twins had a smaller correlation compared to MZ twins, in all of the 5 MZ-selective bands (z-score<-2). To check if this PAC similarity is dependent on image and trial type or not, we repeated the previous steps separately on the following 4 groups: zero-back face, two-back face, zero-back tool and two-back tool trials, and compared MZ twins and UN pairs separately in each group. The difference was significant (z-score>2) in all trial types except for two-back face.
Here, we observed that MZ twins with an almost 100% equivalent DNA show a stronger PAC similarity compared to both DZ twins and UR pairs. This suggests that PAC has underlying genetic mechanisms, especially in the context of its role in WM. Furthermore, comparing trial types show that this effect is particularly evident when lower-level (and less developmentally relevant, as for zero-back trials and tool stimuli) brain functions are involved. Our results are a first step in discovering the genetic factors determining the network dynamics of brain underlying high-level cognitive functions.

Biophysically grounded mean-field models of neural populations under electrical stimulation

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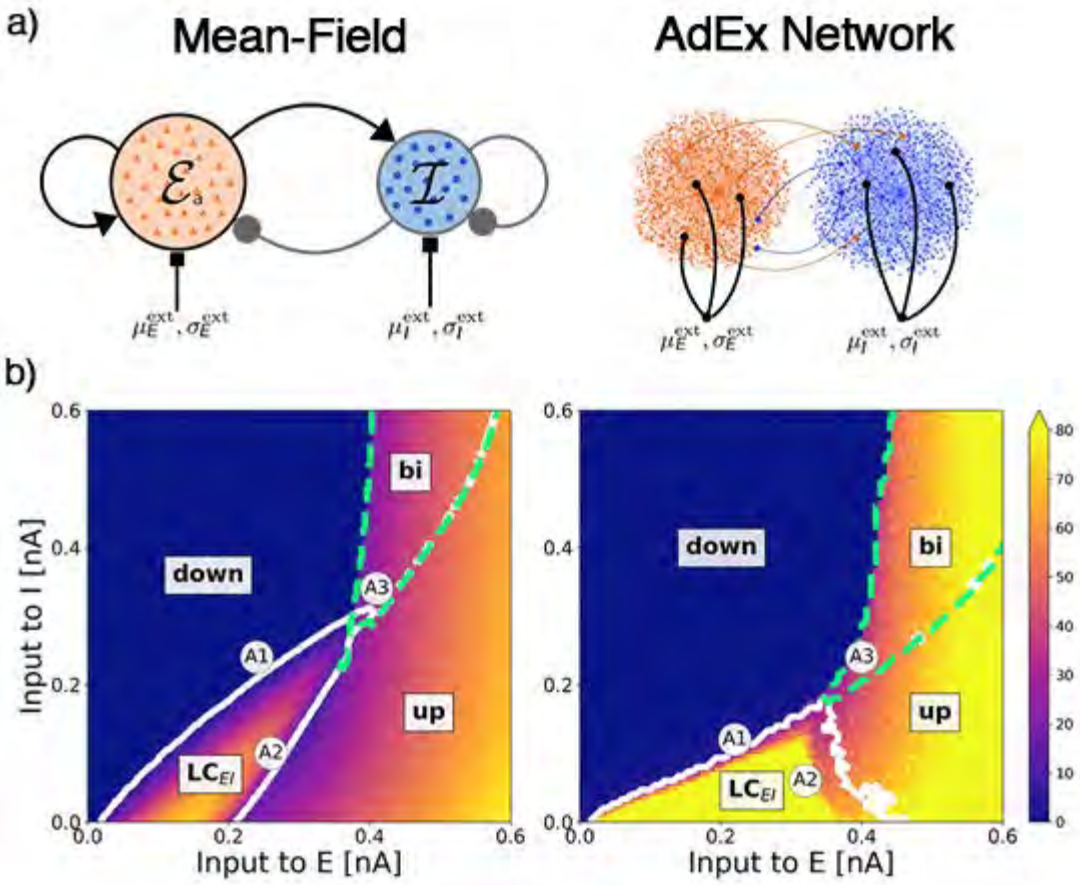
Electrical stimulation of neural systems is a key tool for understanding neural dynamics and ultimately for developing clinical treatments. Many applications of electrical stimulation affect large populations of neurons. However, computational models of large networks of spiking neurons are inherently hard to study and an understanding of how neural populations interact with electrical inputs is lacking.

We present a reduced mean-field model of excitatory and inhibitory adaptive exponential integrate-and-fire (AdEx) neurons (Fig. 1 a) which can be used to efficiently study the effects of electrical stimulation on large neural populations. The rich dynamical properties of this basic cortical model are described in detail and validated using large network simulations. Bifurcation diagrams provide a map of the dynamical landscape. All attractors are retained in the mean-field approximation (Fig. 1 b).

The cortical model occupies several dynamical states with asynchronous *up*- and *down-states*, a bistable regime in which both states coexists, an oscillatory region corresponding to the fast excitation-inhibition loop. When adaptation is turned on, a bifurcation takes place and the bistable region is replaced by a new slow oscillation region due to the interaction of excitation and adaptation feedback. The biophysical parameters of the AdEx neuron can be coupled to an electric field with realistic field strengths which then can be propagated up to the population description.

Our predictions confirm results previously reported in stimulation experiments. Direct external fields can cause attractor switching such as turning on and off oscillations with a field strength of 8-12 V/m. Oscillatory fields can frequency-entrain the population activity at around 1.5-2.5 V/m. Phase-locking of endogenous oscillations can be observed for even weaker fields of around 0.5 V/m. These field strengths have only a very weak effect on a single neuron, indicating that field effects are strongly amplified in the network. The effects of time-varying external stimulation are also well-predicted by the mean-field model.

In summary, our results show that 1) weak fields are able to affect neural population activity 2) the response to stimulation critically depends on the state of the neural system and 3) mean-field models offer an efficient and appropriate framework for investigating the effects of electric fields on a cortical system.


a)
Mean-Field
AdEx Network
$\mathcal{E}_a$
$\mathcal{I}$
$\mu_E^{ext}, \sigma_E^{ext}$
$\mu_I^{ext}, \sigma_I^{ext}$
$\mu_E^{ext}, \sigma_E^{ext}$
$\mu_I^{ext}, \sigma_I^{ext}$
b)
down
bi
up
A1
A2
A3
LC_{EI}
Input to I [nA]
Input to E [nA]
0.0
0.2
0.4
0.6
down
bi
up
A1
A2
A3
LC_{EI}
Input to E [nA]
0
10
20
30
40
50
60
70
80

Modulation of Fronto-Striatal Connectivity by using intermittant Theta Burst Stimulation (iTBS). A 18 Flourine-Desmethoxy Fallypride (DMFP) Positron Emission Tomography (PET) study.

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Background : Fronto-Striatal networks are neural pathways providing connections between frontal lobe regions and the basal ganglia (striatum) that are involved in motor, cognitive, and behavioral processes. It has been shown that Transcranial Magnetic Stimulation (TMS) can modulate connectivity in the human brain. Transcranial Magnetic Stimulation (TMS) with long stimulation protocols to the Pre Frontal Cortex (PFC) has been widely used for clinical purposes such as depression treatments. The combined measurement of PET and TMS technique would help to understand better the dopaminergic activity in the fronto-striatal area.

