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Memory consolidation - synaptic tagging and schemas

Richard Graham Michael MORRIS[1]

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Memory is fundamental to human life. Qualitatively distinct types of memory enable us to change our behaviour in response to experience, to acquire and use a repository of knowledge, to recollect events from the past, and to plan for the future. The use of memory is changing, with an increasing amount of human knowledge now externalised and then sought on-demand via the internet. Human language is replete with aphorisms about knowledge. “Knowledge is power” - “A little bit of knowledge is a dangerous thing” - “Europe needs a knowledge-based society”. These and other assertions reflect the apparently central place that ‘knowledge’ plays in contemporary life. But how much do we understand about the brain mechanisms responsible for the acquisition and use of knowledge?

Neuronal signals for reward, risk and economic decisions

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Rewards induce learning (positive reinforcement), approach behaviour, economic choices and positive emotions (pleasure, desire). We investigate basic neuronal reward and risk signals during learning and decision-making, using behavioural and neurophysiological methods.

We conceptualise rewards as probability distributions of value, which offers a formal approach to the study of value and risk. Key parameters defining probability distributions are expected value and standard deviation (risk). Thus value and risk are fundamental reward characteristics and important variables for economic decision-making. These basic concepts, together with animal learning theory and economic decision theory, allow us to address how reward is processed by neurons in the prime reward structures including dopamine neurons, amygdala, orbitofrontal cortex and striatum.

The phasic dopamine signal is composed of two components, resembling two-component responses in main sensory neurons. The early dopamine response component detects events indiscriminately and is influenced by physical impact, novelty, reward generalisation and reward context. The second component constitutes the reward prediction error response. Although the first component detects punishers by their physical impact, none of the components codes the aversive nature of punishers.

Our behavioural studies reveal that monkeys are risk seeking with small rewards and risk neutral and then risk avoiders with larger rewards. The animals' choices are meaningful in satisfying first and second order stochastic dominance. The forms of the behavioural utility function allow us to assess the relationship of dopamine prediction error responses to economic utility. Indeed, dopamine neurons code marginal utility, the first derivative of utility. Their responses satisfy both forms of stochastic dominance tested. These data unite concepts from animal learning theory and economic decision theory at the level of single reward neurons.

In the amygdala we find reward signals that comply with formal contingency, which is a crucial condition for conditioning according to animal learning theory. Amygdala neurons show also systematic variations with the timing of expected rewards and predict economic decisions in save-spend tasks.

These data reveal how different brain structures contribute specific aspects to the neuronal processing of reward information. The presentation will contain an overview and demonstrate how formal animal learning and economic decision theories lead to a convergent understanding of reward processing in the brain.

A neurogenetic approach to understanding motion computation

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The ability to detect visual motion is critical to the behavior of many animals. To extract motion cues, the nervous system has to compare signals over space and time. Since computational models that describe how such spatiotemporal correlations can be implemented have existed for a long time, it is considered a model for understanding paradigmatic neural computations. How these computations are implemented at the network level remained a mystery for more than 50 years. In order to identify the neurons of motion – detecting circuits, we established a genetic toolbox, the InSITE system, that gave us cell type specific genetic access to in principle every cell in the fly brain, using intersectional strategies. We then used the InSITE toolkit in a forward genetics approach, to identify neurons that are behaviorally required to respond to visual motion in an unbiased manner. This led to some unexpected insights into the neural composition of motion-detecting circuits.

First, the two first order lamina interneurons L1 and L2 had been described to provide inputs to elementary motion detectors. We identified the L3 neurons as a novel, partially redundant input channel. While these cells respond to light signals regardless of their motion, the T4 and T5 neurons of the lobula complex are direction -selective, preferring a particular direction of motion. To identify the cells that connect the lamina inputs to the T4 and T5 outputs and thus perform the computations that are critical to directional signals, we again used a forward genetics approach and identified a behaviorally critical link that was unexpected from anatomical or physiological standpoints. Having genetic access to these identified neurons, we used *in vivo* 2 photon calcium imaging to characterize their physiological properties and understand their roles in motion computation. We identified both neuronal properties predicted by established models as well as novel features that expand our understanding how motion computations are implemented in the brain.

Imaging deep with time-reversed light

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Recent advances in resolution, speed, labelling and the advent of optogenetics have greatly extended the use of optical techniques and enabled many biomedical breakthroughs. Yet, when light propagates through thick biological tissues, refractive index inhomogeneities cause diffuse scattering that increases with depth. This poses a major challenge to optical techniques, limiting their biomedical usefulness in vivo to superficial layers of tissue (in rodents) or to larval stages (in zebrafish). In this talk I will describe several strategies to address this key challenge using techniques based on wavefront engineering and optical time reversal, in order to enable optical imaging at unprecedented depths in biological tissues.

The reciprocal GABAergic septo-hippocampal connection: target selectivity and function

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Subcortical pathways that carry information about motivation, emotions and the autonomic state of the animal can efficiently influence cortical EEG patterns, and thereby modulate learning and memory processes, selective attention, arousal and mood. This lecture will shed light on the neuronal network mechanisms underlying such a powerful control mechanism taking the septohippocampal system as an example. Anatomical and electrophysiological studies of the past decades demonstrate that a reciprocal GABAergic connection exists between the medial septum (MS) and the hippocampus, and the former region likely contains the pacemaker neurons of hippocampal theta activity. In addition to reviewing earlier work on how rhythmic disinhibition can induce theta oscillation in the hippocampus, recent results will be presented on the cholinergic septohippocampal pathway, and the GABAergic feedback from the hippocampus to the medial septal pacemaker circuitry. Selective optogenetic stimulation of cholinergic septohippocampal neurons diminished sharp wave ripples in the hippocampus, and paved the way to theta generation. Selective manipulation of the somatostatin-expressing hippocampo-septal (HS) fibers with a similar optogenetic approach, and recording of the response of medial septal neurons both in urethane-anesthetized and in freely behaving mice showed that activation of the HS feedback robustly altered the firing of MS neurons during spontaneuous theta or non-theta in urethane, as well as activity associated to REM-like states. In contrast, theta bursting discharge patterns coupled to evoked theta in urethane and exploratory theta in freely behaving mice was not affected by stimulating the HS connection. These results shed new light on the role of the reciprocal inhibitory circuitry in generating theta oscillations.

A neural circuit that controls plasticity and the gain of sensory responses in mouse visual cortex

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The brain's response to sensory input is strikingly modulated by behavioral state. In the primary visual cortex (V1) of the mouse, responses are dramatically enhanced by locomotion (Niell & Stryker, *Neuron* 2010), a tractable and accessible example of a time-locked change in cortical state. Selectivity is unaltered, so that the change in cortical state is best described as an increase in the gain of visual responses like that produced by focal attention in primates. We have studied the neural circuits that transmit behavioral state to sensory cortex to produce this modulation. Optogenetic activation of the midbrain locomotor center below the threshold for inducing locomotion enhances V1 visual responses, suggesting that ascending connections to the forebrain are responsible (Lee et al., *Neuron* 2014). In the cortex, calcium imaging of behaving animals revealed that locomotion activates vasoactive intestinal peptide (VIP)-positive neurons in mouse V1 independent of visual stimulation and largely through nicotinic inputs. Optogenetic activation of VIP neurons increased V1 visual responses in stationary awake mice, artificially mimicking the effect of locomotion, and photolytic damage of VIP neurons abolished the enhancement of V1 responses by locomotion (Fu et al, *Cell* 2014). These findings establish a cortical circuit for the enhancement of visual response by locomotion and provide a potential common circuit for the modulation of sensory processing by behavioral state.

We wondered whether the enhanced activity produced by locomotion might also enhance plasticity in the adult cortex, where the recovery of V1 from early sensory deprivation is slow and incomplete. Indeed, visual stimulation during locomotion dramatically enhances recovery in the mouse (Kaneko & Stryker, *eLife* 2014). Excitatory neurons regained normal levels of response, while narrow-spiking (inhibitory) neurons remained less active. Visual stimulation or locomotion alone did not enhance recovery. Responses to the particular visual stimuli viewed by the animal during locomotion recovered, while those to another normally effective stimulus did not, suggesting that exercise promotes the recovery only of the neural circuits that are activated concurrent with the exercise.

These findings suggest that a VIP-SST disinhibitory circuit modulates the global state of cortical activity and adult plasticity. They may provide an avenue for improving recovery from amblyopia and developmental dysfunctions in humans.

Ultrafast endocytosis

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In 1973 John Heuser and Tom Reese demonstrated that neurotransmitter was released from neurons via the fusion of neurotransmitter-filled vesicles with the cell membrane. But at the same time, these experiments launched a controversy that is unresolved today – do vesicles collapse into the membrane and are then recycled slowly on the order of 20 seconds? Or do they retain their existence – and reverse the pore in just 1 second, as proposed in 'kiss and run' endocytosis? Since then, molecular pathways for fusion and recycling have been put forward, but the field remains divided. We have used channelrhodopsin to stimulate neurons in intact nematodes and in cultured hippocampal neurons. The specimen is then frozen 15 ms to 20 seconds after the stimulus. To our surprise, we observed a different form of vesicle recycling that is ultrafast, in which membrane is endocytosed at lateral edges of active zones between 30-100 ms after stimulation. The large endocytic vesicles then fuse to form an endosome and are resolved by clathrin into synaptic vesicles.

"Emerging concepts on the roles of astrocytes"

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We have recently described a macroscopic pathway in the central nervous system – the glymphatic system- that facilitates the clearance of interstitial waste products from neuronal metabolism. Glymphatic clearance of macromolecules is driven by cerebrospinal fluid (CSF) that flows in along para-arterial spaces and through the brain parenchyma via support from astroglial aquaporin-4 water channels. The glymphatic circulation constitutes a complete anatomical pathway; para-arterial CSF exchanges with the interstitial fluid, solutes collect along para-venous spaces, then drain into the vessels of the lymphatic system for ultimate excretion from the kidney or degradation in the liver. As such, this may after circulation represent a novel and unexplored target for prevention and treatment of neurodegenerative diseases. The lecture will discuss the roles of astrocytes in glymphatic functions.

Perceptual and cognitive functions of the thalamus

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The thalamus is classically viewed as passively relaying information to the cortex, while perception and cognition are mediated through computation in cortical networks. However, there is growing evidence that the thalamus actively regulates information transmission to the cortex and between cortical areas using a variety of mechanisms, including the modulation of response magnitudes, firing mode, and synchrony of neurons according to behavioral demands. I will discuss brain imaging and electrophysiology studies performed in two primate brain models, human and monkey. Particularly, I will focus on how the visual thalamus contributes to attention and awareness. Neuroimaging studies on visual awareness as measured in a binocular rivalry task show that the lateral geniculate nucleus, the thalamic relay station of the visual projection, is the first stage of processing that signifies the perceptual experience of an observer. Electrophysiology studies in monkeys demonstrate that the pulvinar, a higher-order thalamic nucleus, controls the information flow through cognitive cortical networks. The evidence that I will discuss in the lecture indicates a general and fundamental role for the thalamus in perception and cognition that has been preserved through evolution.

Symposia

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S2 Neuronal basis of vocal communication in vertebrates - from genes to physiology to behavior

S3 DBS – underlying mechanisms

S4 Timing and valence in associative learning

S5 When the effect determines the cause – sensory consequences of self-action and their relevance for planning, control, and perceptual interpretation of one's behavior

S6 Neural mechanisms underlying spatial orientation in insects

S7 Contribution of astrocyte connexins to neuroglial interaction in the healthy and diseased brain

S8 The ontogeny of entorhinal circuitry and function

S9 Processing of acoustic pulse patterns: Common themes in different brains?

S10 Microcephaly and developmental defects of the brain

S11 Ultramicroscopy for imaging the central nervous system and its pathological alterations

S12 Breaking News I

S13 Functional consequences of sensory loss and restoration

S14 Recent advances in basal ganglia research: action-selection, movement and pathologies

S15 Is insect odor transduction primarily based upon an orco-dependent ionotropic mechanism or on metabotropic cascades?

S16 Molecular, neuronal and behavioral effects of oxytocin: a translational approach

S17 Regeneration in the injured spinal cord - hopes and perspectives

S18 Cellular adaptations for temporal precision in the auditory system

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S28 Processing of temporal stimulus cues in the insect olfactory system

S28/2 Role of glial heterogeneity in brain function

S29 Mechanisms of synchronization and coordination of neural oscillators

S30 Adaptation and plasticity in a distorted sense of hearing during tinnitus and hyperacusis

S31 Integrative study of the social insect brain - combining neuroethological and computational approaches

S32 Microglia and brain tumors: friends or foes?

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S34 Modeling evolution, neuronal development and neurodegenerative disorders using mammalian induced pluripotent stem cells

Symposium

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S1-1 Astrocytes, glutamine synthetase and epilepsy
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S1-3 Stimulation-induced changes of astrocytic Ca2+ signaling during the latent period of mesial temporal lobe epilepsy
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S1-4 Astrocyte uncoupling as a cause of human temporal lobe epilepsy
Peter Bedner, Alexander Dupper, Kerstin Hüttmann, Michel K. Herde, Pavel Dublin, Tushar Deshpande, Johannes Schramm, Christian Henneberger, Martin Theis, Christian Steinhäuser

Astrocytes, glutamine synthetase and epilepsy

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Epilepsy is a chronic brain disorder that affects nearly 50 million people worldwide. The hallmarks of epilepsy are episodic seizures, which usually occur without warning, often resulting in impaired consciousness, and sometimes leading to severe injury or death. The personal and societal impacts of epilepsy are staggering, and the economic burden of the disorder has been estimated to over $10 billion per year in the United States. An important reason for the large negative impact of epilepsy is that up to one-third of all patients cannot control their seizures with current antiepileptic drugs. Due to poor target specificity, many drugs also have moderate to serious side effects that greatly limit their use. Development of more efficacious and well-tolerated (i.e. specific) antiepileptic therapies is therefore necessary. To facilitate the development of such therapies it is important to understand the underlying cellular, molecular and electrophysiological mechanisms of drug-resistant epilepsies. The objective of our research is to investigate the role of astrocytes in epilepsy, particularly how astrocytes regulate brain glutamate homeostasis and neuronal excitability. Our central hypothesis is that astrocytes in the seizure focus of the brain has an abnormal phenotype that facilitates extracellular glutamate excess and recurrent seizures (Eid et al. , 2008). The phenotypic abnormalities include changes in the expression of key glutamate metabolizing enzymes [glutamine synthetase (Eid et al. , 2004) and phosphate activated glutaminase (Eid et al. , 2007)], water channels [aquaporin 4, (Eid et al. , 2005)], potassium channels [Kir 4.1, (Heuser et al. , 2012)] and monocarboxylate transporters [MCTs, (Lauritzen et al. , 2011, Lauritzen et al. , 2012)]. The relevance of these findings with regards to glutamate homeostasis and seizures will be discussed.

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Astrocyte immune responses in epilepsy

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Astrocytes, the major glial cell type of the central nervous system (CNS), are known to play a major role in the regulation of the immune/inflammatory response in several human CNS diseases. In epilepsy-associated pathologies, the presence of astrogliosis has stimulated extensive research on the role of reactive astrocytes in the pathophysiological processes that underlie the occurrence of epilepsy. In epileptic human brain tissue, astrocytes undergo significant changes in their physiological properties including activation of inflammatory pathways. Accumulating experimental evidence indicates that proinflammatory molecules may alter glio-neuronal communications, contributing to the generation of seizures and to seizure-related neuronal damage. In particular, both in vitro and in vivo data point to the role of astrocytes as source and/or targets of pro-epileptogenic inflammatory signaling. In this context, understanding the astroglial inflammatory response occurring in epileptic brain tissue may provide new strategies to target astrocyte-mediated epileptogenesis.

Stimulation-induced changes of astrocytic Ca2+ signaling during the latent period of mesial temporal lobe epilepsy

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Astrocytes display spontaneous Ca2+ elevations, and respond with Ca2+ elevations to applied neuro¬transmitter receptor agonists, and to released neurotransmitters during synaptic transmission. It has been proposed that Ca2+¬dependent gliotransmission directly contributes to the excessive neuronal synchronization that predisposes the brain network to seizures. To study changes of stimulation induced astrocytic network activity in adult mice during the latent period we used the intracortical kainite (KA) model of mesial temporal lobe epilepsy. We performed our experiments with viral delivery of the genetically encoded Ca2+ indicator GCaMP5 into hippocampal astrocytes, which allows spatial and temporal analysis of Ca2+ changes in astrocyte cell bodies, processes and end feet. Neuronal stimulation¬evoked astrocytic Ca2+ signals were recorded by two¬photon microscopy during the latent period at day 1,3 and 7 post KA injection on hippocampal slices prepared from adult mice. The most significant findings were increased amplitude ($\Delta F/F$) in astrocyte soma, processes and endfeet at day 1 post KA injection. We also found significant increase of duration, but decrease of latency in astrocyte endfeet. The observed changes are transient and return to normal at day 7 post KA injection. The changes observed were reversible by application of the non¬selective mGluR antagonist á¬Methyl¬4-carboxyphenylglycine (MCPG), and the selective mGluR5 antagonist 2¬Methyl¬6¬phenylethynyl pyridine hydrochloride (MPEP).
Early changes of astrocyte Ca2+ responses may precipitate other physiological and molecular changes and contribute to epileptogenesis.

Astrocyte uncoupling as a cause of human temporal lobe epilepsy

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Glial cells are now recognized as active communication partners in the CNS, and this new perspective has rekindled the question of their role in pathology. In the present study we analysed functional properties of astrocytes in hippocampal specimens from patients with mesial temporal lobe epilepsy (MTLE) without and with sclerosis (MTLE-HS) combining patch clamp recording, K+ concentration analysis, EEG/video-monitoring, and fate mapping analysis. We found that the hippocampus of MTLE-HS patients is completely devoid of bona fide astrocytes and gap junction coupling, while coupled astrocytes were abundantly present in non-HS specimens. To decide whether these glial changes represent cause or effect of MTLE-HS, we developed a mouse model that reproduced key features of human MTLE-HS. In this model, uncoupling impaired K+ buffering and temporally preceded neuronal death and the generation of spontaneous seizures. Uncoupling was induced through i.p. injection of lipopolysaccharide (LPS), prevented in Toll-like receptor4 knockout mice and reproduced in situ through acute cytokine or LPS incubation. Fate mapping confirmed that in the course of MTLE-HS, astrocytes acquire an atypical functional phenotype and lose coupling. These data further indicate that astrocyte dysfunction may be a prime cause of MTLE-HS and identify novel targets for anti-epileptogenic therapeutic intervention.

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Symposium

S2: Neuronal basis of vocal communication in vertebrates - from genes to physiology to behavior

S2-1 Central Pattern Generator for Vocalization: In Search of a Vertebrate Morphotype.
Andrew Bass

S2-2 The influence of sleep on song-related neuronal activity in RA – what role does Melatonin play?
Susanne Seltmann, Lisa Trost, Andries Ter Maat, Manfred Gahr

S2-3 Motor - auditory interactions for listening and learning
Richard Daniel Mooney

S2-4 About how cortical neurons of bats cope with fast echolocation sequences: Multi-electrode and single-electrode recordings with natural echolocation stimuli.
M. Jerome Beetz, Julio C. Hechavarría, Manfred Kössl

S2-5 Audio-vocal integration and cognitive control of vocal behavior in mammals
Steffen R Hage

S2-6 Neurogenetic contributions to vocal production learning
Constance Scharff

Central Pattern Generator for Vocalization: In Search of a Vertebrate Morphotype.

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Animals that generate acoustic signals for social communication are faced with two essential tasks: generate a temporally precise signal and inform the auditory system about the occurrence of one's own sonic signal. Acoustic behaviors are known for the two major radiations of jawed vertebrates, the ray-finned (Actinopterygii) and lobe-finned (Sarcopterygii) fishes that also include tetrapods. Sonic behaviors are widespread among actinopterygian fishes. Recent neurophysiological and neuroanatomical studies in fishes delineate a hindbrain central pattern generator (CPG) comprised of anatomically distinct compartments that lead to sonic behaviors displaying extreme synchrony and temporal stability on a millisecond timescale. The hindbrain network includes a corollary discharge circuit that informs the auditory system about the occurrence of one's own sonic signal. Although extreme synchrony and temporal precision on a millisecond timescale are characters shared with the sonic systems of tetrapods, little is known about the ancestry of underlying neural mechanisms. Building upon the neurobehavioral phenotype of fishes, we have attempted to reconstruct the developmental and evolutionary origins of the vocal-acoustic phenotypes of jawed vertebrates. We propose the following suite of neural characters for a vocal-sonic CPG morphotype among vertebrates.

1. Developmental origins from a caudal hindbrain-spinal compartment.
2. Population level temporal precision and synchrony on a millisecond timescale established by intrinsic and network properties.
3. Neuronal phenotypes with distinct, genetically specified coding functions such as duration and pulse repetition rate.
4. A corollary discharge circuit linking vocal and auditory networks that codes for call duration.
5. Direct input from vocal midbrain neurons receiving input, in turn, from the preoptic area-anterior hypothalamus.
6. Coordinated activity with pectoral premotor circuitry that shares origins from the same hindbrain-spinal compartment.

The influence of sleep on song-related neuronal activity in RA – what role does Melatonin play?

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Sleep is essential for song learning and song production in birds. The song control nuclei HVC and RA, which both show changes in their neural activity patterns during sleep, play a crucial role in these processes. The presence of Mel-1B receptors in both nuclei suggests that the sleep regulating hormone melatonin may be directly involved. However, it is still largely unknown how melatonin affects song structure and its neuronal correlates. To address this question we used wireless transmitters to record the neuronal activity of individual premotor neurons in the RA of free ranging, socially housed zebra finches while manipulating melatonin levels. Our results suggest a direct influence of melatonin on the activity pattern of premotor neurons in RA. Melatonin was found to increase the interspike interval during the steady firing that RA projection neurons show during non-vocal periods, an effect normally observed at the transition to sleep. Furthermore, we found that melatonin is crucial for the occurrence of an auditory response in RA neurons which occurs spontaneously, or can be triggered by playback of the bird's own song, during sleep. We therefore speculate that the effects of melatonin on RA firing properties are important for sleep related maintenance of the song structure and could as well be involved in song learning.

Motor - auditory interactions for listening and learning

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Auditory-motor interactions are critical to a wide range of behaviors, including those necessary to vocal communication and musical performance. The critical role of auditory experience in the normal development of certain complex behaviors, such as human speech or birdsong, indicates that auditory experience must instruct the vocal motor system. Furthermore, many forms of sensorimotor integration, including those important to the acquisition and maintenance of learned behaviors, are thought to be facilitated by motor to sensory interactions. I will describe studies in both songbirds and mice that delineate circuits that convey information between motor and auditory regions, explore their role in vocal learning, and detail how they function to modulate auditory activity during a variety of movements, including vocalization.

About how cortical neurons of bats cope with fast echolocation sequences: Multi-electrode and single-electrode recordings with natural echolocation stimuli.

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In echolocating bats detailed time processing is fundamental for accurate orientation. Behaviorally, the fruit-eating bat *Carollia perspicillata* is a suitable animal model to investigate temporal processing at short time scale. This species emits extremely short pulses that last between 0.5 and 2 ms and the interpulse time intervals reach minimal values of around 30 ms. For analyzing target distance the animals use the echo delay defined by the time interval from emitting a call until the arrival of the corresponding echo. Combination sensitive neurons tuned to specific echo delays are topographically organized along the rostro-caudal axis of the dorsal auditory cortex (high frequency area) in *C. perspicillata*. These neurons are highly specialized to detect a wide range of different echo delays ranging from 2 to 24 ms.
To assess the impact of interpulse time intervals in the response of the neurons and to describe the activity pattern of the cortex as a whole under naturalistic conditions, we recorded with electrode arrays while the animals were listening to natural echolocation signals. The echolocation signals were taken from the pendulum. Bats are placed in a pendulum and approaching flight behaviour is simulated by swinging them towards a reflecting target. While swinging, the animals emit sequences of pulses that are recorded by a microphone adjacent to the ears.
For electrophysiological experiments, the echolocation sequence and single pairs of pulses and echoes are used as natural stimuli. With multi-electrode recordings covering most of the high frequency area, we investigated the overall activity of that brain region. Our results show that the response to natural echolocation sequences is highly suppressed in comparison to the response to single pulse/echo pairs presented at intervals of 400 ms. Artificial control stimuli with a temporal pattern similar to that of the echolocation sequence (i.e. the same inter-pulse time intervals) lead to a comparable response suppression. The strength of suppression is not equally distributed throughout the sequence, that is, responses are less suppressed at time points at which the neurons are responding to the optimal delays. These results lead to the conclusion that interpulse time intervals have strong suppressive impact in cortical responses, leading to sharp responses to specific parts of the sequence.

Audio-vocal integration and cognitive control of vocal behavior in mammals

Steffen R Hage[1]

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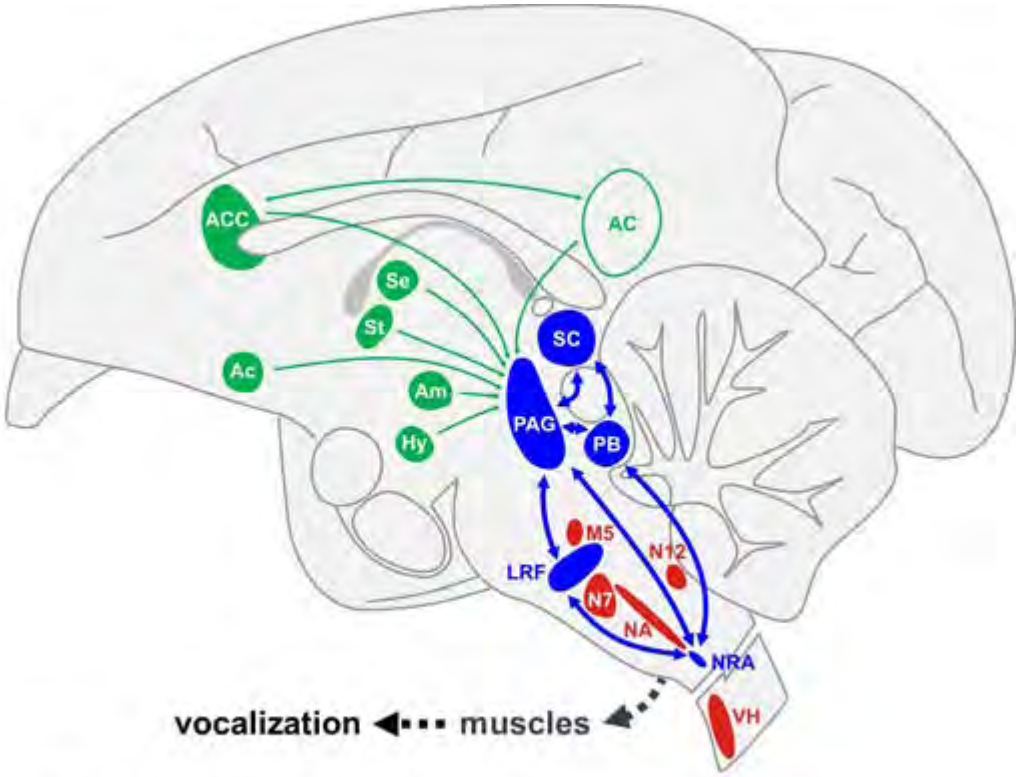
Vocal communication is a complex behavioral pattern that can be found in all mammalian species. It can be subdivided into learned vocal patterns such as human speech, and genetically pre-programmed vocalizations, which include those of non-human primates and most other mammals. One of the general aims of my research is to study and further understand vocal production and audio-vocal integration mechanisms in mammals as a whole, i.e., on each level of the vocal motor system. In my talk, I will give an overview on our recent studies on vocalization-related cognitive processes in the cerebral cortex and on innate brainstem mechanisms involved in audio-vocal integration processes. We performed these studies with a broad range of different methodological approaches including neurophysiological, neuroethological and psychophysical techniques on several animal models such as bats as well as squirrel and rhesus monkeys.

In the studies on vocalization-related cognitive processes in the cerebral cortex, we studied evolutionary aspects of human speech control with our newly established primate model and recorded single neurons in the vocalizing rhesus monkey. We found a neuronal correlate of volitional call initiation in the monkey homologue of human Broca's area that suggests a cardinal role of this structure in vocal planning and call initiation, a putative phylogenetic precursor in non-human primates for speech control in linguistic humans. In several neuroethological and neurophysiological studies in bats and monkeys we studied audio-vocal behaviors in which vocal output is directly affected by ambient noise or species-specific vocal communication. Here, our results indicate different brainstem-based neural mechanisms and/or circuits that are modulating specific call parameters. In a telemetric single-unit recording study in freely moving monkeys, we were able to identify such a region in the brainstem that might potentially be involved in such audio-vocal integration processes.



The vocal motor network

(adapted from Ackermann, Hage & Ziegler, 2014, BBS)

Neurogenetic contributions to vocal production learning

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Abstract body not available

Symposium

S3: DBS – underlying mechanisms

S3-1 Motivational disorders in Parkinson's disease and high frequency stimulation of the subthalamic nucleus: preclinical study in the rat
Sabrina Boulet

S3-2 Deep brain stimulation in psychopathological mouse models of fear- and affective disorders
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S3-3 From rats to men: deep brain stimulation in rodent models of psychiatric afflictions
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S3-5 Effects of different transcranial direct current stimulation (tDCS) polarity on motor learning induced of mental practice
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Motivational disorders in Parkinson's disease and high frequency stimulation of the subthalamic nucleus: preclinical study in the rat

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Beyond motor symptoms, Parkinson's disease (PD) patients also exhibit neuropsychiatric troubles which contribute at least as much as the motor symptoms to patients' morbidity and quality of life. Among these symptoms, apathy is one of the most frequently observed with a prevalence of 40 to 50% of PD patients. Apathy is globally defined as a reduction of motivated goal-directed behaviors and should be considered as a hypo-motivational syndrome. Apathy is also observed in almost fifty percent of parkinsonian patients under high frequency stimulation of the subthalamic nucleus (STN-HFS), the neurosurgical treatment of reference of PD motor symptoms. Even if apathy is probably covered by the dopaminergic treatments administered to patients during numerous years, its resurgence in PD patients under STN-HFS raises relevant questions. The first one aims at clarifying if the post-surgical apathy is the indirect consequence of the reduction of L-Dopa doses (made possible by the motor improvement observed under STN-HFS) or if it is due to a direct effect of STN-HFS on associative -limbic loops ?. The second concerns the neurobiological mechanisms which underlie the expression of this post-surgical apathy which limits the clinical benefits of STN-DBS.

In this context, our work aim at study the neurobiological mechanisms underlying the post-operative apathy in a pathophysiological context similar to those observed in PD patients.

For this, we combine the use of a new model of neuropsychiatric symptoms of PD in rat presenting an apathetic state, and of implantable microstimulator allowing continuous long-lasting stimulation in freely moving animals to determine the direct effects of STN-HFS on motivated behaviors.

We observed that continuous long-lasting STN-HFS can induces decrease in motivated behaviors in normal rats and can exacerbate those already existing in our apathetic rat model and that this STN-HFS induced deficit can be reversed by a chronic treatment with pramipexole.

Regarding previous results of our team, one of the hypotheses that could explain the decrease of motivated behaviors induced by STN-HFS we observed is that STN-HFS may have a direct effect on non-motor circuits, especially decreasing the expression of dopaminergic receptors D2/D3 in limbic structures of the basal ganglia (BG).

Finally, this pre-clinical project should allow us to better understand the complex interactions between STN-HFS and motivated behaviors and thus to improve the treatment and the care of these neuropsychiatric troubles.

Deep brain stimulation in psychopathological mouse models of fear- and affective disorders

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Recent evidence from clinical studies indicates that nucleus accumbens (NAcb) deep brain stimulation (DBS) represents a novel therapeutic intervention for patients suffering from treatment resistant depression or obsessive compulsive disorders. However, the underlying mechanisms of DBS are largely unknown. Here we demonstrate that NAcb -DBS robustly decreases depression - and anxiety-related behaviour in HAB mice, an animal model of high -trait anxiety and comorbid depression, which mimics important features of the human psychopathology including insensitivity to antidepressants targeting the serotonergic systems. In addition DBS rescued impaired fear extinction in an animal model of treatment resistance to exposure therapy. Possible underlying mechanisms will be discussed in this symposium.

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From rats to men: deep brain stimulation in rodent models of psychiatric afflictions

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While DBS serves as successful focal therapeutic strategy in the treatment of otherwise refractory neuropsychiatric syndromes, it can also be used as a powerful experimental tool for mapping brain regions and gaining insight into neural circuits and their interactions. In the recent years various rodent models for psychopathologies were utilized to study the potential of DBS in attenuating or even normalizing aberrant behaviours along with the profound investigation of its effect on the underlying neurobiological processes. The present lecture discusses the use of animal models of psychiatric disorders as an important source of information for such attempts: experiments studying the effects of DBS on behavioural as well as neurobiological deficits in animal models of obsessive compulsive disorders, major depression, alcohol addiction and schizophrenia are presented. The entirety of the presented studies allows statements on optimal stimulation sites, stimulation designs and the putative mechanisms underlying the psychopathologies. However, results are critically depended on the animal model used as well as on the chosen experimental design, calling for a cautious examination of the data before considering its translation into the clinical situation. In conclusion further studies are needed to understand the mechanisms and effectiveness of DBS in the differentially diseased brain.

Effects of different transcranial direct current stimulation (tDCS) polarity on motor learning induced of mental practice

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Background: the technique of mental practice (MP) is defined as an active process through which the individual creates a mental image by means of correlations with perceptual and motor events. Transcranial direct current stimulation (tDCS) is a powerful tool to modulate the excitability of areas involved in mental practice. Objective: the study aim was to verify the effect of the tDCS polarity on motor learning induced by mental practice in healthy subjects. Methods: sixteen right-handed volunteers (aged 18-30 years), through a controlled crossover and double blind study, underwent 4 sessions of MP associated with anode, cathode, bi-hemispheric or sham tDCS (current strength 2mA, duration 13 min, electrodes size 20cm2) over the right primary motor cortex (M1). Each session was separated for at least 48 hours to minimize the cumulative effects. Motor learning was assessed both at baseline and immediately after each session by a blinded rater using non-dominant hand writing time and legibility of the word-list trained mentally. It was assessed using the execution time and the legibility (word length, word size, letter legibility and word legibility). Results: the results suggest that MP associated with anodal tDCS induces motor learning. For writing time, ANOVA revealed a significant main effect for tDCS polarity ($F = 5.045$, $p = 0.016$), but not for time ($F = 1.994$, $p = 0.178$). No effect of type x time interaction ($F = 0.896$ and $p = 0.469$) was found. A post-hoc analysis using paired t test revealed a significant difference when the effect of MP was combined with anodal tDCS compared with the session in which the MP was associated with sham tDCS ($t = 2.41$, $p = 0.02$) . No significant differences were observed on the components of legibility. Conclusions: we conclude that tDCS is a potential tool to maximize the effects of mental practice to promote motor learning.