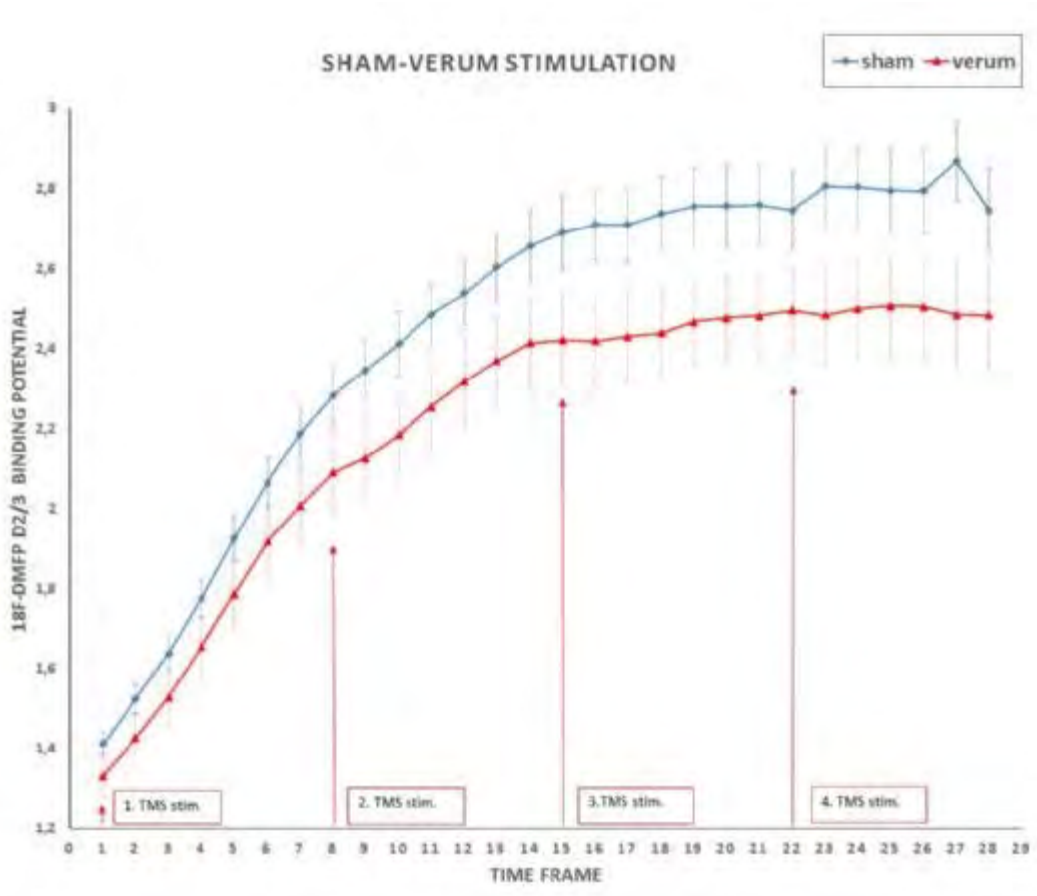
Objective: The aim of the study is to investigate the fronto-striatal connectivity by measuring the release of dopamine in the striatum in response to an excitatory intermittent theta burst stimulation (iTBS) of the Left-DLPFC. A PET measurement was performed by using the 18F-DesmethoxyFallypride(DMFP) radioligand, that is a high affinity receptor-antagonist which competes with endogenous Dopamine neurotransmitters for D2/D3 receptor binding.

Methods:The study was conducted on 23 healthy participants, who underwent iTBS sham (control) and verum (active) stimulations on separate days. The PET scan lasted 120 mins, consisting of 4 iTBS stimulations delivered to the left-DLPFC at 30 mins interval. In verum stimulation, 90% of resting motor threshold (rMT) was used as the stimulation intensity. The excitatory iTBS protocol was delivered in a sequence of trains (burst of 10 pulses in 2 secs having inter train interval of 8 secs), with a total duration of 190 secs. PET dynamic data was analyzed using reference methods in which cerebellum was used as the reference region (due to lack of D2-D3 receptors). Binding Potentials (BP) were used as a measure of concentration of receptors. Mean Binding Potentials in the sub-regions of the striatum (Nucleus Caudate and Putamen) were compared between the sham and verum stimulation using analysis of variance (ANOVA).

Results: iTBS stimulation of the left DLPFC increased the dopamine release in the striatum areas (putamen and nucleus caudate) in the verum stimulation, as compared to sham stimulation (see figure 1). The repeated blocks of iTBS increased connectivity after each stimulation.

Conclusion: Results suggest that the iTBS protocol can effectively enhance the fronto-striatal connectivity. This stimulation protocol can be utilized as a therapeutic therapy for treating major Depression patients by deploying it in the repeated blocks of short intervals. This scheme could prevent the longer stimulation protocols, and be less painful.

figure 1: Mean Binding Potentials at sham and verum TMSs across 28 time frames. We observed lower Binding Potential in the verum stimulation compared to sham stimulation.



Dimensionality of neural circuit manifolds associated with a salt-and-pepper organization of cortical stimulus preferences

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The cerebral cortex is a major learning center in the mammalian brain. Its principal neurons are embedded in dense recurrent networks. Specifically in rodent sensory cortex, cortical circuits include a dense blanket of inhibition (Bopp et al. 2014). If cortical principal cells acquire their stimulus preferences by selecting afferent connections through Hebbian mechanisms then strong feedback inhibition can force neurons to adopt maximally dissimilar selectivities (Rubner & Schulten 1990). Here we introduce and examine a set of toy models to investigate manifolds of stable network configurations in inhibition dominated circuits.

Learning, optimization, or self-organization mechanisms usually do not determine one unique state to which any circuit configuration converges. Rather one expects that there are multiple stable admissible network configurations. For the form vision core circuit of primate/carnivore V1 prior work indicates that stable network configurations form toroid high-dimensional continua (Wolf 2005, Kaschube et al. 2010). The dimensionality of these manifolds is determined by the spatial range of long-range horizontal connections. Similar results for networks that form a salt-and-pepper organization are currently not available. Here we utilize techniques from the study of spin liquid states in solid-state physics (Chalker 2015) to construct mathematically tractable models with salt-and-pepper optimal states.

We demonstrate that these models can exhibit ground state manifolds with extensive dimensionality. This result is consistent with the general expectation that there are a very high number of functionally equivalent salt-and-pepper configurations. Disordered salt-and-pepper states result even for completely dense and uniform connectivity patterns and do not require any structural source of disorder. We also assess how the range connectivity and local uniformity of connectivity within each anatomical cortical column impact on the circuit manifold.

These studies expand the mathematical toolbox for analyzing the multiplicity of stable cortical circuit configurations. Our first results suggest that the evolutionary transition from a rodent-type ancestral circuit configuration of V1 to a primate/carnivore-type V1 architecture was accompanied by a reduction in cortical circuit state dimensionality.