Symposium

S4: Timing and valence in associative learning

S4-1 Relief learning in fruit flies
Ayse Yarali, Christian König, Mirjam Appel, Afshin Khalili, Claus-Jürgen Scholz, Gerald Rubin, Yoshinari Aso

S4-2 Relief and safety learning in rats: Behavioral characterization and neural basis
Markus Fendt

S4-3 Safety signals inhibit fear but are they reinforcing?
Anushka BP Fernando, GU Urcelay, AC Mar, AD Dickinson, TW Robbins

S4-4 Relief and reward in the human brain
Siri Leknes

S4-5 Pain relief learning in humans
Marta Andreatta, Andreas Mühlberger, Paul Pauli

Relief learning in fruit flies

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Fruit flies form two opposite kinds of associative memory from an experience with electric shock (1): Odours that precede shock are later on avoided as signals for punishment; whereas odours that follow shock are subsequently approached as signals for the “feeling of relief” upon shock -offset. This mnemonic opponency is conserved through evolution. In fact, parametric analyses of fly relief learning (2) were instructive for establishing the corresponding paradigms in rats and man (3). Although much is known about punishment learning in a variety of organisms, relief learning is understood very little. We aim at filling this gap, using the fly as a model.

So far, candidate-gene approaches highlighted two fly genes that affect relief learning: (i) Loss of white gene function impairs relief learning, while improving punishment learning (4). This suggests a partially genetically-determined balance between the two opponent memories. (ii) Loss of synapsin function is detrimental for relief learning, just as for punishment learning (pers. comm. B. Gerber, LIN, Magdeburg), arguing for shared molecular end-effectors of synaptic plasticity between the two. To move beyond these candidate gene approaches, and particularly to discover genetic effectors specific to either punishment or relief learning, we opted for a comparative genome-wide strategy, using ~40 nature-driven inbred strains. Genetically, flies within each strain are close to identical; across strains, however, they substantially differ. Correspondingly, punishment and relief learning significantly varied across the strains. This allowed us to look for co-variation between the learning scores and the genome-wide gene-expression levels (5) as well as sequence polymorphisms (6), pointing to candidate genes. We will now analyse these candidates in depth using specific genetic/ transgenic intervention, reasoning that their human orthologues may turn out relevant for psychiatric conditions related to pain and trauma.

In terms of the neural circuits, both fly olfactory punishment and food-reward learning have been studied in beautiful detail. In both cases, the critical synaptic plasticity seems to take place at the output synapses of the “mushroom body” (MB). We found that relief learning relies on MB-function, too. Unlike for punishment and reward learning however, we do not know with respect to relief learning, (i) which neurons may carry a reinforcement signal to the MB (7), (ii) which subsets of MB-neurons may harbour the memory traces, and (iii) which MB-efferent neurons may “read out” these traces. We now address these questions using a novel set of transgenic tools that enables us to express a transgenic blocker of neuronal activity in nearly all MB-associated neurons almost one-at-a-time, screening for effects on relief learning. In the long run, we want to integrate the emerging circuit-account of relief learning with the knowledge on punishment and reward learning for a unified computational model, which can also be implemented in technical devices. These analyses in the fly may point to circuit principles for managing opponent memories, which may apply to evolutionarily higher animals, too.

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Relief and safety learning in rats: Behavioral characterization and neural basis

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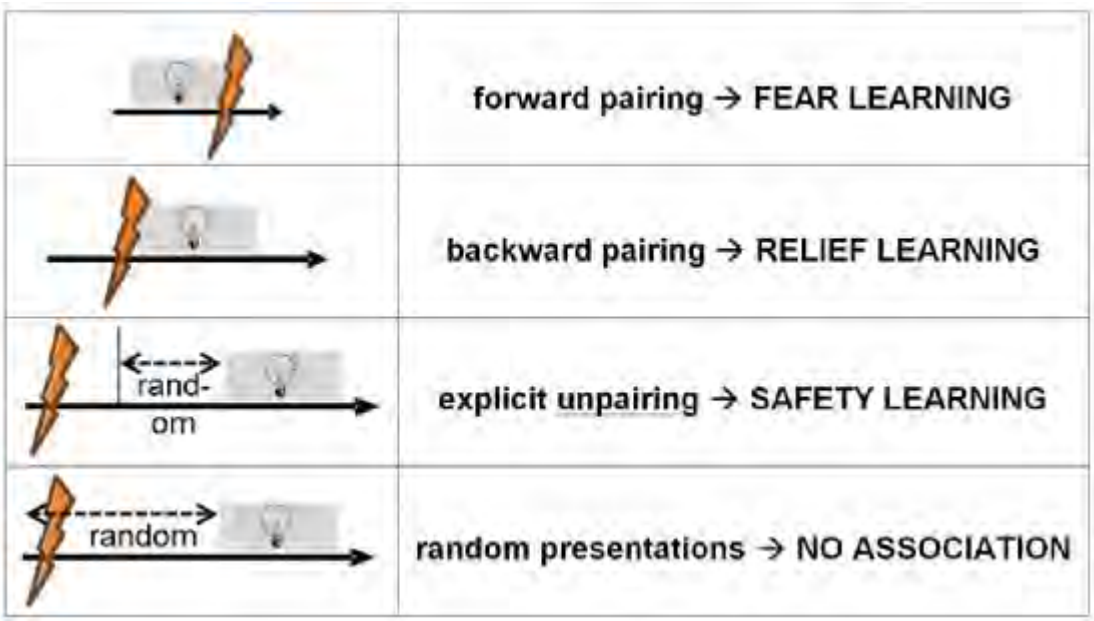
Humans and animals can better cope with aversive events if they have learnt from previous aversive experiences. For example, animals can learn that a particular stimulus predicts an aversive event. This stimulus then becomes a conditioned fear stimulus, i.e. it induces autonomic and behavioral fear responses. Basically, the brain and body are then dedicated to fast and effective responses to aversive events. However, not only stimuli predicting the onset of an aversive event can be learned (fear learning) but also stimuli that are associated with the absence or with the offset of an aversive event (see figure). These processes are called safety learning and relief learning. It is known that patients with anxiety disorders often have exaggerated, i.e. maladaptive fear learning. In contrast, safety learning is attenuated in these patients and there are hints from animal experiments that relief learning might also be diminished. The aim of the presentation is to summarize our recent studies on these learning processes in rodents: The behavioral effects of learned relief and learned safety are very similar. Both support appetitive-like behaviors like approach behavior or an attenuation of the startle response. However, whereas learned safety is dependent of the experimental context, this is not the case with learned relief. In addition, the neural circuitries underlying relief and safety learning are different. The nucleus accumbens is required for relief learning but not for safety learning. Recent studies further demonstrated that relief learning depends on NMDA and dopamine receptors within the nucleus accumbens. This data suggest that the full range of the behavioral consequences of an aversive event, including its pathologies, cannot be appreciated without taking relief and safety learning into account.



Safety signals inhibit fear but are they reinforcing?

Anushka BP Fernando[1], GU Urcelay[1], AC Mar[1], AD Dickinson[1], TW Robbins[1]

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Safety signals provide relief, possible due to their ability to predict the absence of an aversive event, inhibiting fear responses. At issue is whether these signals also act as instrumental reinforcers of avoidance behaviours and if distorted safety learning is at the core of maladaptive avoidance behaviours. In order to test this prediction, rats were trained on a free-operant avoidance task where lever press responses avoided shock and produced a contingent auditory safety signal that marked a 5s shock-free period. The studies presented demonstrate the conditioned reinforcing properties of a safety signal on avoidance behaviour but suggest their neural mediation may differ to that of an appetitive, reward-predicting stimulus. Furthermore, despite avoidance behaviour being reinforced by presentations of the safety signal, this instrumental behaviour is insensitive to changes in value of the safety signal, suggesting avoidance behaviour is habitual with respect to the positive contingency between the avoidance response and safety signal.

Relief and reward in the human brain

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I will discuss recent work on the subjective experience of relief from pain or from the uncertain expectation of pain, with focus on neuroimaging work from my own and other labs.

Pain relief learning in humans

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Avoidance of dangers is crucial for organisms' survival. Classical conditioning elucidates how organisms make associations between an initially neutral stimulus (conditioned stimulus, CS) and biological salient events (e.g. pain; unconditioned stimulus, US). However, each emotionally relevant event has a beginning and an end. Thus, if the onset of an aversive event elicits fear, its offset entails positive properties and may elicit an appetitive response, i.e. relief. It remains unclear whether such relief might work as appetitive US and whether a stimulus associated with relief might elicit conditioned appetitive responses. Because these mechanisms could be implicated in the etiology and maintenance of anxiety disorders or other pathological behaviors, a better understanding of the relief-related processes is crucially important. Our goal was to investigate both the affective properties and the learning mechanisms of the relief. In Study 1 and Study 2, participants were divided into 2 groups. During the learning phase, one group (fear group) saw a visual stimulus (i.e., geometrical shape, fearCS+) followed by a painful electric shock (US) at its offset. The other group (relief group) received first the US unpredictably and then saw a visual stimulus (reliefCS+) after 6s. In Study 3, participants learned both fear- and relief-CSs+. Namely, first the fearCS+ was presented followed by the US at its offset and after 6s the reliefCS+ was displayed. A third stimulus (CS-) was presented, but never in association with the US, to all groups. During the test phase, fearCS+, reliefCS+, CS- and a novel control stimulus were presented and the behavioral (startle response; Study 1 and 3), the physiological (skin conductance response, SCR; Study 3) and the neuro -cortical (blood -oxygen-level dependent, BOLD; Study 2) responses were recorded as learning indices. We also collected cognitive responses (ratings) before and after the learning phase as well as after the test phase. As expected, we found successful fear conditioning as indicated by startle potentiation, amygdala activation, increased physiological (SCR) and verbal arousal, and negative valence to the fearCS+. Interestingly, we found startle attenuation, striatum activation and low physiological arousal to the reliefCS+ indicating implicit appetitive conditioned responses. On the contrary, participants rated the reliefCS+ as aversive (negative valence, high arousal) when the preceding US was unpredictable (Study 1, Study 2), but this stimulus was rated as appetitive (positive valence, low arousal) when the preceding US was predictable (Study 3). In summary, our results suggest that relief is an appetitive response elicited by the termination of an aversive event, and importantly it may be associated with neutral stimuli which afterwards elicited conditioned appetitive responses. This suggests that relief plays a role in learning processes and possibly may be involved in the etiology and maintenance of pathological behaviors, e.g. self-injury or risky behaviors. However, this implicit learning process was not always in concordance with explicit cognitive responses which were strongly modulated by situational factors. Thus, even though the implicit response to the offset of threat was appetitive, it was explicitly evaluated as aversive when the threat was unpredictable. Only when the threat was predictable, the ongoing appetitive relief process was reflected in implicit and explicit responses.

Symposium

S5: When the effect determines the cause – sensory consequences of self-action and their relevance for planning, control, and perceptual interpretation of one's behavior

S5-1 Visual Perception Depends on an Efference Copy for Saccades
Robert H. Wurtz, James Cavanaugh, Rebecca A. Berman, Wilsaan M. Joiner

S5-2 Cerebellar contributions to learning sensory consequences of action
Reza Shadmehr

S5-3 Inverse models for motor control: a songbird perspective
Richard Hahnloser

S5-4 A domain-general role of the cerebellum in fine-tuning sensory predictions
Manuel J. Roth, Axel Lindner

S5-5 Area V3A encodes objective motion velocity regardless of eye movement velocity
Matthias Nau, Didem Korkmaz-Hacialihafiz, Andreas Schindler, Ghazal Darmani, Andreas Bartels

Visual Perception Depends on an Efference Copy for Saccades

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Human vision is a continuous interaction between what is falling on the retina and the saccadic eye movements that change the direction of the eye. The advantage of this system is that the high resolution fovea can be used to examine one object after another because the saccades redirect the fovea to each object. The disadvantage is that each saccade shifts the image on the retina, and we should perceive a series of image jumps with each saccade. Instead we perceive a stable visual scene. The brain must compensate for these problems because we have flawlessly stable vision. The key information needed to solve this problem is the amplitude and direction of each saccade, the vector of the saccade. One frequently proposed possibility is that the vector is obtained within the brain, from an efference copy or corollary discharge (CD) of the impending saccade. Such a CD circuit has been identified in the old world monkey. This circuit extends from the brainstem to the frontal cortex, and has previously been shown to be necessary for controlling sequential saccades. What is not known is whether the CD contributes to perception, specifically to the change in direction of the eye after each saccade. We adapted for the monkey a recently developed human psychophysical method for subjects to report their perceived eye direction, and then tested whether inactivation of the CD brain circuit altered this perception. It did. The monkey's perceived direction of its eyes shifted in both the ipsilateral and contralateral hemifields. This perceived shift occurred in the absence of significant changes in the saccades themselves; the perceived changes could not result from a change in proprioceptive feedback without change in eye position. The change was not due to visual input; the experiments used laser spots in the dark. In summary, we have shown that a CD conveys the amplitude and direction of each saccade. This CD, which is available before the saccade, can be the input for the subsequent brain mechanisms that compensate for image movement on the retina due to the saccade. The CD related to perception is the same as that providing the guidance for a rapid sequence of saccades. Thus the signals driving the saccades that generate the problems of the moving eye are the same signals producing the CD that solves those problems.

Cerebellar contributions to learning sensory consequences of action

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Execution of accurate eye movements depends critically on the cerebellum, as evidenced by the deficits observed in patients and lesion studies. These observations have suggested that Purkinje cells (P-cells) predict the state of the eye (e.g. its direction or velocity) during a saccade. Yet, this encoding has remained a long-standing puzzle because P-cell firing shows little modulation with respect to saccade velocity or direction. Here, we analyzed data from P-cells in the oculomotor vermis of behaving monkeys during saccades and estimated the inhibition that these cells produced at the caudal fastigial nucleus (cFN). We uncovered a time course of synaptic input at cFN that precisely predicted the real-time velocity of the eye. When we aligned the simple spikes of each P-cell to a coordinate system that depended on that cell's complex spike field (CS), the result unmasked a pattern of inhibition at cFN that encoded both saccade velocity and direction via a multiplicative coding, i.e., a gain-field. Therefore, our results suggested two new principles regarding function and anatomy of the cerebellum: the encoding of state of the eye does not occur in the firing rates of individual P-cells, but via synchronized inputs to cFN; the anatomical projections of P-cells to cFN neurons is not random, but organized by the CS fields of the P-cells.

Inverse models for motor control: a songbird perspective

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Which computational rules underlie vocal learning in birds and imitation learning in general? One possibility is that vertebrates construct internal models of their sensory -motor apparatus using information gained during motor exploration. We have recently shown how simple eligibility-weighted Hebbian learning rules can lead to formation of inverse models, which are specific mapping from sensory to motor neurons. Inverse models can support imitation behaviors because they allow transformation of an experienced sensory pattern into the motor commands required for producing that pattern, for example a particular birdsong. We recently showed existence of a conceptual link between inverse models and mirror neurons, which are neurons that display similar activity during motor behavior and during replay of the sensory consequence of that behavior.

I will present the arguments why precise mirroring as seen in HVC, the main premotor area of adult birdsong, evidences predictive inverse models and why our more recently observed temporally offset mirroring in LMAN, the main premotor area of juvenile subsong, evidences causal inverse models. The key point to note is that predictive inverse models have an uncertain role in learning of new imitation behaviors whereas causal inverse models are a possible substrate for their rapid learning.

Despite the correlative evidence of causal inverse models there is currently no behavioral evidence that songbirds use inverse models for making targeted changes to their songs. In the final part of the talk I will show our preliminary data in search of such evidence.

A domain-general role of the cerebellum in fine-tuning sensory predictions

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Perception and action are not only informed by current sensory input but in addition by predictions about upcoming sensory events. These 'sensory predictions' are based on internal models reflecting the statistics of an organism and its sensory environment. Formulating such predictions can, thereby, allow one, for instance, to react more rapidly to predictable stimuli. Furthermore, sensory predictions are crucial to distinguish sensory information that was self-caused from externally caused sensations. Towards this end, efferent information from one's own motor commands is fed into internal models in order to predict the resulting sensory consequences. These predicted consequences are then compared to the actual sensory input, allowing one to isolate the sensory component that was produced by its own actions (e.g for the sake of sensory attenuation etc). In order to be accurate, however, such predictions need to undergo constant recalibration to match the changing properties of one's own motor system and/or those of the environment. This recalibration is accomplished by reducing prediction errors.
Compatible with its important role in motor control, the cerebellum seems to be the major site involved in the prediction of the sensory consequences of one's own actions and their recalibration on a short time scale. Thus, cerebellar patients are impaired in recalibrating sensory predictions about their own actions in response to an altered interrelation between motor commands and their sensory consequences. However, the need for predicting upcoming sensory information is not restricted to cases of self-action. Sensory predictions are rather believed to reflect a general principle implemented in our nervous system. Therefore, it seems likely that also in cases of learning sensory predictions about external sensory events – independent of self-action - the existing cerebellar circuitry that, in principle, carries all necessary sensory inputs, is being utilized.
Addressing this notion in a psychophysical experiment, we show that cerebellar patients, in contrast to healthy controls, are significantly impaired in learning to perceptually predict the time of reappearance of a moving visual target that temporarily disappears behind an occluder. Taking these findings and other results into consideration, we propose that the cerebellum serves as a neuronal machine responsible for the fine-tuning of predictive models. Importantly, we further suggest that this fine-tuning is performed in a domain-general fashion, irrespective of whether sensory predictions built on efference copies or on sensory information, and irrespective of whether sensory predictions support action, perception, or both.