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Interneuron Inhibition Stabilizes Pyramidal Neurons against Cortical Spreading Depression

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Migraine is neurological disorder that affects an estimated 1 billion people worldwide, yet its root cause remains a mystery. There is evidence that migraine is preceded, and perhaps initiated, by a slowly traveling wave of inactivation in cortical pyramidal neurons referred to as cortical spreading depression (CSD). However, the mechanisms that cause CSD are unknown. A small subset of migraineurs possess a known genetic mutation for familial hemiplegic migraine (FHM3) that predisposes them to the disease due to a mutation of sodium channels that are prevalent in cortical interneurons. This mutation leads to extremely high firing frequencies and an accumulation of extracellular potassium that results in a spike block in the pyramidal neurons.

Through computational modeling of an interneuron-pyramidal neuron feedback circuit, we present evidence for a new mechanism of CSD initiation in FHM3, as indicated by spike block in the pyramidal neuron. Increased extracellular potassium levels have been shown to be important in the initiation of spike block, but the influence of ionic concentrations on the interneuron itself has not been explored. We show that the interneuron can play a crucial role in initiation of the pyramidal neuron's spike block and that when potassium feedback to the interneuron is included, spike block in the pyramidal neuron is delayed or prevented. This indicates that while high levels of extracellular potassium make the system more prone to spike block, this effect is moderated by the interneuron and helps to stabilize the circuit against spike block. Moreover, when spike block occurs in the pyramidal neuron, the loss of inhibition from the interneuron facilitates this spike block. In all of our simulations in which the pyramidal neuron exhibits spike block, it was preceded by spike block in the interneuron. This feature is robust and can be controlled in the simulation by either sending the interneuron into spike block prematurely or preventing it from exhibiting spike block at all. In the first case, spike block in the pyramidal neuron occurs prematurely and the time of the pyramidal neuron spike block is linearly correlated with the time of the interneuron spike block. In the latter case, the time of spike block in the pyramidal neuron is delayed. These results show that the interneuron's inhibitory properties serve to maintain the cortical circuit's stability and when this inhibition is lost, spike block in the pyramidal neuron occurs.

Poster Topic

T27: Techniques and Demonstrations

T27-11 Application of a novel potassium channel-based optogenetic silencer *in vivo*
Piret Kleis, Enya Paschen, Ute Häussler, Yinth Andrea Bernal Sierra, Peter Hegemann, Carola A. Haas

T27-12 White matter stroke as a model for post stroke spasticity
Frederique Wieters, Matthias Gruhn, Ansgar Büschges, Gereon R Fink, Markus Aswendt

T27-13 Novel method to produce a layered 3D scaffold for human pluripotent stem cell-derived neuronal cells
Laura Elina Honkamäki, Tiina Joki, Nikita Grigoryev, Kalle Levon, Laura Ylä-Outinen, Susanna Narkilahti

T27-14 A simple, fast and easy to use correlation-based multi-channel spike sorter
Markus Chi-Haw Chou, Bagas Danu Wijaya Marwan, Pavel Larionov, Thomas Schanze

T27-15 Home-enclosure based behavioural and wireless neural recording setup for unrestrained rhesus macaques
Laura Hansmeyer, Pinar Yurt, Naubahar Agha, Attila Trunk, Ralf Brockhausen, Michael Berger, Antonino Calapai, Stefan Treue, Alexander Gail

T27-16 Unravelling the synaptic interactome: Advances in synaptosomal cross-linking mass spectrometry
Cong Wang, Miguel A Gonzalez-Lozano, Frank Koopmans, Patrick F Sullivan, Jonas Protze, Gerd Krause, Matthijs Verhage, Ka Wan Li, August B Smit, Fan Liu

T27-17 The Cone Method: Inferring Target Selection Times from Single-Trial 3D Movement Trajectories in Choice Behavior
Philipp Ulbrich, Alexander Gail

Efficient research data management for reproducible research, collaboration and data publication

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Research data management tasks, including organizing reproducible data workflows in the lab, keeping relevant data and metadata accessible, or exchanging data for collaboration or publication, are becoming increasingly demanding. To reduce the workload associated with data management, we present tools and services for research that are designed to seamlessly integrate with and support the scientist's lab data workflows. Specifically, these tools address key components of efficient data management: Metadata collection, data organization and storage, as well as sharing and publication.

To facilitate the collection of metadata, the lightweight odML[1] format provides a flexible solution to store any kind of metadata, enabling automated metadata collection and analytics[2] as well as conversion to other formats such as RDF for utilizing semantic web technologies.

To organize and integrate data and metadata, the NIX[3] data format offers a unified way of storing and linking data, metadata, and analysis results, which enables keeping all relevant data of a study in a coherent representation. It supports a wide range of data types, including electrophysiology and imaging data. NIX uses the odML metadata format and is integrated with the Neo[4] Python package for electrophysiology, enabling Neo users to store their data in a common open format.

To manage data and workflows, the GIN[5] services track changes and provide secure access, making it convenient to work from multiple workplaces while keeping all data available and in sync. Data can be managed from web and file browsers or the command line, enabling integration into data acquisition or analysis procedures. GIN works with any kind of directory structure and file types, it uses established versioning technology[6,7] to keep previous versions available when datasets are updated. This makes it straightforward to share data within a lab or with off-site collaborators and to work on it together. GIN can be deployed locally in the lab and its microservice architecture makes it easy to rapidly develop and deploy services to meet new data management requirements. G-Node's public GIN services include data format validation[8] and provide persistent identifiers (DOIs) for dataset publication [9].

The tools presented are easy to use, interoperate with other tools supporting reproducibility and data sharing[10,11,12], and enable efficient data management in line with the FAIR principles[13].

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Quantitative Magnetic Resonance Imaging for Segmentation and White Matter Extraction of the Hypothalamus

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The hypothalamus is a small diencephalic brain structure consisting of approximately 15 distinct nuclei. These are, to a large extent, functionally separable and involved in a variety of metabolic, endocrine, and psychiatric diseases. Hence, it has become increasingly important to distinguish different hypothalamic regions in neuroimaging research to better characterize disease-induced tissue damages and abnormalities. In the last years, segmentation of the hypothalamus was mostly based on T1- and T2*-weighted magnetic resonance images (MRI) using anatomical landmarks identified in histological examinations. However, image contrast of conventional anatomical MRI lacks morphological detail, resulting in inaccurate segmentations and inter-rater bias. Additionally, the hypothalamus’ position lateral to the third ventricle and close proximity to white matter tracts including the fornix and mammillothalamic tract display one of the remaining shortcomings of hypothalamic segmentation, as reliable exclusion of white matter is not yet possible.

Recent studies found that quantitative magnetic resonance imaging (qMRI), a method to compute standardized tissue contents including myelin, iron, and water, improved segmentation of cortical and subcortical brain regions. So far, this has not been tested for the hypothalamus. Therefore, in this study, we investigated the usability of qMRI and diffusion MRI for the purpose of detailed manual segmentation and automated data-driven parcellation of the hypothalamus and compared our results to recent state-of-the-art segmentation techniques.

Our results show that qMRI and diffusion parameters indeed differ between hypothalamic subunits, and that qMRI is helpful for hypothalamic segmentation especially with respect to enhanced tissue contrast in lateral hypothalamic regions and grey-white boundaries. In addition, we were first to reliably exclude white matter from hypothalamic tissue including the fornix and mammillothalamic tract using an automated clustering procedure as compared to other recent approaches. We propose that qMRI can improve hypothalamic segmentation and parcellation and possibly enhance inter-study comparability and interpretability with particular focus on the fornix and other surrounding white matter that, to date, display one of the biggest weaknesses in detailed hypothalamic analyses.