Area V3A encodes objective motion velocity regardless of eye movement velocity

Matthias Nau[1], Didem Korkmaz-Hacialihafiz[1], Andreas Schindler[1], Ghazal Darmani[1], Andreas Bartels[1]

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It is still not very clear how the visual system compensates for self-induced visual motion. This mechanism is crucial to convey visual stability, and also to recognize motion in the external world. There are two possibilities how an object can change its position in your visual field. Either it moves in the outside world or we move our eyes. In both cases its image will move across the retina. The mechanisms enabling us to discriminate between these two options are still not well understood. It is thought that efference copies of eye movement commands are integrated with visual input, allowing to separate self-induced retinal motion from external objective motion. A recent fMRI study showed that area V3A encodes visual motion almost exclusively in world-centered (objective) coordinates, while being almost unresponsive to retinal motion per se. Conversely, the human motion complex (V5/MT and MST) encoded both, objective and retinal motion with equal strength (Fischer, Bülthoff, Logothetis and Bartels, 2012). In the present study we asked two related questions. First, we asked whether human motion regions differentiate between outside objective motion being faster or slower than eye movements with different speeds (i.e. resulting in either positively or negatively signed retinal motion). Second, do these regions encode retinal and objective motion in absolute units or in units relative to the velocity of eye movements? To answer these questions, we created 2D random dot stimuli that moved either slower or faster than a fixation dot. All velocities were chosen so that we could examine neural responses to slower, matched and faster background motion relative to 0°/s, 2°/s and 3°/s eye movement speed. Moreover, we ran a functional localizer scan for each subject, allowing us to identify areas V5/MT, MST, V3A, V6, and CSv for region of interest (ROI) analyses. In our analysis, we tested each ROI's response using a separate set of general linear models (GLM). The GLMs incorporated each of the above hypothesized response properties, and F-tests were used to identify which of the competing models accounted for significantly more variance. We found that all regions encoded both, retinal and objective motion in absolute, not in relative units, and that V3A, but not CSv or V5/MT does differentiate between negatively and positively signed retinal motion. These results suggest that motion is encoded in absolute units throughout the visual motion system, and that V3A has indeed a special role among human motion processing regions in that it represents motion signed with respect to eye movement direction.

Symposium

S6: Neural mechanisms underlying spatial orientation in insects

S6-1 Central Complex Activity Associated with Context and State Spatial Orientation
Roy E. Ritzmann

S6-2 Short-lived and long-term memories improve spatial orientation in *Drosophila*
Roland Strauss

S6-3 Perceptual Hypotheses in *Drosophila* Vision
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Central Complex Activity Associated with Context and State Spatial Orientation

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The capacity to move efficiently through complex environments toward goals or away from threats is a hallmark of animal behavior. In order to accomplish these tasks, animals must stay on target even while negotiating barriers to forward locomotion. Decisions that are made are affected by the context of the animal's immediate surroundings as well as the animal's internal state. Furthermore actions that are made by a hungry individual may be very different from that of a satiated animal. In insects, the central complex (CX) is in an excellent position to play a critical role in this context and state dependent spatial orientation. It receives numerous forms of sensory information monitoring surrounding context and contains receptors for numerous neuromodulators that could alter descending commands as internal state becomes modified.

In this talk, I will describe recent work from our laboratory that monitors behavior and CX activity in tethered and freely moving cockroaches as they negotiate barriers. We have demonstrated that, in the cockroach, neurons within the CX respond to antennal as well as visual stimuli, including directional motion cues. Lesion or reversible blockade of CX regions have negative impacts on various behaviors. Moreover, multi-unit recording in tethered or freely moving insects reveals single units that are strongly correlated with walking speed. These correlations are enhanced before the cockroach executes a turn or climbing movement. In climbing, several units have been found where the relationship between walking speed and unit activity is distinctly altered prior to a climb.

I will also describe activity recorded in the CX of praying mantises as they stalk and track prey. This predatory relative of the cockroach affords particular advantages for analyzing the spatial control provided by the CX. Since the mantis must track and accurately strike its prey, we can predict the exact direction of the insect's movements. We have found units that monitor both the mantis's movements as well as those of its target prey. These tracking actions are altered as the mantis feeds and reduces its stalking behavior.

Short-lived and long-term memories improve spatial orientation in *Drosophila*

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Man like animals require path planning and recall for proper orientation and navigation in complex environments. *Drosophila melanogaster* flies memorize the direction towards their target in order to ensure continued navigation when the target gets temporarily out of sight. Even after a detour they can use idiothetic information to recall their former orientation towards the target to resume the initial approach. The visual orientation memory for attractive targets is localized in the ellipsoid body of the central complex in the adult brain. Neurogenetic studies reveal the underlying neurons and the molecular machinery for this working memory.

In a virtual-reality environment we presented flies also with two alternatingly visible targets of the same kind. Wild-type flies took decisions for one of the two objects and stuck to their decision when the objects kept flipping visibility. In contrast to wild-type flies, structural mutants of the ellipsoid body kept going with their immediate sensory input and therefore showed zigzag behavior. The properties of the underlying integration process in wild-type flies have been studied. Implementation of the proposed algorithm on a roving, camera-equipped robot demonstrates the benefits of such an integration process for consistent goal-oriented behavior in noisy environments.

In contrast to these short-lived memories flies can establish and keep also a long-term, probably life-long memory for their own body size. Size is not just genetically determined but dependent on environmental factors like food quality and temperature regime during larval stages. Flies learn their body size after hatching and show size-dependent decisions at chasms in the walkway. At a decisive width of the gap in their way small flies refrain from costly climbing behavior whereas their larger siblings engage in climbing and try to surmount the gap. The default state of flies before calibrating their size memory is “very large”. The information is gathered from visual feed-back of the retinal images of contrast edges during normal locomotion (parallax motion). Learning requires particular sets of neurons in the protocerebral bridge of the central complex.

Perceptual Hypotheses in *Drosophila* Vision

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Visual perception is an active process. One way to demonstrate activity in visual behavior is to present ambiguous visual stimuli that elicit several equally adaptive responses. A well known example from psychophysics is the Necker cube which is seen in two rivaling perspectives. The brain is said to respond to one of two equally valid perceptual hypotheses which subsequently may be confirmed (or not) by further sensory information. If a hypothesis is not confirmed, the brain tries the alternative one.
We use the flight simulator to expose *Drosophila* to ambiguous stimuli. The fly is surrounded by two identical, concentric dot patterns (p1; p2). Both can be driven by the fly's yaw torque and we measure the fly's ability to fly straight (optomotor balance). We now add to the angular velocity of each pattern a constant rotatory bias, clockwise for p2 and counterclockwise for p1. If the fly brain would simply process the summed output of its motion detectors, it would have only one yaw torque value to obtain optomotor balance. However, *Drosophila* occasionally adjusts its mean yaw torque to the two yaw torque levels which compensate for the rotatory bias of one of the two patterns separately. We show that the percentage of time spent with this 'stabilization behavior' is strongly influenced by the pattern contrast, although the flies ability to perceive the single patterns independently is not influenced by the contrasts used. Furthermore, we investigated the temporal dynamics of the stabilization behavior as well as the behavior of *Drosophila* in this paradigm without feedback (open loop).
The findings imply that from each set of dots moving coherently the fly can derive a separate 'hypothesis' about self-rotation, and tries to confirm it with optomotor balance.

Processing of chromatic and polarized light stimuli in the central brain of the bumblebee

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Bees possess a time-compensated sky-compass and use it for spatial orientation and navigation. When sunlight enters the earth's atmosphere, it is scattered by gas molecules, which results in a pattern of polarized light and a chromatic gradient along the celestial sphere. Bees were the first animals in which perception of the sky polarization pattern and its use in orientation tasks was shown. Since this seminal discovery by Karl von Frisch in 1949, a wealth of studies have been undertaken to investigate behavioral aspects of bees' orientation with regard to polarized light. It has also been shown that bees are able to use chromatic cues to distinguish between the solar and the antisolar hemisphere.

On the other hand, our knowledge about the neuronal representation and the processing of sky-compass cues in the bee brain is extremely limited. Here we present data from our morphological and electrophysiological studies of the polarization vision pathway in bees.

Using dye injections, we were able to trace the polarization vision pathway from the dorsal rim area of the compound eye via the medulla, the anterior optic tubercle and the bulbs of the lateral complex into the central complex. The neurons within this pathway, as well as 9 types of neuron that we stained within the central complex were strikingly similar to polarization sensitive neurons in locusts and monarch butterflies.

Using intracellular recordings, we investigated the responses of central-complex neurons to polarized light and to the azimuth and wavelength of an unpolarized light spot. Several types of neuron in the central complex were sensitive to the plane of polarized light and, in addition, to the azimuth of an unpolarized light spot.

Our study shows, that both the morphology and the physiology of neurons within the polarization-vision pathway is highly conserved between different insect species.

Neural coding of the hierarchy of celestial compass cues in an insect brain

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Despite their tiny brains, many insects are remarkable navigators. Of these, some diurnal (*Scarabaeus lamarcki*) and nocturnal (*S. satyrus*) dung beetles have developed a unique orientation behavior to avoid competition for food at the dung pat. They cut off a piece of dung, form it into a ball and roll it away along straight paths. To keep a straight path, both species rely on celestial compass cues such as the sun, the moon or the skylight polarization pattern. We analyzed the hierarchy of these cues in both species in the field by setting the sun position in conflict with polarization pattern. We found that the sun is the main reference for the internal compass in diurnal beetles, whereas in nocturnal beetles the lunar polarized light is the dominant cue. Interestingly, if we force nocturnal beetles to roll during the day, they switch to the celestial body (the sun) as primary cue, while the diurnal species continues to use a celestial body compass (the moon) during the night.

To formulate an understanding of the neuronal mechanism behind this flexible cue hierarchy system neurons from the beetles' putative internal compass network, the central complex are analyzed through intracellular recordings in the presence of an artificial sun, moon (green light spot moving on a circular path around the beetle's head) or skylight polarization (dorsally rotating polarizer). We found that all cells responded to both stimuli when stimulated in sequence, and thus encoded both celestial cues. When presented with both cues simultaneously, neurons in the diurnal species were tuned only to the light spot independent of the light intensity. Interestingly, the same neurons in the nocturnal species responded only to the light spot at high light intensities but switched to an exclusive response to polarized light when the light spot intensity fell to lunar levels. Thus, in the beetle central complex single neurons encode a hierarchy of different celestial cues and can alter this hierarchy according to the ambient light conditions, accurately reflecting our behavioral findings. In summary, our data clearly show that the visual ecology of an animal dictates the neural activity and defines how a nocturnal visual system is adapted to dim light orientation.

Context-dependent signaling of sky-compass cues in an insect brain

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The polarization pattern of skylight can provide a compass-cue that is used for allocentric orientation by various insect species, e.g. to steer a steady course during long-distance migrations. Studies in the desert locust *Schistocerca gregaria* have identified ascending pathways of polarization-sensitive neurons which converge in the central complex of the brain. The central-complex network involves neurons tuned to the electric field vector (*E*-vector) of polarized light with preferred *E*-vector angles varying along columns of neuropils in a compass-like fashion (polarotopy).

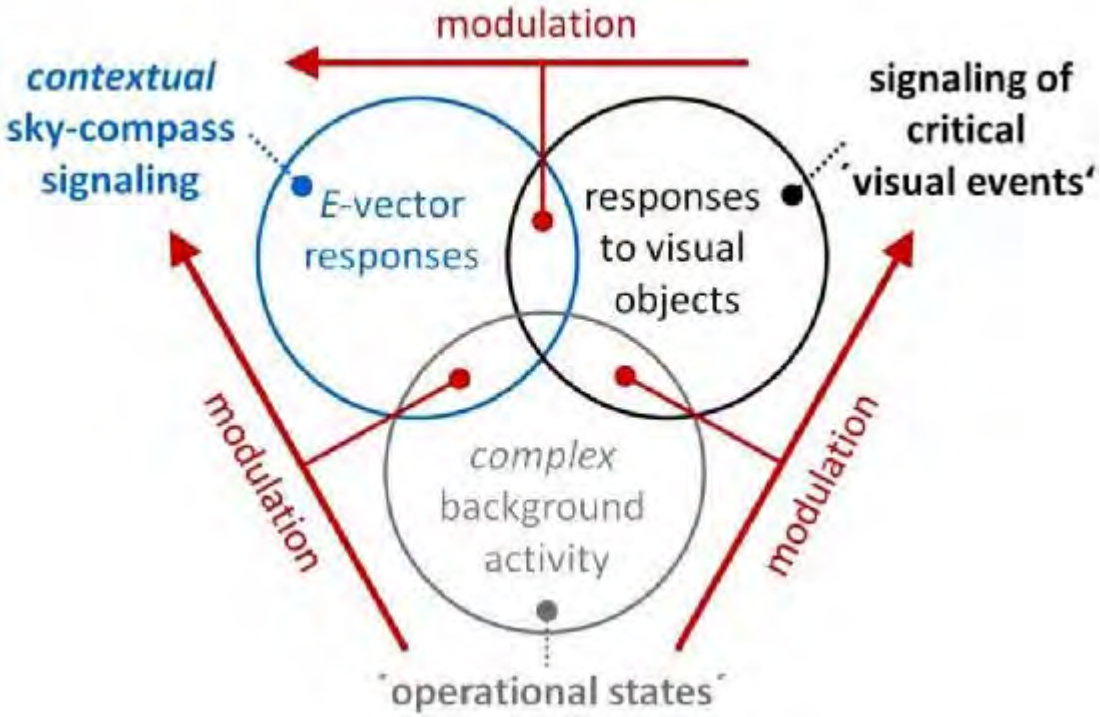
We strive to elucidate how activity in this polarotopic population is modulated and possibly integrated with representations of additional cues to control compass-guided locomotion. Here, we report on three phenomena suited for this task (summarized in the figure below): 1.) the adaption of responses to stationary *E*-vector angles, 2.) their disadaptation by salient events of visual motion and 3) the modulation of visual responsiveness by states of background activity.

Adaptation may correlate with the tendency to steer a steady course which was observed in tethered flying locusts.

The disadaptation of responses by events of sudden small-field motion against the visual background might serve to prepare for spatial re-orientation in the near future, e.g. following escape maneuvers in response to approaching objects.

A masking of *E*-vector responses by increased background activity could serve to 'ignore' compass information in the planning of motor actions other than compass-guided locomotion. In vertebrates, co-shaping by dynamical background – activity was shown to explain the large variability of both neuronal responses (V1) and to correlate with changes in perceptual state and behavioral responses to visual cues.

Together, these processes can serve for a context-dependent signaling of compass cues as they may link polarization-signaling to behavioral goals and to critical events in the visual scenery.


modulation
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complex
background
activity
'operational states'

Symposium

S7: Contribution of astrocyte connexins to neuroglial interaction in the healthy and diseased brain

S7-1 Characterization of Panglial Gap Junction Networks in the Murine Brain
Stephanie Griemsmann, Simon P. Höft, Peter Bedner, Jiong Zhang, Elena von Staden, Anna Beinhauer, Joachim Degen, Pavel Dublin, David W. Cope, Nadine Richter, Vincenzo Crunelli, Ronald Jabs, Klaus Willecke, Martin Theis, Gerald Seifert, Helmut Kettenmann, Christian Steinhäuser

S7-2 Deletion of astrocytic connexin 43 causes a narcolepsy-like phenotype
Philip, G. Haydon, Jerome Clasadonte

S7-3 Relevance of the interaction between Connexin43 and c-Src in astrocytoma cells
Arantxa Tabernero, Ester Gangoso, Ana González-Sánchez, Myriam Jaraíz, José M Medina

S7-4 Chronic hemichannel activation in astrocytes contributes to neuronal suffering in a murine model of Alzheimer's disease.
Chenju YI, Christian GIAUME, Annette KOULAKOFF

S7-5 Calcium signalling and vesicle-related proteins in different astrocyte culture types
Anne C. Wolfes, Saheeb Ahmed, Camin Dean

S7-6 FUSION PROPERTIES OF GLIOTRANSMITTER VESICLES IN CULTURED ASTROCYTES
Alenka Gucek, Jernej Jorgacevski, Priyanka Singh, Claudia Geisler, Nina Vardjan, Marko Kreft, Alexander Egner, Robert Zorec

Characterization of Panglial Gap Junction Networks in the Murine Brain

Stephanie Griemsmann[1], Simon P. Höft[1], Peter Bedner[1], Jiong Zhang[1], Elena von Staden[1], Anna Beinhauer[1], Joachim Degen[2], Pavel Dublin[1], David W. Cope[3], Nadine Richter[4], Vincenzo Crunelli[3], Ronald Jabs[1], Klaus Willecke[2], Martin Theis[1], Gerald Seifert[1], Helmut Kettenmann[4], Christian Steinhäuser[1]

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Glial cells are connected with each other via gap junction channels. For this purpose they express distinct sets of connexin (Cx) proteins. Astrocytes express Cx26, 30 and 43, while oligodendrocytes express Cx29, 32 and 47. In this study, we focused on glial cells in the thalamus, which plays important roles as a relay station for sensory information in the central nervous system. Although glial cells participate in this activity, little is known about their properties. Experiments were performed in wild type and transgenic mice with glia specific fluorescence labelling as well as in Cx knock-out mice. Astrocytes were investigated between postnatal days 9-60.

The formation of coupled networks between astrocytes and oligodendrocytes in the murine ventrobasal thalamus was investigated and their properties compared with those in the hippocampus and cortex. Biocytin filling of individual astrocytes or oligodendrocytes revealed large panglial networks in all three gray matter regions. In the thalamus panglial networks were already established in juvenile mice. Network formation was exclusively between astrocytes and oligodendrocytes, while other glial cells or neurons were not involved.

Combined analyses of mice with cell type-specific deletion of Cx, semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) and western blotting showed that Cx30 is the dominant astrocytic Cx in the thalamus. Many thalamic astrocytes even lack expression of Cx43, while in the hippocampus astrocytic coupling is dominated by Cx43. Deletion of Cx30 and Cx47 led to complete loss of panglial coupling, which was restored when one allele of either Cxs was present. Immunohistochemistry revealed a unique antigen profile of thalamic glia and identified an intermediate, mature cell type expressing both Olig2 and Cx43.

Together, these results indicate that thalamic astrocytes differ in various aspects from their counterpart in other brain regions and support the emerging concept of astrocyte heterogeneity.

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Deletion of astrocytic connexin 43 causes a narcolepsy-like phenotype

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Astrocytes express connexin 43 although its role in the regulation of brain circuits is unclear. Using astrocyte specific cre recombinase we have performed astrocyte specific deletion of the Cx43 gene (Cx43 KO) and determined the behavioral consequences. Using EEG and EMG recordings we monitored the sleep phenotype of these mice and found that during the light phase, wakefulness, rapid eye movement sleep (REM) and non REM (NREM) sleep were unaffected. However, during the dark phase, the subjective daytime of the mice, we found excessive daytime sleepiness and fragmented wakefulness characteristic of narcoleptic phenotype. Because of the importance of orexinergic neurons of the lateral hypothalamus in narcolepsy we asked whether their activity is altered in astrocytic Cx43 KO mice. Compared to littermate controls we found that the activity of orexinergic neurons in Cx43 KO mice was significantly reduced. Because of previous studies that have linked Cx43 dysfunction with altered astrocyte-to-neuron lactate shuttling we asked whether lactate might contribute to this phenotype. In preliminary studies we have found that addition of lactate to Cx43KO mice restored the normal tonic activity of orexinergic neurons raising the possibility that an astrocytic source of lactate is critical for the normal activity patterns of orexinergic neurons and as a consequence for normal daily cycles of wakefulness.

Relevance of the interaction between Connexin43 and c-Src in astrocytoma cells

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Connexin43 (Cx43), the main gap junction channel-forming protein in astrocytes, is down-regulated in malignant astrocytomas. These tumors are composed of a heterogeneous population of cells that includes many with stem-cell-like properties, called glioma stem cells (GSCs), which are highly tumorigenic and lack Cx43 expression. Interestingly, restoring Cx43 reverses GSC phenotype and consequently reduces their tumorigenicity. In this study, we investigated the mechanism by which Cx43 exerts its antitumorigenic effects on GSCs. We have focused on the tyrosine kinase c-Src, which interacts with the intracellular carboxy tail of Cx43. We found that Cx43 regulates c-Src activity and proliferation in human GSCs expanded in adherent culture. Thus, restoring Cx43 in GSCs inhibited c-Src activity, which in turn promoted the down-regulation of the inhibitor of differentiation Id1. Id1 sustains stem-cell phenotype since it controls the expression of Sox2, responsible for stem cell self-renewal, and promotes cadherin switching, which has been associated to epithelial-mesenchymal transition. Our results show that both the ectopic expression of Cx43 and the inhibition of c-Src reduced Id1, Sox2 expression and promoted the switch from N- to E-cadherin, suggesting that Cx43, by inhibiting c-Src, down-regulates Id1 with the subsequent changes in stem-cell phenotype. Based on this mechanism, we found that a cell-penetrating peptide, containing the region of Cx43 that interacts with c-Src, mimics the effect of Cx43 on GSC phenotype, confirming the relevance of the interaction between Cx43 and c-Src in the regulation of the malignant phenotype and pinpointing this interaction as a promising therapeutic target.

Chronic hemichannel activation in astrocytes contributes to neuronal suffering in a murine model of Alzheimer's disease.

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We have previously shown that the expression of astroglial connexins, proteins forming gap junction channels and hemichannels, increased close to amyloid (Aß) plaques in brains from Alzheimer's disease (AD) patients and from APPswe/PS1dE9 mice, a murine model of AD. Since, in brain slices, acute Aß application triggers astroglial Cx43 hemichannel activity leading to neuronal degeneration, we have investigated hemichannel function in APPswe/PS1dE9 mice (9 months) that exhibit Aß plaques. Ethidium bromide (EtBr) uptake in acute brain slices was used as index of hemichannel activity and was quantified in GFAP-immunostained astrocytes. The respective contribution of connexins (Cx) and pannexins (Panx) to the hemichannel activity was analyzed by using selective blockers of each family.
We have shown that hemichannels were activated in all hippocampal astrocytes with a higher EtBr uptake in reactive astrocytes contacting Aß plaques than in distant ones. While Cx43 was the major hemichannel contributor in the overall population of astrocytes, a minor Panx1 component (about 25%) was solely identified in astrocytes contacting plaques. Distinct pathways were involved in Cx and Panx hemichannel activation. Inflammation triggered Panx1 hemichannels since their activity was inhibited by minocycline or TNFa+IL1ß antagonists, but did not affect Cx43 hemichannels. The latter were triggered by the high astroglial [Ca2+]i measured in fluoro-4AM loaded slices. Reducing [Ca2+]i to physiological level by blocking mGluR5, purinergic or IP3/RyR receptors inhibited Cx43 hemichannel activation but not Panx1. Then we have shown that astroglial hemichannel activation allowed for ATP, glutamate and D-serine release. Finally we have shown that the lack of astroglial Cx43 prevented neuronal damage in APPswe/PS1dE9 mice. Indeed in APPswe/PS1dE9 with an astroglial targeted knocking out of Cx43 gene, the level of oxidative stress in pyramidal neurons and the abundance of neuritic dystrophies associated with Aß plaques were strongly reduced. Hence, blocking astrocyte hemichannels could represent an alternative therapeutic strategy in AD.
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Calcium signalling and vesicle-related proteins in different astrocyte culture types

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Astrocytes are multi-taskers: Not only are they vital for brain homeostasis, nutrient supply to neurones, and protecting against pathogens via the blood-brain barrier, but they also influence signalling at neuronal synapses. Astrocytes are hypothesized to release gliotransmitters (e.g. glutamate, ATP, D-serine) at so-called “tripartite synapses” – connections between a pre- and post-synaptic neurone and an astrocytic process. If this involves exocytosis, identifying the molecular mechanism is key to understanding how astrocytes communicate with neurones. In neurones, SNARE complexes steer exocytosis, and synaptotagmins (syts) promote vesicle fusion upon calcium influx.

Interestingly, astrocytes were reported to express specific isoforms of the SNARE proteins, and to further contain mRNA of distinct syt isoforms (Mittelsteadt *et al*. J Comp Neurol, 2009), which hints at functional similarities in vesicle fusion. Yet, much of this data comes from astrocyte monocultures that differ greatly from those *in vivo*. Growing *in vivo*-like astrocyte monocultures is now possible using immunopanning (Foo *et al*. Neuron, 2011), although this is also not ideal due to time, cost and a relatively low yield. To circumvent these problems and investigate astrocytic signalling and vesicle protein distribution in *in vivo*-like astrocytes, we created a simple protocol by which *in vivo*-like astrocytic monocultures can be generated quickly and easily by growing them in neuronal medium to mimic the environment in the brain.

Using this protocol, we tested for the presence of three vesicular glutamate transporter isoforms in astrocytes, and found differences in expression depending on the protocol used to generate astrocytes. We further confirmed that several of the 17 syt isoforms are expressed in astrocytes. These isoforms responded differently to stimulation (with KCl and glutamate) in astrocytes transfected with pHluorin-syt reporters of vesicle fusion. Thus, different syt isoforms may regulate distinct types of fusion events in astrocytes. Our data indicate that syt-7 and syt-17 are present in discrete puncta in astrocytic processes, including those adjacent to synapses between neurones in astrocyte/neurone co-cultures. These syt isoforms may thus play a role in signalling via exocytosis at tripartite synapses.

In addition, we found differences in calcium signalling between distinct astrocyte and astrocyte/neurone co-cultures infected with Gfap promoter-driven GCaMP3 (to express GCaMP3 specifically in astrocytes). *In vivo*-like astrocytes maintain thin, branched processes, which actively transmit localized calcium transients within and between astrocytes.

Our data suggest that syts and SNARE complex proteins may mediate exocytosis in *in vivo*-like astrocytes. Future experiments may reveal mechanisms by which factors are released from astrocytic vesicles to affect synaptic transmission and circuit function in the brain.

FUSION PROPERTIES OF GLIOTRANSMITTER VESICLES IN CULTURED ASTROCYTES

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Astrocytes, the most abundant type of glial cells, actively participate in brain signalling by releasing a plethora of gliotransmitters which can modulate synaptic transmission. Mechanisms of release are under debate. In addition to non-vesicular mechanisms, the vesicular release of gliotransmitters appears to have many advantages in terms of “economy” and spatial signalling versatility. However, the anatomy and nature of exocytotic vesicle interaction with the plasma membrane is still unclear. Using stimulated emission depletion (STED) and structured illumination (SIM) super-resolution microscopies, we studied the morphology of gliotransmitter vesicles, whereas the interaction between a single vesicle with the plasma membrane was monitored by the high-resolution cell-attached patch-clamp measurements of membrane capacitance (C_m), a parameter linearly related to the surface area of the plasma membrane. Immunolabelling of vesicles containing D-serine, glutamate, atrial natriuretic peptide (ANP) and brain derived neurotrophic factor (BDNF) yielded their diameter to be ~70 nm, whereas ATP was found in larger vesicles (~200 nm diameter). High-resolution cell-attached C_m measurements revealed that the predominant mode of vesicle interaction with the plasma membrane consisted of reversible capacitance steps, reflecting transient exocytosis, whereas irreversible capacitance steps, reflecting full-fusion exocytosis, were less abundant. The diameters of vesicles fusing with the plasma membrane ranged from 35 - 800 nm. Reversible unitary exocytotic events exhibiting transient fusion were found in two different populations, the first corresponding to smaller vesicles with diameters around 70 nm and the second corresponding to larger vesicles with the median diameter at 200 nm, consistent with the STED and SIM measurements. Upon stimulation with ATP, which increases intracellular Ca^{2+} concentration, the smaller vesicles persisted in transient fusion mode, whereas larger vesicles proceeded to full-fusion exocytosis. This was interpreted previously to be due to different SNARE protein densities in different sized vesicles and was tested here by astrocyte treatment with botulinum neurotoxin D (BotD), which significantly reduced the occurrence of unitary exocytotic events for both vesicle types. Additionally, when we expressed the dominant-negative SNARE (dnSNARE; a truncated cytosolic SNARE domain of synaptobrevin 2) in astrocytes, the fusion-pore diameter was narrower in both vesicle types. Taken together, this study shows that vesicle content discharge is modulated by vesicle size, however independently of the functional integrity of the SNARE proteins.

Symposium

S8: The ontogeny of entorhinal circuitry and function

S8-1 Postnatal development of parahippocampal-hippocampal connectivity.
Menno Peter Witter

S8-2 Searching for feedback: contribution of the entorhinal processing to the development of prefrontal-hippocampal communication
Ileana L. Hanganu-Opatz

S8-3 Pacemakers and waves: developing pre-grid cell networks of rodent medial entorhinal cortex
Rhiannon Meredith, Julia Dawitz, Johannes Hjorth, Tim Kroon, Valery Dassen, Huibert Mansvelder, Marta Ruiperez-Alonso

S8-4 The development of the neural map of space in the hippocampal formation
Francesca Cacucci

S8-5 Optogenetic control of hippocampal theta oscillations reveals their function in locomotion via hippocampus to lateral septum pathway.
Franziska Bender, Maria Gorbati, Marta Carus Cadavieco, Natalia Denisova, Xiaojie Gao, Constance Holman, Tatiana Korotkova, Alexey Ponomarenko

S8-6 Backpropagating Action Potentials mediate plasticity of spine calcium dynamics in the medial entorhinal cortex
Anne-Kathrin Theis, Ulrike Pannasch, Martin Rückl, Sten Rüdiger, Dietmar Schmitz, Friedrich Johenning

Postnatal development of parahippocampal-hippocampal connectivity.

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The discovery of a variety of spatially modulated neurons in the entorhinal cortex has triggered an interest to unravel the functional relationships between these cells in entorhinal cortex and those found in other parahippocampal structures, such as the pre- and parasubiculum on the one hand and the hippocampus on the other hand. Understanding development of these networks may provide hints on how the functionality of individual neurons or the networks they form is correlated with the presence or absence of functional connectivity. With the use of anatomical anterograde and retrograde tracing and in vitro slice physiology we studied the development of connections in the network and observed that all connections appear at around postnatal day 0 (P0). Although projections are initially sparse, they develop according to topographical patterns seen in the adult brain. We did not observed unspecific distribution of projections and subsequent pruning, rather projections increase in density and level of branching within a restricted adult-like pattern. Connections seem initially not very active, but an abrupt change in activity at specific postnatal days characterizes each connection. In case of projections from entorhinal cortex to hippocampus, we noticed that a dorsolateral-to-ventromedial axis of origin in the entorhinal cortex maps onto a dorsoventral axis of termination already in the first week postnatally. Likewise, projections from subiculum to entorhinal cortex, pre-and parasubiculum develop early and follow a clear transverse gradient of origin that relates to complex but adult like termination patterns. Finally, projection patterns from pre- and parasubiculum to medial entorhinal cortex look adult like from P0 onwards. When stimulated in slices, these projections start to evoke excitatory postsynaptic potentials only around P8/9.

The present data thus indicate the areas within the parahippocampal-hippocampals system structurally wire together in an early postnatal period in line with adult patterns. This connectivity becomes active again early on, in all instanced before eye opening. These findings are corroborated by in vivo electrophysiological recordings, showing that around eye opening at P15, when rats start to actively explore their environment, their navigational system is likely to be fully functioning.

Searching for feedback: contribution of the entorhinal processing to the development of prefrontal-hippocampal communication

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Precise information flow during mnemonic and executive tasks requires the co-activation of prefrontal and hippocampal networks in oscillatory rhythms. This interplay emerges early in life with hippocampal theta bursts driving the generation of prefrontal theta-gamma oscillations. In the absence of monosynaptic reciprocal communication, the question arises whether indirect feedback interactions control this early drive. Here, we demonstrate that prefrontal -hippocampal activation couples the discontinuous theta oscillations and neuronal firing in both entorhinal cortex and ventral midline thalamic nuclei of neonatal rats. However, these two areas have a different contribution to the neonatal long-range communication. The entorhinal cortex mainly acts as a hippocampal modulator and phase-locks the hippocampal firing via direct axonal projections. In contrast, the thalamic theta -band entrainment is controlled by mutual interactions with the prefrontal cortex and drives the hippocampal activation. Thus, the neonatal prefrontal cortex controls the level of hippocampal activation by directed feedback interactions.

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Pacemakers and waves: developing pre-grid cell networks of rodent medial entorhinal cortex

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Mature medial entorhinal cortex (MEC) contains grid cells whose firing occurs in regular grid-like patterns across an environment. Grid cells emerge during the third week of postnatal development in rodents. It is hypothesised that grid cells develop from topographic modules early in development. However, activity of MEC networks prior to grid cell formation is largely unknown. Using a combination of multiphoton calcium imaging and cell-specific electrophysiology, we show a highly synchronous module, that includes stellate cells, developing during the second postnatal week. Synchrony is strongly developmentally-regulated and intrinsic to MEC, with sparse distribution of potential pacemaker-like cells within superficial layers. Both superficial and deep layer activity is tightly-correlated. Unlike mature MEC, network activity is dominated by glutamatergic excitation and not driven by neighbouring hippocampal regions. An intrinsically-generated, synchronous module dominates activity of immature MEC prior to eye-opening and navigation: we propose that it plays a key role in establishing circuitry necessary for adult grid cell function.

The development of the neural map of space in the hippocampal formation

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The hippocampal formation contains several classes of neuron which respond to an animal's position and orientation in space. To discover how this neural representation of space develops, we recorded single neurons in the hippocampal formation, in freely-moving rat pups, from postnatal day 12 (P12) onwards.
Place cells in the hippocampus are present from at least P14, whereas grid cells emerge later, at around P20. We therefore set out to investigate which spatial inputs drive place cells in young rat pups, in the absence of grid cells in the entorhinal cortex.
We find evidence that boundary-responsive cells are present in the hippocampal formation from at least P16, and furthermore, that the recording arena walls stabilise hippocampal place fields in pre-weanling pups. Environmental boundaries may therefore be a foundational input for the development of the hippocampal neural representation of space.

Optogenetic control of hippocampal theta oscillations reveals their function in locomotion via hippocampus to lateral septum pathway.

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Hippocampal theta oscillations (5-12 Hz) support encoding of animal's position during spatial navigation. Yet, longstanding questions about the role of theta rhythm in initiation and maintenance of locomotion remained unanswered. In this study we implemented optogenetic control of hippocampal theta oscillations by combining ChR2 -driven theta frequency stimulation of projections of the theta rhythm generator, medial septum, to hippocampus, with electrophysiological monitoring of hippocampal network oscillations and neuronal activity. We found that the regularity of theta amplitude and frequency stabilizes running speed during exploratory behavior, i.e. an increased temporal regularity of the theta-rhythmic hippocampal output determined a more regular and slower running speed. Hippocampal theta oscillations were coordinated with theta -rhythmic activity in the lateral septum (LS), main subcortical output of hippocampus. Inhibition of this pathway, using chemo (DREADDs) - or optogenetics (eNpHR3.0), revealed its necessity for theta-rhythmic control of running speed. Theta frequency stimulation of LS projections to lateral hypothalamus, where neurons displayed locomotion-dependent firing, replicated the reduction of running speed by increased regularity of hippocampal theta oscillations.Thus hippocampus to LS pathway translates changes of hippocampal theta synchronization into rapid adaptive adjustment of running speed.