Deciphering spatio-temporal fluorescence changes using multi-threshold event detection (MTED)

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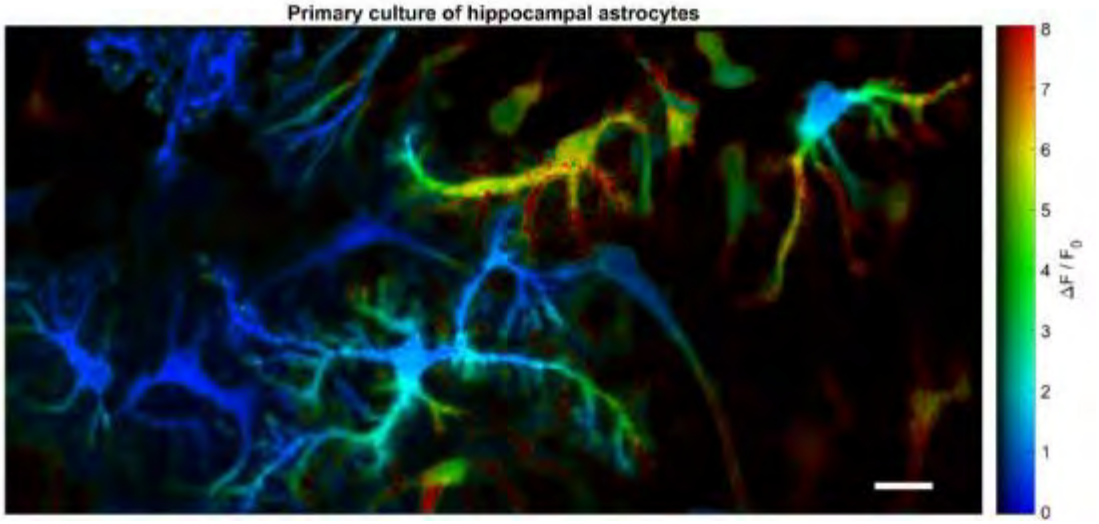
Recent achievements in indicator optimization and imaging techniques promote the exploration of Ca^{2+} activity patterns. Astrocytes are important regulators of the brain network and well known for their highly complex morphology and spontaneous Ca^{2+} activity (Fig. 1). However, the astrocyte community is lacking standardized methods to analyze and interpret Ca^{2+} activity recordings, hindering global comparisons. Here, we present MTED (Multi-Threshold Event Detection), a biophysically based concept to analyze astrocytic Ca^{2+} activity, which includes multiple thresholds and allows a differentiated and in-depth characterization of Ca^{2+} signal complexity. We analyzed various ex vivo and in vivo imaging datasets and verify the validity of our algorithm across Ca^{2+} indicators, imaging setups, and model systems from primary cell culture to awake, head-fixed mice. We found the Ca^{2+} activity patterns to be temperature-dependent across models and defined the subset of Ca^{2+} events shaped by neuronal impact. Applying our concept enables standardized analysis and advances astrocyte research for decrypting brain function.

Fig1: Primary culture of hippocampal astrocytes expressing the Ca^{2+} indicator GCaMP6s. Shown is the maximum projection of endogenous Ca^{2+} activity of a 10 min recording, visualized as $\Delta F/F_0$.



Passive Current Subtraction: A new method for online compensation of voltage clamp artefacts.

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Voltage clamp experiments are commonly used to assess properties of ionic currents. In order to properly analyze voltage clamp recordings, artefacts have to be removed analytically. These artefacts are composed of several passive currents that are caused by imperfections in the experimental setup like an incomplete seal of the patch clamp and capacitive currents of the cell as well as the recording pipette. The usual approach to remove these passive currents is P/N leak subtraction. The same stimulus but with a decreased amplitude is applied to the cell repeatedly, usually four times, in such a way that the voltage dependent ionic currents of the cell do not activate. The resulting currents are subtracted from the original trace and only the actual ionic currents remain. The P/N leak subtraction protocol prolongs the recording time five-fold, which is a severe disadvantage for long protocols (e.g. recovery from inactivation). In addition, errors between membrane and command voltage caused by ionic currents of the cell and scale with their amplitude can only roughly be quantified and set a limit to the maximum currents the experimenter is willing to tolerate. This leads to a trade-off between higher signal-to-noise ratios and smaller voltage errors.

In this work we developed a short stimulus (250ms) consisting of a few voltage steps followed by a chirp stimulus. This stimulus is used to parameterize the passive properties of cell and pipette. From these parameters we can construct the passive currents from any voltage clamp protocol and subtract them from the measured voltage clamp currents (PCS: Passive Current Subtraction), which only leaves the ionic currents. As for the P/N subtraction this returns the ionic currents of interest, but with a significantly reduced temporal overhead, because the duration of the PCS stimulus is independent of the duration of the voltage clamp protocol.

Based on the parameters obtained we can also make an estimate of the membrane potential from the measured pipette potential. We intend to fit conductance based models directly to the compensated ionic currents and the estimated membrane potential. This way we take the voltage error into account and therefore will be able to maximize the signal-to-noise ratio and employ cells with strong ionic currents.

The Passive Current Subtraction is a potent method that significantly cuts down measurement time, improves analytical accuracy and potentially allows to optimize experimental procedures for stronger ionic currents with higher signal-to-noise ratios.

Advanced optogenes for optogenetic stimulation

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The biophysical characterization of microbial rhodopsins, which have been recently identified by metagenomic approaches, has led to the discovery of variants that are potentially useful for optogenetic applications. Xenorhodopsins (XeR´s) allow the cation-gradient independent optogenetic activation of excitable cells[1], due to their inwardly-directed proton pumping activity[1,2], which is a unique feature among light-driven ion pumps. We show that the L-type calcium channel Cav1.3 can be conveniently activated by *Nanosalina* XeR, thereby demonstrating a minimal invasive optogenetic stimulation approach, which precludes additional calcium concentration changes due to the optogenetic stimulus. Further examples for variants with advanced properties are Chrimson and Chronos[3]. Chronos enables neural photostimulation of fast-spiking neurons with high temporal fidelity due to its fast channel-closing kinetics. Chrimson variants have the most red-shifted action spectra to date and therefore bear a much reduced risk of phototoxicity. We demonstrated that mutations in helix 6, a segment of channelrhodopsin, which is apart from the ion conducting pathway and which moves during the open to closed transition, are accelerating channel closing[4]. The fast Chrimson mutants are of special interest as they enable optogenetic stimulation with red light pulses at high stimulation rates. We recently generated a fast Chronos mutant (f-Chronos) by helix F modification, which is to our knowledge the fastest channelrhodopsin variant to date. The biophysical properties of f-Chronos corroborate our hypothesis that helix 6 modification can accelerate protonation reactions, which govern the channel open time. The ultrafast closing kinetics of f-Chronos will likely expand the frequency range of high fidelity neuronal photostimulation to the kHz range, which is of interest for remote control of fast spiking neuronal subtypes such as the spiral ganglion neurons of the auditory system.