Backpropagating Action Potentials mediate plasticity of spine calcium dynamics in the medial entorhinal cortex

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Backpropagating action potentials are known to calcium transients in dendritic spines. We show an enhancement of these calcium transients that is mediated by activity. Our experiments were performed in the medial entorhinal cortex of rats with an age between P16 and P25 - an age were the spine number increases and grid cell firing develops. We compared calcium transient enhancement in entorhinal cortex spines with the enhancement in CA1 pyramidal cell spines. Entorinal cortex spines have a higher initial calcium transient amplitude than in CA1 spines and more spines are sensitive to enhancement. Comparison of morphological parameters revealed that spines in the entorhinal cortex have longer necks compared to CA1 spines but head size is the same. We propose the plasticity of spine calcium transients to constitute a storage mechanism of neuronal suprathreshold activity patterns that is more pronounced in entorinal cortex spines than in CA1 spines.

Symposium

S9: Processing of acoustic pulse patterns: Common themes in different brains?

S9-1 Neural network and mechanism for sound pattern recognition in the cricket brain
Stefan Schöneich, Konstantinos Kostarakos, Berthold Hedwig

S9-2 Temporally selective processing of communication signals by frog auditory midbrain neurons
Jakob Christensen-Dalsgaard

S9-3 Keeping time: Processing of auditory communication cues in the brainstem
Anna Katarina Magnusson

S9-4 Chronic calcium imaging of neuronal ensembles in the mouse auditory cortex
Dominik Florian Aschauer, Jens-Bastian Eppler, Matthias Kaschube, Simon Rumpel

S9-5 How to measure signal periodicity, if you must?
J. Leo van Hemmen

Neural network and mechanism for sound pattern recognition in the cricket brain

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From human language to birdsong and the chirps of insects, acoustic communication is based on amplitude and frequency modulation of sound signals. Whereas frequency-processing starts at the level of the hearing organs, temporal features of the sound amplitude like rhythms or pulse rates require processing by central auditory neurons. Besides several theoretical concepts, the brain circuits that detect temporal features of the signal are poorly understood. Here we show how five neurons in the brain of female field crickets form an auditory feature-detector circuit for the pulse pattern of the male calling song. Auditory responses to the calling song are forwarded towards the brain via a single ascending interneuron. A small circuit of identified local brain neurons recognizes the species-specific sound pulse pattern and exhibits properties fundamental to a feature detection circuit based on delay-line and coincidence-detection mechanism. A constant internal delay that matches the pulse period of the calling song is provided by a non-spiking brain neuron. Upon acoustic stimulation it receives a transient inhibition that triggers postinhibitory rebound depolarization. Direct (ascending neuron) and delayed (non-spiking neuron) excitatory responses converge in a coincidence detector neuron. The coincidence detector neuron responds best to the pulse pattern of the species-specific calling song when the rebound activation of the non-spiking neuron temporally coincides with the response of the ascending interneuron to the subsequent sound pulse. The output of the coincidence detector neuron is further processed by a feature detector neuron that receives an additional inhibitory input to suppress unselective responses and background activity. The sparse but highly pattern selective spike response of the feature detector neuron closely matches the pulse period tuning of the phonotactic behavior. The circuit provides the basis for auditory mate recognition in field crickets and reveals principal mechanisms of sensory processing underlying the perception of temporal patterns. (The research was supported by the BBSRC and the Isaac Newton Trust)

Temporally selective processing of communication signals by frog auditory midbrain neurons

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For anurans as for other sound communicating animals temporal processing is important for species recognition. In most species, vocal communication consist of relatively simple signals with a regular temporal structure, and animals have been shown to be sensitive to alterations of temporal structure in behavioral experiments, such as call rate and call duration. In a few species, part of the neural processing of temporal structure has been identified, and neurons selective for AM-rate, call duration and number of pulses have been described.

We have investigated the processing of temporal patterns from the auditory nerve to the midbrain (inferior colliculus)in the aquatic frog Xenopus, and will highlight the changes from representation of call patterns in auditory discharges to temporal selectivity and describe models for this selectivity.

Keeping time: Processing of auditory communication cues in the brainstem

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The temporal structure of slow sound amplitude fluctuations (sound envelope) is an important cue for the perception of complex acoustic stimuli. For instance, perception of human speech relies heavily on accurate processing of the sound envelope. The neuronal mechanisms by which the sound envelope is extracted and conveyed to higher auditory areas are unknown.
The Superior Paraolivary Nucleus (SPON) is a prominent structure in the mammalian auditory brainstem, which is characterized by its ability to fire with high precision to the sound envelope or sound gaps. I will present results which reveal how SPON neurons are equipped to accomplish this task. Using a combination of electrophysiology and mathematical modelling, we show that interplay between four ion channels tune these neurons to extract temporal information contained in behaviorally relevant communication sounds. Subtle differences in the channel combinations creates variability that ensures coverage of the entire temporal register but that emphasizes tuning for species-specific calls This is an example of how the brain transforms a temporal pattern of periodic signals into a neural representation of the acoustic envelope, important for perceiving speech and music.

Chronic calcium imaging of neuronal ensembles in the mouse auditory cortex

Dominik Florian Aschauer[1], Jens-Bastian Eppler[2], Matthias Kaschube[2], Simon Rumpel[1]

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Recently (Bathellier et al., 2012), we found that local neuronal ensembles of the auditory cortex encode broad sound categories into non-linear population response modes and generate a basis set of perceptual categories that can be used to drive behavioral decisions. To understand how such local neuronal ensembles emerge in the network, the key requirement is to monitor neuronal population activity chronically over extended periods of time. Classical neurophysiological approaches, like extracellular recordings of action potentials, have largely not been able to provide chronic observations due to technical limitations. Towards this goal we tested and characterized several genetically encoded calcium indicators in vivo, and established a procedure for awake, chronic two-photon recordings of sound responses. Using AAV-mediated expression of the genetically encoded calcium indicator GCamP6m under the transcriptional control of the pan-neuronal synapsin1 promoter enabled measurements of a few hundred individual cells simultaneously. Alignment of neuronal populations within a recording session to compensate for movements of the animal and alignment across imaging sessions at a two day interval is greatly facilitated by the virally mediated co-expression of a nuclear marker (H2B::mCherry) under the same promoter that can be spectrally unmixed from the calcium indicator. We developed automated image analysis routines that allow compensation for movement and detection of frames that cannot be properly aligned. Our dataset will serve to quantitatively analyze the stability of functional neuronal ensembles putatively forming auditory representations over the period of several days to more than a week. This question is of fundamental interest given the volatility in structural connections between neurons of the neocortex even during adulthood as observed in chronic imaging studies of dendritic spines.

How to measure signal periodicity, if you must?

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Given a sequence of *n* spike times, how can their periodicity, if any, be measured ? Simple as the question sounds, it hits a key problem both in nature and in neuroscience. An explanation will be given of how the simple geometric algorithm of (resonating) vector strength does the job. As for the animals that must discern periodicity, it will be shown how balanced inhibition can serve to identify low-frequency signal periodicity and how variation of a single parameter, the inhibitory time constant, can tune the system to different frequencies.

Symposium

S10: Microcephaly and developmental defects of the brain

S10-1 NEURAL STEM AND PROGENITOR CELLS AND NEOCORTEX EXPANSION IN DEVELOPMENT AND EVOLUTION
Wieland Bernhard Huttner

S10-2 Mechanisms of microcephaly, and their links to development and evolution of the human brain
Pierre Vanderhaeghen

S10-3 Modeling human brain development and disease in 3D culture
Magdalena Renner, Madeline A. Lancaster, Juergen A. Knoblich

S10-4 Microcephaly – from bedside to bench
PD Dr. Angela M. Kaindl

NEURAL STEM AND PROGENITOR CELLS AND NEOCORTEX EXPANSION IN DEVELOPMENT AND EVOLUTION

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Our group studies the molecular and cellular mechanisms of neurogenesis in the developing neocortex in the context of mammalian brain evolution, specifically the various types of cortical stem and progenitor cells and their modes of division. With regard to (i) the site of mitosis along the apical-basal axis of the cortical wall and (ii) the absence or presence of ventricular contact at mitosis, three principal classes of cortical stem/progenitor cells can be distinguished. First, stem/progenitor cells that reside in the ventricular zone (VZ) and that contact the ventricle where they undergo mitosis, i.e. the neuroepithelial cells, apical radial glial cells and apical intermediate progenitor cells, collectively referred to as apical progenitors (APs). Second, stem/progenitor cells that reside in the subventricular zone (VZ) where they typically undergo mitosis and that have delaminated from the ventricle, i.e. the basal (or outer) radial glial cells and basal intermediate progenitor cells, collectively referred to as basal progenitors (BPs). Third, stem/progenitor cells that undergo mitosis in the basal VZ or in the SVZ and that retain ventricular contact, called subapical progenitors.

Our group has been studying the following issues related to these stem/progenitor cells in the developing mouse, ferret, marmoset, macaque and human neocortex: (1) the various lineages from APs to BPs; (2) the machinery underlying BP delamination; (3) symmetric versus asymmetric cell divisions; (4) the microcephaly gene Aspm; (5) the AP marker prominin-1/CD133; (6) membrane particles released into the ventricle; (7) extracellular matrix, integrins, and progenitor self-renewal; (8) cell cycle length; (9) transcriptomes of embryonic mouse and fetal human neocortical germinal layers and specific progenitor subpopulations.

Recent insights into neural stem/progenitor cell biology and the development and evolution of the neocortex will be presented.

Mechanisms of microcephaly, and their links to development and evolution of the human brain

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Human primary microcephaly (MCPH) is an autosomal-recessive neurodevelopmental disorder which affects only the brain with a major reduction in the size of the cerebral cortex. Mutations in the abnormal spindle-like microcephaly associated (ASPM) gene are the most common cause for primary microcephaly. So far, different animal models failed to faithfully recapitulate the human disease phenotype, so that the function of the protein still remains elusive in this context. We developed a human model to study cortical development in vitro and to model MCPH. We generated induced pluripotent stem (iPS) cells from skin fibroblasts obtained from four MCPH patients displaying different mutations in the ASPM gene. By developing a simple three-dimensional (3DM) differentiation system in growth factor-reduced conditions, we generated 3DM neuroepithelial structures displaying appropriate apico-basal polarity and dorsal telencephalon/cortical identity, which can differentiate into cortical neurons with layer-specific identity. This early cortical 3D model revealed several several abnormalities in the specification and differentiation of ASPM-mutated cortical progenitors, which sheds new light on the function of ASPM and the mechanisms of human MCPH.

Modeling human brain development and disease in 3D culture

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The human brain is highly unique in size and complexity. While many of its characteristics have been successfully studied in model organisms, recent experiments have emphasized unique features that can not easily be modeled in animals. We have therefore developed a 3D organoid culture system derived from human pluripotent stem cells that recapitulates many aspects of human brain development. These cerebral organoids are capable of generating several brain regions including a well-organized cerebral cortex. Furthermore, human cerebral organoids display stem cell properties and progenitor zone organization that show characteristics specific to humans. Finally, we use both RNAi and patient specific iPS cells to model microcephaly, a human neurodevelopmental disorder that has been difficult to recapitulate in mice. This approach reveals premature neuronal differentiation with loss of the microcephaly protein CDK5RAP2, a defect that could explain the disease phenotype. Our data demonstrate an in vitro approach that recapitulates development of even this most complex organ, which can be used to gain insights into disease mechanisms.

Microcephaly – from bedside to bench

PD Dr. Angela M. Kaindl[1]

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Microcephaly is defined as a reduction of the head circumference. It is often associated with intellectual disability and can be caused by environmental and/or genetic factors. In corresponding pedigrees, a multitude of mutant genes has been identified in the past decades, but further genetic heterogeneity exists, and mechanisms by which these regulate cognitive function and brain size remain to be elucidated. Despite the vast increase of known hereditary microcephaly disorders, the diagnosis remains unclear in about 40% of patients with this clinical sign. I will present out approach to the diagnosis of rare genetic microcephaly disorders and present some recently identified novel hereditary microcephaly entities. Identifying genes linked to microcephaly can improve our understanding of the function of the respective genes and their role in nervous system development.

Symposium

S11: Ultramicroscopy for imaging the central nervous system and its pathological alterations

S11-1 Technical advances in ultramicroscopy and their application for investigating neuronal development and diseases.
Nina Jährling, Klaus Becker, Bettina Wegenast-Braun, Stefan Grathwohl, Mathias Jucker, Edgar Kramer, Saiedeh Saghafi, Christian Hahn, Hans-Ulrich Dodt

S11-2 Visualizing neuronal structures in translucent adult mouse brain with light sheet fluorescence microscopy
Guenter Giese, Annemarie Scherbarth, Rolf Sprengel, Johann Engelhardt, Patrick Theer, Martin K. Schwarz

S11-3 RNA-Seq screening identifies critical regulators of axon growth and regeneration
Andrea Tedeschi, Sebastian Dupraz, Claudia S. Laskowski, Jia Xue, Thomas Ulas, Marc Beyer, Joachim L. Schultze, Frank Bradke

S11-4 Imaging of whole-mount samples with 1 µm resolution using light-wedge- microscopy
Ulrich Leischner

S11-5 Imaging and quantification of dopaminergic neurons of the mouse using ultramicroscopy
Edgar Richard Kramer, Karsten Tillack, Helia Aboutalebi, Nina Jährling, Hans-Ulrich Dodt

Technical advances in ultramicroscopy and their application for investigating neuronal development and diseases.

Nina Jährling[1,2], Klaus Becker[1,2], Bettina Wegenast-Braun[3,4], Stefan Grathwohl[3,4], Mathias Jucker[3,4], Edgar Kramer[5], Saiedeh Saghafi[1,2], Christian Hahn[1,2], Hans-Ulrich Dodt[1,2]

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Alzheimer Disease AD is a progressive neurodegenerative disorder, which is characterized by a variety of neuropathological abnormalities, such as the extracellular deposition of aggregated amyloid-beta peptide (Aß) in the brain parenchyma. In brains of transgenic APPPS1 mice these ß-amyloid deposits can be visualized and quantified by ultramicroscopy (UM) after staining by systemic dye loading and chemical clearing. We measured the total number of amyloid plaques, the amyloid load (volume percent) and the amyloid plaque size distributions in a defined volume in the frontal cortex of two age groups (2.5 versus 7.-8.5 mo). We demonstrate that UM is an easy, relative quick and direct method for counting uncropped ß-amyloid deposits in 3D.

Axons of motor and sensory neurons span long distances towards their targets. Tracing of these long nerves is a difficult task, as they are orientated in 3D, frequently split and generally do not take the shortest path to their target. In a neurodevelopmental project we applied UM to analyze axon guidance by growth factors as RET/GDNF. For this we visualized the topographic projections of motorneuron axons in the hind limb of mouse embryos.

In the recent years UM has been subject of various histological and optical developments. We present a new way of signal preserving using a particular resin, latest improvements in light sheet generator optical unit, and final developments of imaging optics corrected for refractive index mismatch.

Visualizing neuronal structures in translucent adult mouse brain with light sheet fluorescence microscopy

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Neuronal populations projecting directly onto defined source cells in the entorhinal cortex of adult mice were labeled with green fluorescent protein using a Rabies virus-based technology. Due to the properties of our modified Rabies virus, EGFP positive neurons are classified to be monosynaptically connected to the source cell populations. In search for a simple and straightforward sample preparation which allows visualization of neurons and their neurites throughout the entire mouse brain, we developed a modification of the standard BABB (Murray's Clear) optical clearing procedure. Both level and long-term stability of fluorescent protein fluorescence were significantly improved, in combination with excellent clearing of adult mouse brains. For detection and imaging of fluorescent structures in whole brain preparations, we built a light sheet microscope suitable for recording from small animal brain samples. This system allowed us to repetitively image neuronal structures labeled with different fluorophores, even allowing detection of single axons and axon bundles throughout the uncut adult mouse brain. Imaging was possible down to several millimeters in depth, at almost confocal resolution. Fluorescent labels and tissue geometry were well preserved even after two years in clearing solution. In addition, we performed a comparative quantitative analysis of the differential distribution of Rabies virus -infected, EGFP-expressing neuronal cells. By 3D reconstruction we were able to generate a spatial map of brain regions monosynaptically connected to the entorhinal cortex area. *(Rabies virus technology by Martin K. Schwarz[1,5])*

RNA-Seq screening identifies critical regulators of axon growth and regeneration

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Traumatic central nervous system (CNS) injuries often result in permanent disabilities due to axon regeneration failure. Mechanistically, both non-permissive environment and reduced intrinsic growth ability have been proposed to account for the regenerative failure in the adult. While progress has been made in characterizing extracellular growth inhibitors expressed in the adult CNS, our current understanding of the molecular mechanisms that lead to neurons losing their ability to regenerate is still fragmentary. Here we used a systematic and unbiased approach to identify genes whose expression correlated both positively with loss of axon elongation during later stages of embryonic development, and negatively with conditions necessary to gain growth competence in the adult. RNA-Seq screening identified a developmental switch gene that limits axon growth and regeneration. *In vitro* and *in vivo* silencing or pharmacological blockade following administration an important therapeutic class of drugs promoted axon growth. Using three-dimensional imaging of the unsectioned spinal cord, we showed that the same pharmacological approach is sufficient to promote axon regeneration *in vivo*. Given that our pharmacological approach is already used clinically to manage a wide range of neurological disorders, our results could significantly impact on the development of therapies aimed at promoting structural plasticity and regeneration following a variety of CNS trauma.