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3 Klapoetke, N. C. et al. Independent optical excitation of distinct neural populations. Nat Methods 11, 338-346, doi:10.1038/nmeth.2836 (2014).
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Capturing detailed provenance information in the analysis of electrophysiology data

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The analysis of electrophysiology data typically comprises multiple steps. These often consist of several scripts executed in a specific sequence that take different parameter sets and use distinct data files. As the researcher adjusts the individual analysis steps to accommodate new hypotheses or additional data, the resulting workflows may become increasingly complex, and undergo frequent changes. Therefore, robust tools forming the workflows are necessary to fully document the workflow and improve the reproducibility of the results.

Provenance refers to the characterization of data manipulations and corresponding parameters throughout the analysis.[1] It is possible to use workflow management systems to orchestrate the execution of the scripts and capture provenance information at the level of the script (i.e., which script file was executed, and in which environment?) and data file (i.e., which input and output files were supplied to that script). However, the resulting provenance track does not automatically provide details about the actual analysis carried out inside each script. Thus, analysis results can only be understood by source code inspection or trust in the correctness of any accompanying documentation. Here, we aim to improve existing tools by implementing a data model that captures detailed provenance information and by accurately representing the analysis results in a systematic and formalized manner.

We focus on two open-source tools for the analysis of electrophysiology data. The Neo (RRID:SCR_000634) framework provides an object model to standardize neural activity data acquired from distinct sources.[2] Elephant (RRID:SCR_003833) is a Python toolbox that provides several functions for the analysis of electrophysiology data.[3] We implemented prototypes of two complementary solutions to extend the functionality of Neo and Elephant to (i) automatically capture provenance information at the function-execution level inside a Python script, and to (ii) support the standardization of the analysis results together with the storage of relevant information describing their generation.

The first solution is a set of data analysis objects that standardize the output of Elephant functions. They encapsulate all relevant parameters used by the function to generate the output, such that they can be easily re-used or shared. The second solution maps function inputs, outputs, and parameters throughout the execution of the Python analysis script, and builds a representation of the relationships between the different steps of the analysis within the script (i.e., the provenance trace). The captured information can be used to build a graph to visualize the steps followed in the script, and that can be stored together with the results as metadata. We compare the results obtained with or without the use of the two solutions on the basis of a

realistic analysis scenario of electrophysiology data, showing the potential benefits for reproducibility, interoperability, discoverability, and re-use of analysis results.

1. Ragan et al. (2016) IEEE Trans Visual Comput Graphics 22:31.
2. Garcia et al. (2014) Front Neuroinform 8:10.
3. http://python-elephant.org

This work is performed as part of the HDS-LEE and receives funding from the Helmholtz Association of German Research Centres, EU Grant 785907 (HBP) and EU Grant 945539 (HBP), and the Helmholtz Association Initiative and Networking Fund (ZT-I-0003). We thank Prof. Stefan Decker for valuable input.

Software for reliable, flexible simultaneous data acquisition from diverse sources and closed-loop intervention protocols

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Acquisition of data from a variety of heterogeneous sources with accurate, aligned timestamps is a requirement for many kinds of *in vivo* experiments. In addition, it is often necessary to manipulate experimental settings based on the animal's state or behavior, and to store acquired data in a standardized format to simplify subsequent analysis. To address these requirements, we developed a new, integrated software solution capable of simultaneous acquisition of data from an arbitrary amount of sources, e.g. multi-channel electrophysiological recordings, conventional video images, high-speed camera data, serial interfaces or Miniscopes. The software guarantees aligned timestamps for all inputs, and makes use of the parallel-processing capabilities of modern CPUs to effectively run many tasks simultaneously. New data sources can be integrated and adjusted for new experimental setups without or with very little programming skills. For more advanced applications, the modular software design facilitates extension with new modules written in either C++ or Python.

All data generated from a given experiment is stored in a well-defined, comprehensive structure, making it easy to compare, pool or share data between experimentalists with different research questions.

With these abilities, our software enables reliable closed-loop experiments for many different (neuro)scientific questions. Tests with different experiment setups in multiple groups show the successful performance and easy-to-learn structure of the program.

On the objectivity, reliability, and validity of deep learning enabled bioimage analyses

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Significant advances in fluorescence imaging techniques enable life scientists to gain insights into biological systems at an unprecedented scale. The interpretation of image features in such bioimage datasets and their subsequent quantitative analysis is referred to as bioimage analysis. A substantial proportion of bioimage analyses is still performed manually by a human expert - a tedious process that is long known to be subjective. Particularly in tasks that require the annotation of image features with a low signal-to-noise ratio, like in fluorescence images of tissue samples, the inter-rater agreement drops. However, like any other scientific analysis, also bioimage analysis must meet the general quality criteria of quantitative research, which are objectivity, reliability, and validity. Thus, the automation of bioimage analysis with computer-aided approaches is highly desirable.

Recently, deep learning (DL) has enabled impressive advances in computer vision research. The predominant difference between DL and conventional algorithms is the capability of DL models to learn the respective task on the base of an annotated training dataset, instead of following user-defined rules for feature extraction. Lately, DL has also been deployed for bioimage analyses. However, in absence of ground truth annotations, DL models need to be trained on manual and thus subjective annotations, which could cause the model to incorporate such a bias. Moreover, model training is stochastic and even training on the same data could result in models with divergent outputs. Both, the training on subjective annotations and the model-to-model variability could impair the objectivity and reliability of DL-based bioimage analyses, yet neither has been evaluated systematically.

This study first assessed the impacts of both limitations experimentally by analyzing the abundance of the activity-dependent transcription factor cFOS after fluorescent labeling in mouse brain sections. Since the abundance of cFOS can be manipulated by mouse behavior, we used behavioral experiments and their analyses to evaluate the bioimage analysis results. Moreover, we show that pooling the input of multiple human experts during model training as well as integration of multiple trained models in a model ensemble can mitigate the impact of these limitations. Overall, this study establishes guidelines for how DL can be used to increase the general quality of bioimage analyses (Segebarth & Griebel et al. 2020).

Segebarth, D., Griebel, M., Stein, N., von Collenberg, C. R., Martin, C., Fiedler, D., ... Flath, C. M. & Blum, R. (2020). On the objectivity, reliability, and validity of deep learning enabled bioimage analyses. Elife, 9, e59780.

A low-cost, open source, fully customizable 5-choice serial reaction time task apparatus for automated behavioral training

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The five-choice serial reaction time (5-CSRT) task is a widely used behavioral paradigm to study visuospatial attention and motor impulsivity in rodents. The task requires animals to make a nose poke into one of five small apertures to get a reward at a remote location. The target aperture is indicated by a light source, and task difficulty can be controlled by task timing and, in principle, by the number of apertures offered to the animal. Chambers equipped with computer-controlled light-gated apertures, light sources, and food trace are available from various manufacturers, and usually come together with software to specify task parameters and analyze behavioral performance. They have, however, the limitations that first, they are expensive and second, they provide only limited freedom for experimental design, due to fixed hard- and software. We here introduce a low-cost and fully customizable alternative based on Arduino-equipment and standard electrotechnical components, open Arduino-scripts for hardware control, and a Matlab-toolbox for behavioral task specifications, including a staircase procedure to allow for automated training. All hardware equipment can be mounted to customized chambers and might be used either as a low-cost alternative for implementing the 5-CSRT standard procedure, or for adapting chamber- and task-design for non-standard approaches. We explain the design of the system and outline the code for control of both the Arduino board and the experiment, and present training data as a proof of principle for reliable functioning of the system.