Imaging of whole-mount samples with 1 µm resolution using light-wedge- microscopy

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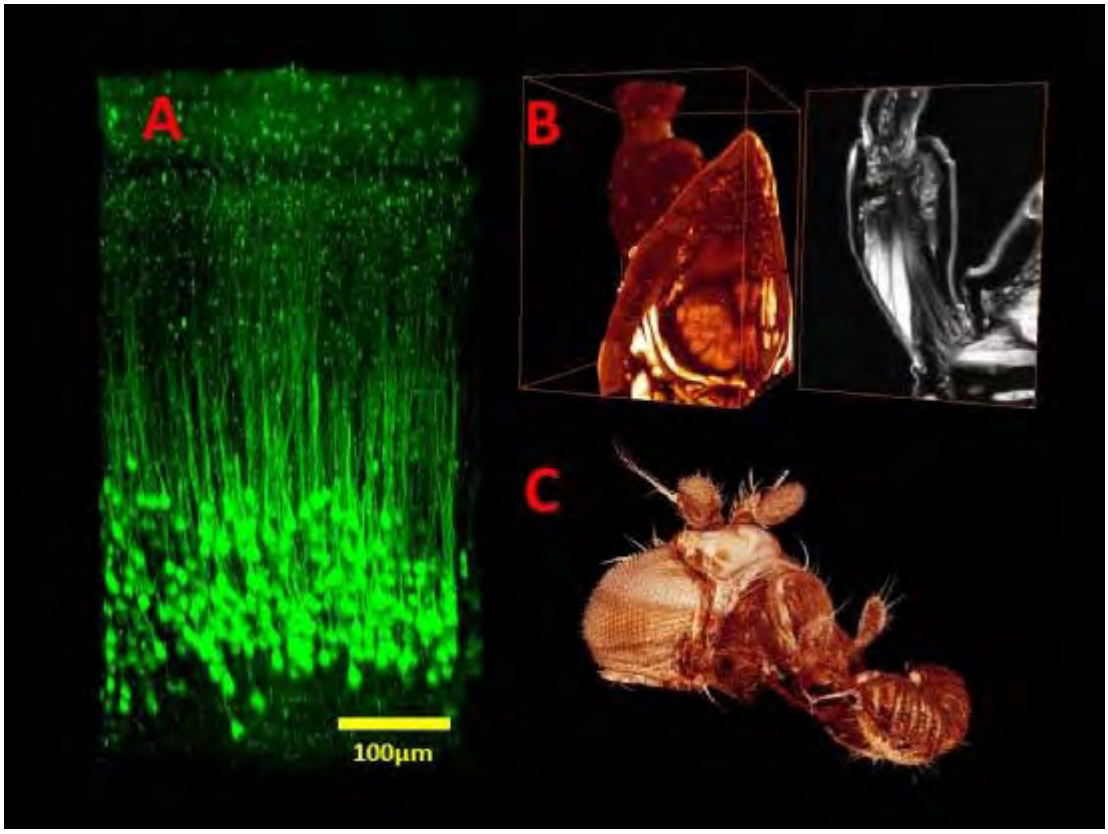
Imaging large objects with light-sheet microscopy is facing two major drawbacks: light sheets for a homogeneous illumination for millimeter-large fields of view are quite thick, resulting in a poor depth resolution of several micrometers(1, 2). This is about 5-10 times worse than the x-y resolution, making the overall data difficult to analyze and to display. The other disadvantage is a technical constrain: imaging deep inside large objects requires long working distances, both for the illumination- and for the imaging optics. These long working distances are very sensitive to aberrations due to refractive index mismatch, making the use of different embedding media(3-5) for increasing the transparency more difficult.

We addressed these problems by developing a custom optics for the generation of ultra-thin light-sheets that can be adapted to most of the refractive indices of the utilized embedding media. The illumination optics also includes a scanning technique that allows for placing the µm-thin focus line at any lateral position in the field of view, allowing for the acquisition of several light-sheet images with the thin illumination focus at different positions. These images can be combined to one overall image of high quality. In the imaging path, we compensated the image distortions due to aberration by a custom deconvolution.

These modifications of light-sheet microscopy allow for the generation of 3D fluorescence images in a different domain: It gathers data with an isotropic resolution of ~1µm in all 3 dimensions of objects with a cubic size of 2-3mm, not possible with other techniques. The micro-CT technique is imaging with a bit worse resolution and similar object size, but it does not allow for the use of fluorescent tags, nor is it suited for displaying non-absorbing substances like fat tissue. We show the advantages of this technique on image data cortex with YFP-labeled cells, as well as several insects (e.g. Drosophila melanogaster, and Zorotypus weideneri).

1. H.-U. Dodt et al., Ultramicroscopy: three-dimensional visualization of neuronal networks in the whole mouse brain. Nat Methods 4, 331 (Apr, 2007).
2. U. Leischner, W. Zieglgänsberger, H.-U. Dodt, Resolution of Ultramicroscopy and Field of View Analysis. PLoS ONE 4(6), 1 (June 2009, 2009).
3. K. Becker, N. Jährling, S. Saghafi, R. Weiler, H. U. Dodt, Chemical clearing and dehydration of GFP expressing mouse brains. PloS one 7, e33916 (2012).
4. K. Chung et al., Structural and molecular interrogation of intact biological systems. Nature 497, 332 (2013).
5. M.-T. Ke, S. Fujimoto, T. Imai, SeeDB: a simple and morphology-preserving optical clearing agent for neuronal circuit reconstruction. Nature neuroscience 16, 1154 (2013).

Figure 1: Images from this light-wedge microscopy. A: Cortex of a mouse with YFP-labeled cells.
B: Antennal connection of the insect Zoraptera weideneri with olfactory glomeruli (160x210 µm).
C: Head of Drosophila melanogaster (after deconvolution, length: 1100µm)


A
B
C
100µm

Imaging and quantification of dopaminergic neurons of the mouse using ultramicroscopy

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Currently, estimating the number of dopaminergic neurons in the mouse brain by stereology is the gold standard in the field to get quantitative data about the dopaminergic cell number.
Here we developed a new absolute quantification method for fluorescently labeled cells and other structures by performing ultramicroscopy on whole glass-body brains, and applied that for imaging and counting dopaminergic neurons. We made use of the genetic Cre-lox system in mice to strongly fluorescently label specifically dopaminergic neurons which is nicely compatible with the generation and analysis of conditional knockout mice and transgenic mice. This method is not only more precise than stereology but also much faster, less cumbersome and less artefact-producing since no standard histological techniques are required like embedding, cutting, staining, mounting and re-aligning of the sections for 3D representation.
A commercial ultramicroscope setup with a light sheet producing laser, an object table and a camera with objective lenses can replace the need for a microscope for stereology requiring a motorized stage, a stereology program and a fast camera. Rehydration of the tissue after ultramicroscopy preserves the tissue integrity and fluorescent signal in dopaminergic neurons which allows performing classical histology and immunohistochemistry to co-localize other proteins in dopaminergic neurons or label and quantify additional cell types and structures in this tissue.

Symposium

S12: Breaking News I

S12-1 *Conditional mutants of Bassoon in excitatory forebrain synapses and dopaminergic synapses, to study its contribution in learning and memory processes*
Anil Annamneedi, Horst Schicknick, Anna Fejtová, Wolfgang Tischmeyer, Oliver Stork, Eckart D Gundelfinger

S12-2 Learned Helplessness in *Drosophila melanogaster*- does it transfer to other behavior?
Sophie Batsching, Alice Weiglein, Reinhard Wolf, Prof. Martin Heisenberg

S12-3 Exploring the effects of ß2-adrenergic receptor agonists in DOK7 congenital myasthenic syndrome
Lisa K J Clausen, Judy Cossins, David Beeson

S12-4 Chronic study of spontaneous activity and orientation selectivity in visual cortex around eye opening
Bettina Hein, Klaus Neuschwander, Gordon B. Smith, David E. Whitney, David Fitzpatrick, Matthias Kaschube

S12-5 Opposing effects of cAMP-effectors PKA and Epac on activity-dependent BDNF secretion in dissociated hippocampal neurons
Jan-Hendrik Heyne, Petra Lichtenecker, Tanja Brigadski, Volkmar Leßmann

S12-6 Influence of mtDNA single nucleotide polymorphisms on age dependent changes of memory function
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Conditional mutants of Bassoon in excitatory forebrain synapses and dopaminergic synapses, to study its contribution in learning and memory processes

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Abstract: Bassoon, a large scaffolding protein, is one of the core components of the cytomatrix at the active zone (CAZ) of neurotransmitter release at excitatory, inhibitory and modulatory presynapses, which plays an important role in various aspects of presynaptic plasticity[1]. Bassoon involves in regulated neurotransmitter release from glutamatergic synapses[2] and the regulation, specifically of P/Q-type Ca2+ channels[3]. We have generated two conditional Bassoon mutants, one lacking the protein in excitatory synapses of the forebrain and another lacking the protein in dopaminergic synapses. We used Emx1-Cre mice and DAT-Cre mice, to specifically inactivate floxed exon 2 of the Bassoon gene in forebrain excitatory neurons (forebrain Bsn conditional knock-out: *B2E cKO*) and midbrain dopaminergic neurons (dopaminergic Bsn conditional knock-out: *B2D cKO*) respectively. Immunohistochemical stainings confirmed the lack of Bassoon in excitatory synapses of *B2E cKO* forebrain and dopaminergic terminals in the striatum of *B2D cKO* mice. We show that *B2E cKO* mice, when compared to the controls, display a stronger fear response (measured as high freezing behavior) towards the shock context, during hippocampus-dependent contextual fear memory retrieval and hyperactive behavior in home cage activity test. Interestingly, *B2D cKO* mice also display hyperactive behavior in home cage activity test, specifically during dark phase. In addition, *B2D cKO* mice display anxious behavior during exploration in open field test. Previously it was shown that, glutamate and dopamine receptor activation is required[4,5] in auditory cortex dependent learning paradigm-FMTD, which in turn causes changes at the presynaptic protein regulation[6]. Current studies are designed to elucidate the role of Bassoon in FMTD paradigm and furthermore dissect the role of Bassoon in excitatory hippocampal synapses during fear memory processes and in dopamine mediated functions, utilizing both *B2E* and *B2D cKO* mice respectively.

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Learned Helplessness in *Drosophila melanogaster*- does it transfer to other behavior?

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If external conditions change for an animal in such a way, that innate behavior for controlling the situation (e.g. an escape reflex) does not help anymore, the animal may learn to suppress the behavior.
It is well known from vertebrates that exposure to uncontrollable stress can lead to changes in behavior that do not occur, if the stress is controllable. For instance Seligman & Maier (1967) showed that dogs first exposed to unescapable electric shock, later fail to learn to escape in a shuttle box.
We study how the fly *Drosophila melanogaster* copes with uncontrollable stress. We use the shock box (Kapustjansky, 2012) to apply electric shocks as aversive stimuli and to monitor a single fly's behavior in a restrained and stressful situation. Recently Yang et al. (2013) showed that flies reduce walking speed and take longer rests if they are exposed to uncontrollable heat pulses.
In the present study we expose flies for 20 minutes to a random sequence of shock pulses (80V AC) and investigate their behavior afterwards. For instance, we measure their free walk in an open field and their courtship behavior.

Exploring the effects of ß2-adrenergic receptor agonists in DOK7 congenital myasthenic syndrome

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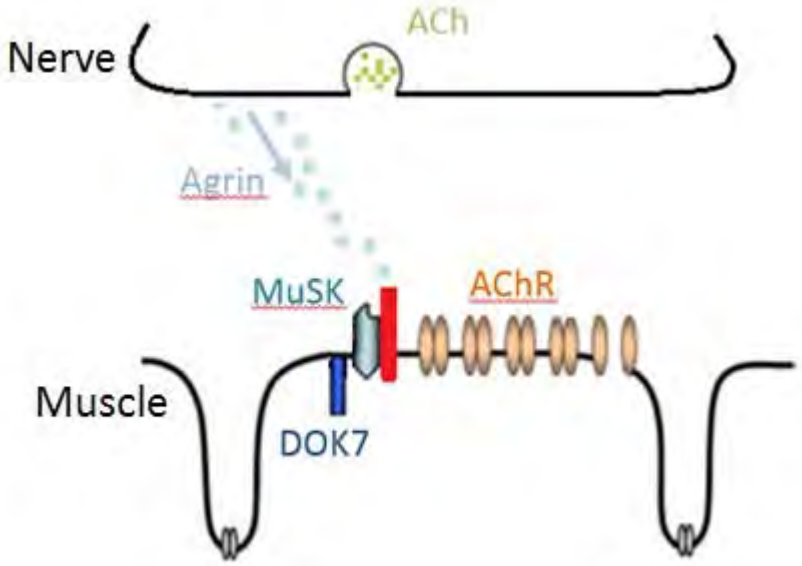
Congenital myasthenic syndrome (CMS) is a group of inherited disorders characterised by muscle weakness. In order for a muscle to contract, signal transmission needs to occur between nerve and muscle at specialised synapses called neuromuscular junctions. Acetylcholine is released from the nerve terminal and binds to acetylcholine receptors (AChRs), which have to be densely clustered on the muscle surface. The key player in the AChR clustering pathway is muscle specific kinase (MuSK) which is activated through the nerve derived protein agrin. In about 20% of cases CMS is caused by mutations in the gene DOK7. DOK7 protein is a muscle intrinsic activator of MuSK and essential for sufficient clustering of AChRs. Mutations in DOK7 have been shown in cultured cells to reduce clustering.

Clinical studies of DOK7 CMS have revealed a beneficial response to ß2-adrenergic receptor agonists, such as salbutamol. Since it is only poorly understood how salbutamol increases muscle strength in patients, we conducted experiments on the underlying molecular mechanisms. We hypothesise that cAMP/PKA mediated second messenger signalling of the ß2-adrenergic receptor might increase stabilisation of the synaptic structure and/or feed into the impaired DOK7 signalling pathway to provide a compensatory signal. To investigate this hypothesis, we used AChR clusters formed on C2C12 mouse myotubes, which provide a model of the postsynaptic synapse. AChR clustering was induced by overexpression of DOK7 or by incubation of wild type myotubes with agrin.

We first investigated whether salbutamol increases AChR cluster numbers in DOK7 mutant cells. The common c.1124 -27dupTGCC mutation was overexpressed by retroviral infection and myotubes were incubated with salbutamol. The results indicate a dose - dependent increase in cluster numbers. Interested in the optimisation of treatment strategies, we tested novel, previously uncharacterised ß2-agonists. To investigate whether salbutamol enhances the stabilisation of AChR clusters on the membrane, the effects of salbutamol incubation during dispersal of clusters were studied. Agrin-induced clusters break down following the wash-off of agrin. We demonstrated that salbutamol significantly reduces the dispersal of clusters. These effects could be abolished through selective blocking of the ß2-adrenergic receptor.

We recently adapted the CRISPR/Cas9 gene editing technology as a novel and exciting approach to modeling disease mechanisms in CMS. CRISPR genome engineering enables stable expression of variants found in patients without overexpression of the affected protein. Mutations can be introduced to neuromuscular proteins endogenously expressed by C2C12 cells. Unlike with retroviral overexpression of DOK7, where AChR clustering occurs in the absence of agrin, it is now possible to study the effects of DOK7 mutations and their treatment strategies on agrin induced clustering. First results from this novel and more physiological model of DOK7 CMS will be reported.

In summary, the results obtained so far provide first evidence that ß2-adrenoreceptor agonists directly affect proteins involved in neuromuscular transmission. The effects shown in vitro provide insights into the mechanism causing destabilisation at the neuromuscular junction in patients and will contribute towards the development of more potent treatment strategies for patients suffering from CMS.


ACh
Nerve
Agrin
MuSK
AChR
Muscle
DOK7

Chronic study of spontaneous activity and orientation selectivity in visual cortex around eye opening

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Spontaneous activity within the cortex potentially has a significant impact on the wiring of neuronal circuits during early developmental stages. However, at present we know very little about the structure of spontaneous activity in developing cortical circuits, and how this activity shapes sensory representations. Here we use longitudinal epi-fluorescence imaging of GCaMP6 population activity in ferret visual cortex to characterize the visually evoked and spontaneous patterns of activity prior to and after eye opening (between PD20 and PD40) and to assess their relation to the mature orientation preference map.
At the earliest times we see relatively little spontaneous activity, consisting of isolated activity blobs, which typically last a few seconds and occur with a frequency as low as <1/min. Closer to eye opening, events become more frequent and often large regions are active during an individual event. This tendency continues after eye opening. Moreover, both prior to and after eye opening, spontaneous activity typically exhibits a modular spatial structure, resembling the activity patterns evoked by moving gratings after eye opening. A cross-correlation analysis reveals that spontaneous activity patterns are highly diverse. Two distinct patterns can occupy almost complementary domains on the cortical surface. Starting prior to eye opening, spontaneous patterns of activity are often correlated with the mature orientation preference map and this correlation increases with age. Intriguingly, if a spontaneous pattern resembles a given single condition map, it often does so throughout the imaged region. Consistently, the cross-correlation shows little change when varying the size of the area over which it is computed.
We conclude that columnar patterns of spontaneous activity emerge and increase in frequency prior to eye opening, are highly structured, and potentially comprise templates later used by the mature cortex to represent sensory input.

Opposing effects of cAMP-effectors PKA and Epac on activity-dependent BDNF secretion in dissociated hippocampal neurons

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The mammalian neurotrophin brain-derived neurotrophic factor (BDNF) is an important modulator of a variety of brain functions. The protein is secreted in an activity-dependent manner and is involved in synaptic plasticity processes. The second messenger cAMP is another important modulator of synaptic plasticity. While involvement of cAMP in the secretion of peptides in neuroendocrine cells as well as neurons has been studied, little is known about the influence of cAMP and its downstream pathways on fusion pore opening of BDNF-containing secretory granules.