Image recognition and augmented reality-based technology for data presentation

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Data presentation and scientific exchange are essential parts of scientific work. Knowledge is gained in discourse and thus, successful research needs exchange of data and opinions. Despite substantial progress in technology and the use of digital possibilities in large parts of society and hands-on research, data presentation has not changed much within the last decades. Researchers still present printed and digital posters, papers and research proposals that do not offer possibilities to include multidimensional content, such as videos, 3d objects, audio and interactive content.

The use of interactive digital posters and documents is not popular and utilizing tablets and laptops causes high costs and is sometimes inconvenient. This often limits exchange and presentation of research outcome to two dimensions and, thus, does not fit to requirements of today's research data that often exist as digital, multidimensional content (e.g., time series, 3d structures). When presenting and discussing data this current limitation can cause loss of information and reduced power of persuasion and plausibility. Today, there is no practical easy-to-use solution for this deficiency which fulfils the needs of scientists.

We decided to fill this gap and pushed a challenging side project that provides a new data presentation tool for the scientific community. The tool is called “Augmented Science” and consists of an application (App, available in the App Store for iOS and in the Google Play Store for Android) and a web-based content management platform. It functions by using image recognition, allowing any 2d image to be linked to video content. In an easy-to-use way, researchers can connect any image with video content on the web platform at www.augmentedscience.io. With the freely available App, the assigned digital content can be retrieved and displayed just by pointing the smartphone at the target such as an image depicted on a poster. In addition, if desired by the responsible researcher, it is possible to easily get in contact with the author by integrated email function. The Augmented Science App and Content Management System is free for use for scientists.

In the near future we plan to integrate the possibility to display interactive 3D objects and to provide a handy functionality for context-related scientific exchange and networking.

Application of a novel potassium channel-based optogenetic silencer *in vivo*

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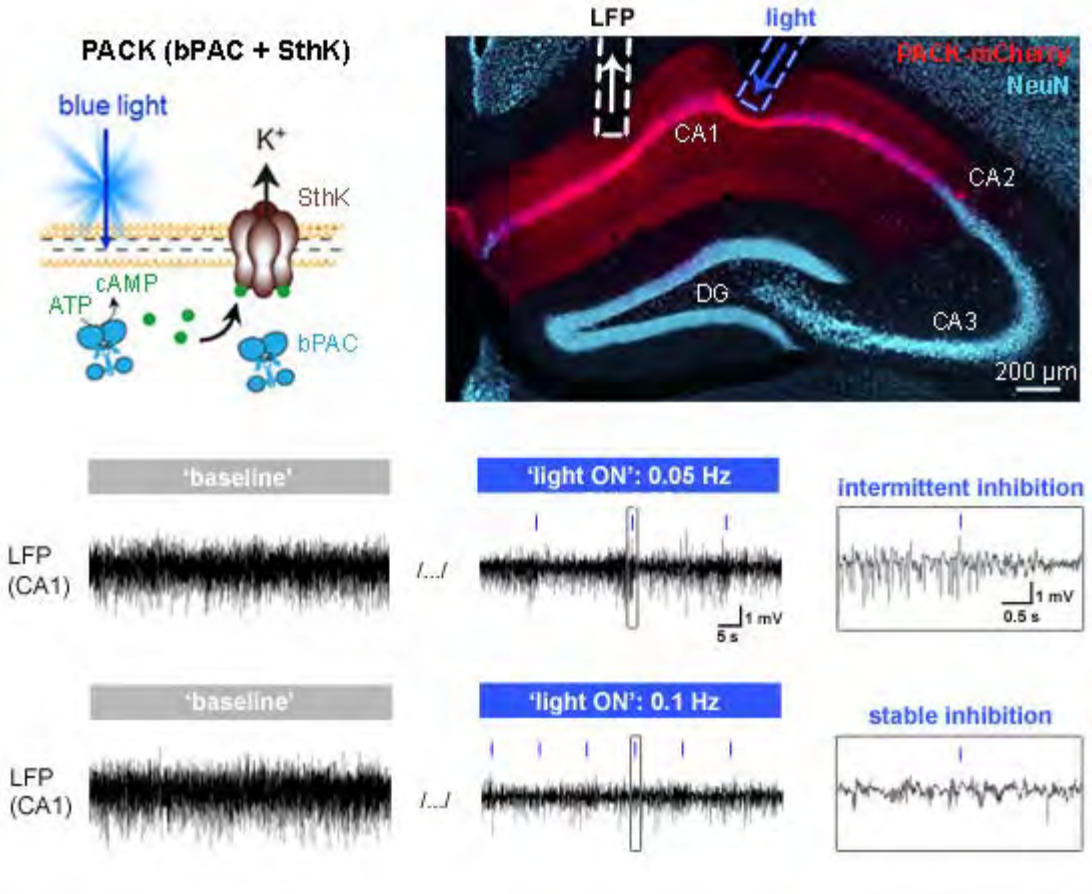
Optogenetic silencing takes advantage of genetically encoded light-sensitive proteins, allowing to switch off the neurons of interest with a high temporal and spatial precision. However, the performance of currently available inhibitory optogenetic tools remains insufficient due to low light sensitivity, unanticipated changes in ion distributions, and strong rebound responses. Recently a new inhibition approach has been developed, which exploits potassium-conductance as the hyperpolarizing factor. The two-component optogenetic tool (PACK), comprising a photoactivated adenylyl cyclase (bPAC) and cAMP-dependent potassium channel (SthK), has been identified as a promising silencer in various mammalian cell cultures and *in vivo* settings (Bernal ***et al.***, Nat. Commun. 2018). However, the performance of PACK has not yet been assessed in chronic experiments with awake rodents.

Here, we aim to validate the inhibitory action of the PACK silencer in hippocampal neurons of freely moving mice. We targeted CA1 pyramidal cells with PACK using a stereotactic injection of AAV9.CaMKII vector and enabled photoinhibition of these cells via an implanted optic fiber. Local field potential (LFP) recordings revealed notably reduced activity in CA1 during a 50-minute illumination phase compared to the baseline. In most mice, 10 ms light pulses (80 mV/mm^2) applied at 0.1 Hz provided a stable inhibition, whereas 0.05 Hz resulted in an intermittent reduction of LFP amplitude. Surprisingly, several side effects were associated with PACK expression, such as chronic neuroinflammation, CA1 pyramidal cell dispersion, and spontaneous generalized seizures. These effects were also present in mice, which were expressing bPAC without the potassium channel. Furthermore, in bPAC-expressing mice, illumination with blue light resulted in elevated LFP amplitude and frequency, as well as increased the occurrence of generalized seizures. Our results suggest that utilizing cAMP signalling as an actuator in an optogenetic tool provides sufficient light sensitivity but is not an optimal approach. A further control group injected with AAV9.CaMKII.mCherry will be included in the study to determine if the vector and reporter gene contribute to the side effects.


PACK (bPAC + SthK)
blue light
K+
SthK
cAMP
ATP
bPAC
LFP
light
PACK-mCherry
NeuN
CA1
CA2
DG
CA3
200 µm
'baseline'
'light ON': 0.05 Hz
intermittent inhibition
LFP
(CA1)
1 mV
5 s
1 mV
0.5 s
'baseline'
'light ON': 0.1 Hz
stable inhibition
LFP
(CA1)

White matter stroke as a model for post stroke spasticity

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Spasticity is one of the most common causes of motor disability worldwide. Besides traumatic brain injury, amyotrophic lateral sclerosis, multiple sclerosis and traumatic brain injury, spasticity can also be caused by stroke. The cause of spasticity is a damage of the upper motor neurons by lesions or inflammatory reactions, which leads to an imbalance of inhibitory and excitatory neural circuits.

Previous stroke models have focused primarily on cortical lesions (photothrombosis) or large-scale lesions (thromboembolism, Middle Cerebral Artery Occlusion (MCAO)), but these do not result in a targeted lesion of the corticospinal tract (CST). In patients, a lesion within the CST leads to an increased incidence of spasticity and may then result in post stroke spasticity (PSS), which is one of the most common causes of physical limitation after stroke. Despite the high incidence, to date there are few effective treatments for PSS and no reliable animal model to replicate this condition. However, a reliable PSS model in mice is imperative to better understand the morphological, electrophysiological, as well as cellular alterations of PSS in order to develop new therapies based on these findings.

For this reason, we have established a PSS model in the mouse, which leads to spasticity by means of a local lesion via a modified photothrombosis within the internal capsule. Using weekly behavioral tests (rotating beam, grid walk, grip strength, cylinder test, neuroscore) and electrophysiology (longitudinal & terminal H-reflex measurement), the animals were analyzed over 56 days after lesion. In addition, histological staining was used to characterize lesion size/localization, astrogliosis, secondary neurodegeneration, and change in myelination.

The animals showed an increased Hoffmann reflex as early as 14 days after induction of local ischemia, which was interpreted as a sign of spasticity. Over the 56-day period, a further increase in the H-reflex was measured by repetitive electrophysiology, which was further confirmed by additional terminal H-reflex measurements at the ulnar nerve on days 28 and 56. This increase in H-reflex, as a sign of spasticity, also manifested in a worsening of the sensorimotor behavioral test used.

These results demonstrate the possibilities of using this model to better understand the pathophysiological changes after stroke that lead to PSS. This PSS model is characterized by its high reproducibility, reliability and low mortality compared to other lesion models like thromboembolism or MCAO.

Novel method to produce a layered 3D scaffold for human pluripotent stem cell-derived neuronal cells

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INTRODUCTION: Degenerative disorders or traumas in the central nervous system (CNS) occur often as a consequence of damage and loss of axons. The adult human brain and spinal cord have limited ability to regenerate after injury [1] but regeneration and restoration of the function of injured neural tissue requires regrowth of axons. In vivo, axons form aligned bundles between different areas of the brain and in innervation to target tissues. The formation of this kind of organized structure is enabled by guidance cues which include extracellular matrix (ECM), surrounding cells and tissue structures which in in vitro models is mimicked with biomaterials. Ability to offer guiding cues for the cells and thus direct neuronal growth three-dimensionally (3D) in vitro opens new possibilities to model defected tissue in CNS in the future. Here, we describe a method to create 3D, layered fiber-hydrogel scaffold for in vitro cell culture by combining electrospun, align oriented poly (D,L-lactide) (PLA) fibers [2] and collagen I hydrogel. The aim of the work is to study parallel alignment of neuronal cells relative to fibers in 3D composite scaffold.
METHODS: In this work 3D composite scaffold consists of electrospun fiber layers and hydrogel layers. The scaffold was created by novel method of alternating electrospinning of aligned oriented PLA fibers [2] and cell laden collagen hydrogel gelation in one process. Cultured cells were human pluripotent stem cell (hPSC) derived neuronal cells.
Diameter and surface topography of the fibers were studied using scanning electron microscope (SEM). Expression of neuron specific proteins were investigated using immunocytochemical staining. Scaffolds were imaged using confocal microscope. Data obtained from the images were analysed with ImageJ software. 3D reconstructions of the layered structure were made with Imaris software.
RESULTS: Optimization of the electrospinning process has been successfully performed. We have managed to produce fluorescent PLA fibers (600 nm in diameter) in order to help visualisation 3D layered structure of the scaffold.
We have managed to construct layered scaffold of three fiber layers and two hydrogel layers, in which neuronal cells grow in every layer.
Neuronal orientation of hPSC derived neurons along fibers was observed after two weeks culturing on 2D align oriented fiber layer and in 3D composite scaffold.
DISCUSSION & CONCLUSIONS: We successfully prepared 3D layered nanofiber-hydrogel scaffold for neural cell orientation. Our results indicate the ability to guide neuronal orientation in relation to fiber orientation. This electrospinning method enables us to spin fibers on freshly gelated, cell laden collagen hydrogel, therefore distributing fibers better into the hydrogel and bringing fibers and cells to closer contact to each other.
This novel method allows to create 3D scaffold containing fibres as guiding component for neuronal in vitro models that better mimic 3D fibrous nature of native ECM.
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A simple, fast and easy to use correlation-based multi-channel spike sorter

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Spike sorting is to detect and to classify action potentials present in extracellular recordings. This can be done by simple but often inaccurate thresholding methods or by using more sophisticated ones, like principal component or wavelet analysis. We developed an easy to handle and fast spike sorting software that uses correlation for detection and *k*-means for classification of spike-waveforms into classes representing activities of different neurons.

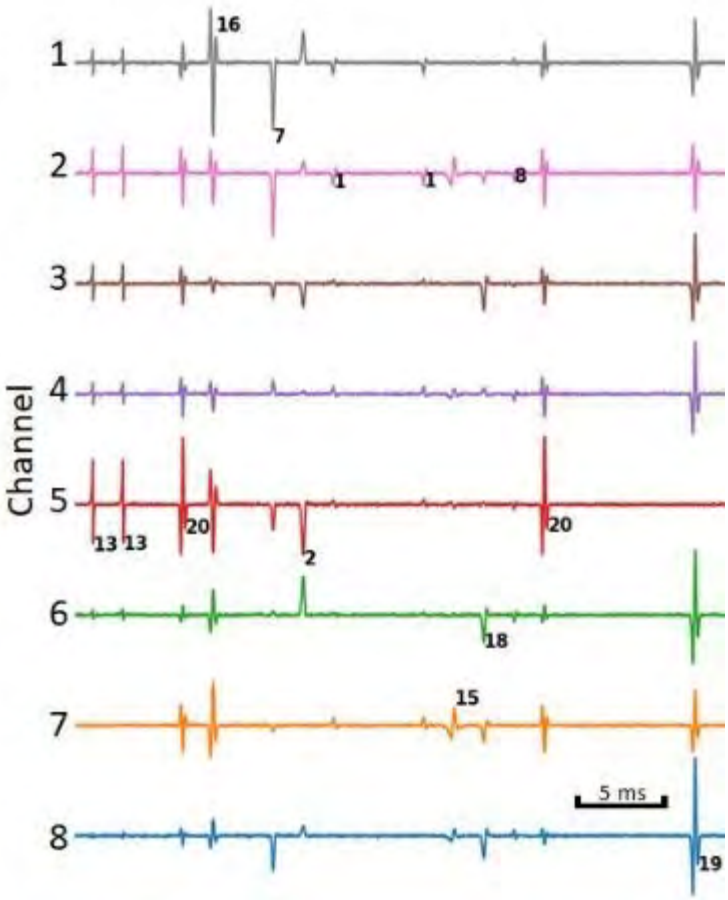
Spikes candidates are detected by finding local extrema with predefined prominence and threshold for each bandpass filtered signal-channel separately. The next steps are the elimination of duplicated peaks and the cut out of spike waveforms. After concatenation of related spikes to calculate spike waveform reference by averaging, cross-correlation of concatenated spike candidates with the reference is done. The spike waveforms are then parametrized by extrema in the correlograms. To classify these data into groups or classes (units) we selected *k*-means algorithm. To estimate the optimal *k* we developed a compute-intensive approach: Run *k*-means for given *k* and then calculate for every class the mean distance in relation to the respective centroid, summing up yields D_k, do this *n*-times for $1 < k \leq K$, find the lowest average D_k, the associated *k* is an estimate for the optimal number of classes. Thus, *n* times *K* runs of *k*-means are necessary.

It is well known that the usefulness of software also depends on its usability. Thus, we have developed a simple and somehow intuitive user interface that supports the user in a targeted manner. In addition, it allows the user to check and, if desired, to improve spike sorter's cluster assignments by visually driven interventions, i.e. setting of *k*-means parameter *k* and initial cluster centroids by hand. The algorithm and the user interface are written in Python.

Using simulated data we found that our spike sorting approach works well for single- and multi-channel signals. E.g., the analysis of 8 channels with 100,000 data points each (5 s) and 20 different spike waveforms and a total of 911 spikes took, on a mean office PC with AMD FX-6300 CPU, about 3 s for known *k* required for *k*-means algorithm, and about 32 s when *k* had to be estimated by our compute-intensive approach. Thus, multi-channel real-time spike sorting seems to be feasible with a PC with a current high-performance CPU. To test spike sorter’s detection capability, simulated data with Gaussian white noise were used. Fig. 1 shows detected spikes in a section of a simulated multi-channel signal. We found that the error was about 5 % when the standard deviation of the noise was about 10 % of the mean spike amplitude calculated from the spikes on all channels. Errors were due to noise. In the low-noise regime and without overlapping spikes, the detection performance was almost perfect.

We have developed an easy to use, fast and efficient spike sorting software for single- and multi-channel extracellular neural recordings. The challenge of correctly sorting data with overlapping spikes and the

testing of our spike-sorter with real data will be addressed in future work.



Home-enclosure based behavioural and wireless neural recording setup for unrestrained rhesus macaques

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Neurophysiology studies in awake behaving monkeys usually require separation from the social groups and partial movement restraint during the experiment. Recent developments allow animals to perform advanced cognitive tasks in a self-paced manner while remaining within their home environment using an eXperimental Behavioural Instrument (XBI) (Calapai et al. 2016; Berger et. al 2018). Moreover, we developed a ReachCage experimental setting to study neural correlates in freely moving animals using wireless recordings (Berger et al. 2020). Here, we combine both approaches and incorporate wireless neural recording into our XBI to achieve synchronised behavioural and neural recordings in the home environment of the animal while avoiding movement restraints. We recorded broad-band neural data from 192- channels in parallel from three different brain areas at single unit resolution while the animals were engaged in a visually instructed goal-directed, memory-guided reach task on a cage-mounted touch screen. The collected neural data is of high transmission rate and has low number of artefacts. Furthermore, we were able to extract neural features in correlation to task parameters, such as spatial tuning during movement planning. In conclusion, the current system allows for conducting neurophysiological experiments in the home environment of the animal, enabling the animal to stay in vocal and visual contact with its conspecifics and alleviating movement restraints. With this, we achieved system-level neural recordings during trained cognitive task performance.

Unravelling the synaptic interactome: Advances in synaptosomal cross-linking mass spectrometry

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The functionality of the brain highly depends on the intercellular communication of specialized cells called neurons. Information transmission between neurons can be of either chemical or electrical nature and are carried out by synapses. These synapses form junctions with each other, each of which consist of a presynapse, a synaptic cleft and a postsynapse, all containing complex molecular machineries acting via stable or transient protein-protein-interactions (PPIs). To decipher the complex synaptic PPI, we combined cross-linking mass spectrometry (XL-MS) with biochemical and computational approaches using cerebellum and hippocampus obtained from 8 to 10 week old mice. From this dataset, we obtained 11,999 unique lysine-lysine cross-links, representing interactions within and between 2362 proteins. This extensive collection led to the detection of novel interaction partners and provided information which helped to model protein conformational dynamics as well as to determine the main components of synaptic specific protein complexes. Despite the high number of cross-links identified, the yield of crosslinks from synaptic specific proteins, especially from the post synaptic density, can still be improved. To tackle this challenge, we aim to separate different compartments of the synaptic junction biochemically and perform XL-MS analysis for them individually. Furthermore, we will employ an enrichable cross-linker namely DSBSO to improve the sensitivity and dynamic range of cross-link detection.

The Cone Method: Inferring Target Selection Times from Single-Trial 3D Movement Trajectories in Choice Behavior

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When interacting with their environment, animals and humans continuously process novel sensory information and rapidly adjust ongoing goal-directed movements accordingly, e.g. when chasing pray or foraging. Consequently, due to its high ecological validity, studying sensory integration and decision-making processes during ongoing movements (including unconstrained, free behavior) has gained increased scientific attention. Studying ongoing choice behavior additionally provides rich, time-continuous behavioral data that allows deeper insights into the underlying sensorimotor transformation processes than the behavior measured in static “first decide, then act” behavioral paradigms does. Yet, existing methods of movement analysis, developed mostly in the field of psychology, are often based on statistically comparing two groups of trial-averaged trajectories and are not easily applied to three-dimensional data, preventing them from being applicable to natural free behavior.

We developed and tested the *cone method* [1] to estimate the *timepoint of overt commitment* (TOC) along a single two- or three-dimensional trajectory, i.e. the time where a movement is adjusted towards a newly selected spatial target. We conducted two experiments in humans and applied the cone method to arm movements that were tracked using a haptic manipulandum. In Experiment 1, we established a “ground truth” data set in which the cone method successfully identified the experimentally constrained TOCs across a wide range of all but the shallowest adjustment angles. In Experiment 2, we demonstrate the power of the method in a typical decision-making task with expected decision time differences known from previous findings. The TOCs identified by the cone method matched these expected effects. In both experiments, we compared the cone method’s single trial performance with a trial-averaging method and obtained comparable results.

The cone method provides a distinct addition to existing tools used to study decisions during ongoing movement behavior, demonstrated here for 3D arm movements in humans, but applicable to any similarly structured goal-directed movement behavior. Potential novel applications may include target selection behavior in freely moving nonhuman primates, rodents, insects, and other animal models, and are not restricted to limb movements but may also be applied to head or full body movements. Due to its capability to estimate target selection times from single-trial 3D movements, we consider the cone method particularly promising towards a wide range of studies of non-repetitive free behavior.

[1] Ulbrich & Gail (2020). Preprint on bioRxiv. https://doi.org/10.1101/2020.08.01.232314

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