In our study, we now have investigated the relevance of the second messenger cAMP and its downstream pathways for the release of BDNF-containing secretory granules in dissociated hippocampal neurons from mice. Neurons were transfected with GFP-tagged BDNF and fusion pore opening of BDNF-containing secretory granules was analysed by combining whole cell patch-clamp recording and live cell imaging. Measurements were performed in the presence of the fluorescence quencher bromphenol blue to investigate fusion pore dynamics of BDNF-containing secretory granules. Fusion pore opening was induced by repetitive backpropagating action potential (bAP) firing upon electrical stimulation. While bAP-induced fusion pore opening of BDNF -containing secretory granules was facilitated by simultaneous electrical stimulation and application of cAMP analog 8 -Br-cAMP, fusion pore opening of BDNF-containing granules was inhibited by 8-Br-cAMP application, when delivered before electrical stimulation. This reduction of fusion events was also observed upon application of the selective Epac agonist 8-Br-2-O-Me-cAMP-AM which was applied before electrical stimulation. Simultaneous electrical stimulation and application of the Epac agonist as well as application of the selective PKA agonist 6-Phe-cAMP irrespective of timing of application increased release probability of BDNF-GFP.

Our results show that the cAMP-effectors PKA and Epac can exert opposing effects on bAP-induced BDNF-release from hippocampal neurons and that the negative effect mediated by Epac signaling is critically dependent on timing of the Epac activation.

Influence of mtDNA single nucleotide polymorphisms on age dependent changes of memory function

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Mitochondrial metabolism plays a pivotal role especially in organs of high energy demand such as brain. Age dependent alteration of organ function / memory decline is assumed to be linked to mitochondrial dysfunction. In fact, a number of studies indicate surreptitiously generated mitochondrial reactive oxygen species (ROS) as a possible driving force of the ageing process. Ageing basically constitutes an accumulation of diverse pathological changes of an organism during life. Among other, successive changes of mitochondrial DNA e.g. single nucleotide polymorphisms (SNPs) also are suspected to underlie the ageing process. As part of a collaborative project, our group focused on the neurophysiological implications of altered respiratory chain enzymes. We used mouse strains with stable SNPs, representative for mutated OXPHOS enzyme complexes. We gauged memory function and ROS production at defined time points (3, 6, 12 and 24 months), covering most of the expected mouse life span. Spatial learning ability was analyzed *in vivo* using Morris Water Maze tests, and synaptic plasticity i.e. long-term potentiation (LTP) was determined in hippocampal field-EPSP (fEPSP) recordings *in vitro*. Furthermore, superoxide anion levels were measured using MitoSOX and confocal microscopy. Mutations in complex I and IV were associated with memory dysfunction, with an early-onset memory decline in complex I mutated mice, and, by contrast, a severe late-onset memory impairment in complex IV mutated mice. Increased ROS levels were not directly linked to these changes, and their impact on memory function is, if at all, delayed by months.

The Impact of MicroRNAs in Memory Formation Processes in the Honeybee (*Apis mellifera*)

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Understanding the molecular processes during the training phase is an important aspect of explaining the fundamentals of memory formation. In addition to the well understood functions of the second messenger cascades in memory formation, recent investigations proved microRNAs to be important players in these molecular processes. These short (19- 25 nt), non-coding ribonucleic acids regulate processes like degradation of mRNA and silencing of gene expression at the post-transcriptional level. Because little is known about microRNAs in learning, this work aims to identify microRNAs involved in memory formation and synaptic plasticity in the honeybee brain. Of special interest are microRNAs that change their levels due to associative learning. The role of these learning modulated microRNAs will be analyzed by impairing their function via application of appropriate tools as antisense oligomers and chemicals. The molecular targets of the microRNAs and their function in mediating the behavioral outcome will be analyzed. Focusing on the microRNAs that are regulated by learning we expect to gain a better understanding in learning induced effects on microRNA function and their implication in memory formation.

Identification of novel spinocerebellar ataxia disease genes using next generation sequencing approaches

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To date, 37 different dominantly inherited spinocerebellar ataxia (SCA) types are known. Currently, genetic testing of the most frequent SCA genes via routine DNA diagnostic screening leaves 30% of the cases genetically undiagnosed. To further elucidate the genetic basis of this disorder, we performed whole exome sequencing (WES) to identify the disease-causing mutation in 20 Dutch families with hereditary cerebellar ataxia without a genetic diagnosis, followed by targeted sequencing of all candidate genes obtained via WES in 96 seemingly independent cerebellar ataxia cases that were send in for regular diagnostic screening. The sequencing array also contained all known rare SCA genes that are not included in current diagnostic analysis.

We performed WES on 40 individuals preferably from two affected cousins per multiplex family if possible, or parent-child trios, with one affected parent and one affected child in simplex families. The data was prioritized using Ingenuity software focusing on the damaging variants including truncating-, missense- and splice site variants. All variants with a minor allele frequency higher than 0,1% were excluded. The remaining variants were prioritized for cerebellum and/or Purkinje cell expression. Finally, all selected variants were validated by Sanger sequencing and co-segregation analysis.

Using WES, we could identify a single genetic cause in 2 families, and identified 5 mutations in genes known to be involved in SCA6 (*CACNA1A*), SCA14 (*PRKCG*), SCA21 (*TMEM240*), and SCA40 (*CCDC88C*). However, we identified multiple plausible candidates (n=39) in the remaining families. Upon targeted sequencing of the 96 cases, we identified again mutations in *CACNA1A* and *PRKCG*, and 11 not previously reported, *in silico* predicted likely pathogenic variants in the genes causing SCA5, 11, 15/16, 28 and 35, revealing the genetic basis of 16% of these cases. In 8 WES candidate genes, we identified one or multiple additional cases that carried likely pathogenic variants. These genes play a role in pathways involved in cerebellar neurodegeneration and include neurogenesis, synaptic transmission and cell-cell adhesion. Additionally, chromatin remodeling seems to be important in the etiology of SCA.

Currently, we are functionally validating these novel genes in vitro and in vivo to gain novel insights in the disease mechanisms underlying cerebellar neurodegeneration and ataxia. Combining WES with targeted sequencing is an excellent method to identify new disease genes.

Computational modeling of lesion induced dendritic reorganization

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In response to the loss of inputs, deafferentated neurons retract and remodel their dendritic tree. Here we used computer models to predict the electrical responses of dentate granule cells after lesioning their inputs. We show that lesion induced retraction of granule cell dendrites leads to a precise adjustment of the excitability enabling the remaining synapses to drive the neuron. In addition, our models predict that dendritic retraction selectively enhances action potential backpropagation in the retracted dendritic area, which is likely to affect synaptic plasticity. Both these features are exquisitely homeostatically tuned as a result of alterations in the general electrotonic passive properties of dentate granule cells.

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The olfactory hole-board test: A new paradigm to study behavior to biologically-relevant odors

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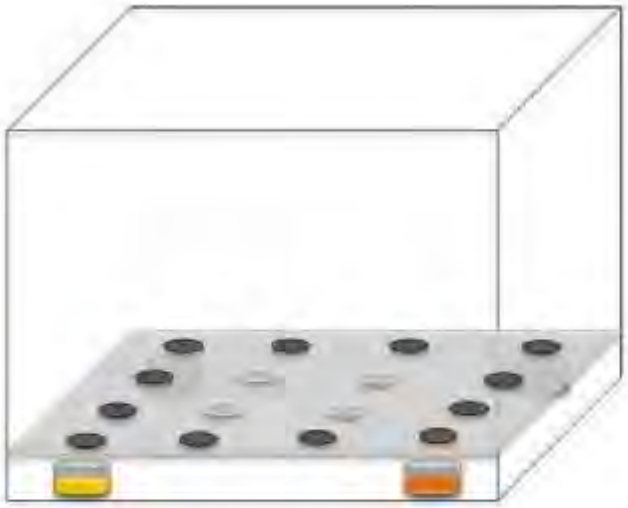
Rodents, like most mammalian species, rely on their olfactory sense to guide their behaviors. In this context, especially odors of biological relevance (e.g. predator odors, sex odors, odors of spoiled food) are known to effectively influence basic survival needs such as mating behaviors, anti-predatory defensiveness and foraging abilities. However, living in an odor-enriched environment also necessitates the capability of the animals to precisely identify and discriminate single odors from odorant mixtures. Research focused on the effects of biologically-relevant odors on rat behavior, often include multi-trial paradigms where animals experience a sequence of single odor exposures.

Here, we introduce a novel experimental setup, the olfactory hole-board test, that allows to study the effects of different olfactory cues on rat's behavior within a single trial. Very shortly, the hole-board apparatus consists of an open field chamber with 12 holes (four corner holes, eight border holes) in the floor into which an animal can poke it's head, referred to as head-dipping behavior. During testing, different odor samples are placed in each corner hole.

Our first experiment demonstrates that the number of head dips into holes containing a predator odor sample is remarkably reduced when compared to the number of visits of the control odor hole. At the same time, animals visited more often the hole with a urine sample of an estrous rat. To determine whether the observed lack of predator odor hole visits is simply the result of the repugnant properties of urine itself rather than its fear-inducing properties, we simultaneously exposed rats in a second experiment to urine samples of a carnivore, herbivore and omnivore and to the control odor. Our data show, that rats clearly discriminate between carnivore urine and herbivore/omnivore urine samples. However, animals still visited the control odor hole more often than the holes containing herbivore and omnivore urine. In a third experiment, using urine samples of female rats differing in the estrus cycle-phase, we observed that sexually-naive rats did not differentiate between estrus cycle-phase specific urine samples while ca. 40% of the sexually-experienced rats preferred urine from the proestrus phase. The remaining 60% preferred all odor samples equally.

To test whether anxiolytic-like treatments specifically modulate the visits of predator odor-containing holes, but not of control/ female odor-containing holes, we pretreated rats with the benzodiazepine midazolam (0, 0.19, 0.38 mg/kg) or the 5-HT1A receptor agonist buspirone (0, 0.1, 1 mg/kg). The predator odor-induced reduction of hole visits was completely abolished by pretreatment with buspirone with minor effects on total locomotor activity, while midazolam dose-dependently sedated rats resulting in decreasing numbers of head dips in total.

Taken together, our results demonstrate that the olfactory hole-board test is a valuable tool for studying olfactory preferences for biologically-relevant odors and thereby the link between olfaction and emotional behavior, in rats. This new olfactory paradigm is broadly applicable and also sensitive to anxiolytic treatment.



Schematic drawing of the *olfactory hole-board* testing apparatus.

Symposium

S13: Functional consequences of sensory loss and restoration

S13-1 DEAF AUDITORY CORTEX MEDIATES ENHANCED FACE PERCEPTION IN THE CONGENITALLY DEAF
Stephen Lomber, Alex Meredith, Andrej Kral

S13-2 Plasticity with single-sided deafness: representational maps and binaural interactions
Andrej Kral, Jochen Tillein, Peter Hubka

S13-3 Sensitive phases for the development of multisensory processes
Brigitte Röder

S13-4 The neural correlates of hearing colors and shapes: insights from darkness on brain plasticity and stability
AMIR AMEDI

S13-5 Cortical plasticity following sensory deprivation: Characterizing the patterns of thalamocortical and corticocortical projections in early- and late-deaf cats
Blake Edward Butler, Stephen Gordon Lomber

DEAF AUDITORY CORTEX MEDIATES ENHANCED FACE PERCEPTION IN THE CONGENITALLY DEAF

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When the brain is deprived of input from one sensory modality, it often compensates with supernormal performance in one or more of the intact sensory systems. In the absence of acoustic input, it has been proposed that “deaf” auditory cortex may be recruited to perform visual cognitive functions. To test this hypothesis we examined the visual capabilities of four adult congenitally deaf cats and four adult hearing cats on a battery of visual cognitive tasks to define which visual abilities are involved in cross-modal compensation. The animals were tested on their abilities to both learn and recall seven different pattern or object discriminations: simple patterns (geometric black shapes), complex patterns (geometric black shapes with borders or overlays), simple objects (geometric black objects), junk objects, natural scenes (2-dimensional pictures), and faces (both human and conspecific). Both the deaf and hearing cats learned to discriminate the simple patterns, complex patterns, simple objects, junk objects, and natural scenes at similar rates. However, the deaf cats were significantly faster at learning (fewer trials and errors to criterion) both the human and conspecific faces compared to the hearing cats. Abilities to recall any of the visual discriminations were no different between the hearing and deaf cats. These results demonstrate that deaf subjects possess enhanced visual cognitive abilities compared to hearing subjects. The second part of this study was to examine if cross-modal reorganization in auditory cortex may be contributing to the superior cognitive capabilities of the deaf cats. To accomplish this, we bilaterally placed cooling loops on A1, A2, the temporal auditory field (TAF), and insular cortex (area IN) to permit their individual deactivation. Bilateral deactivation of A1, A2, or area IN, did not alter learning rates for either the human and conspecific faces. However, bilateral deactivation of TAF resulted in the elimination of the enhanced face (both conspecific and human) discrimination learning capabilities of the deaf cats and resulted in performance similar to hearing cats. Unilateral deactivation of right TAF resulted in no decrease in the enhanced face learning abilities of the deaf cats, while unilateral deactivation of left TAF resulted in a partial, but significant, decrease in the enhanced face learning abilities of the deaf cats. These results provide evidence of a lateralization in the enhanced face learning abilities of the deaf cats. Overall, our results show that enhanced visual cognition in deaf cats is caused by cross-modal reorganization within “deaf” auditory cortex and that it is possible to localize individual visual functions within reorganized auditory cortex.

Plasticity with single-sided deafness: representational maps and binaural interactions

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Previous studies have shown that early single-sided deafness leads to a plastic reorganization of aural preference in the brain (Kral et al., 2013, Brain; Kral et al., 2013, Front Syst Neurosci). The representation of the hearing ear is stronger (larger local field potentials and higher evoked neuronal firing rates) and the evoked responses appear earlier than with stimulation of the deaf ear. Here the sensitivity to binaural cues has been investigated in the auditory cortex and compared binaural and monaural properties of neurons with cochlear implant stimulation in adult normal hearing cats (HCs), congenitally deaf cats (CDCs) born deaf on both ears, and cats born with unilateral deafness but normal hearing on the other ear (uCDCs). In CDCs the monaural response thresholds, dynamic ranges and spontaneous activity were significantly reduced compared to HCs. There were fewer excitatory-excitatory (EE) responses and more 0E responses, but fewer binaural facilitation in CDCs. uCDCs showed weaker responses to the deaf ear compared to the hearing ear. The monaural and binaural responsiveness depended on the relation of the recorded cortex and the hearing ear in uCDCs. The cortex ipsilateral to the hearing ear reorganized extensively, with more EE and less E0 responses. The cortex contralateral to the hearing ear demonstrated more E0 responses and more suppressive interactions. Facilitatory binaural interactions were similarly reduced in CDCs and uCDCs. ITD sensitive units were rare in uCDCs and mostly observed in the contralateral cortex. The ipsilateral cortex had more flat or non-classified ITD responses. In total, unilateral deafness prevented nonspecific deficits in responsiveness, but reorganized the hemispheres differently, with more extensive reorganizations at the cortex ipsilateral to the hearing ear. Finally, binaural interactions and ITD sensitivity were extensively reduced in unilateral deafness. The data on one hand support the absence of compensatory changes in binaural cue representation following single-sided deafness. The results demonstrate significant loss of sensitivity to binaural interactions and a hemisphere-specific reorganization as a consequence of the adaptation to single-sided deafness.

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Sensitive phases for the development of multisensory processes

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Studies on multisensory development have shown that some multisensory capabilities exist from early on and that multisensory development consists not only of an extension of multisensoy processes but additionally comprises a loss and refinement of sensory functions. Whether or to which degree multisensory development depends on experience and whether sensitive periods exist in humans is not yet known. Investigating individuals who had suffered a period of congenital total blindness from birth due to cataracts after sight restoration provides a unique model to test this question. At the behavioral level we found reduced or a lack of multisensory interactions in these individuals except for simple crossmodal stimuli which are integrated predominantly based on synchrony detection. A first fMRI study analyzed brain activity during lip-reading (visual stimulation). Results suggested that the lack of audio-visual speech gains in the cataract-reversal group might be predominantly due to the lack of visual input to auditory areas. In a second fMRI investigation we analyzed the BOLD response to crossmodal audio-visual speech stimuli. While we found crossmodal interactions in auditory areas in normally sighted controls, this effect was absent in the cataract -reversal individuals. Importantly, we observed in the cataract-reversal individuals a supressed activity to audio-visual compared to visual stimulation in visual brain regions. We speculate that the suppression is due to a reorganization of visual areas during the period of blindness causing a change in the weighting of auditory and visual input, possibly an inhibition of noisy visual input from the deprived retina to reduce interference during auditory processing.

The neural correlates of hearing colors and shapes: insights from darkness on brain plasticity and stability

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Preferential response to visual number, letter symbols and visual body shapes were discovered in the last decade in the right inferior temporal gyrus (rITG) and visual word form area (VWFA) and extra striate body area (EBA) respectively. It remains unclear how such a preference emerges, what is the contribution of shape biases to its formation, and does visual input and experience is critical to its formation. To study these questions, we used congenital blindness as a model for brain development without visual experience. During fMRI, we presented blind subjects with shapes encoded using a novel auditory sensory-substitution device (SSD) called the EYEMUSIC or using veteran SSDs like the vOICe. In sensory substitution devices input originally processed in one sense (e.g. vision for SSDs used by blind) are encoded and trained using a different sense. Results indicate greater rITG activation during numeral identification than during control tasks using identical stimuli, greater VWFA to reading and greater EBA activation to body images. Using resting-state fMRI in the blind and sighted, we further show that the distinct areas with preference for numerals and letters present distinct patterns of functional connectivity with quantity and language processing areas, respectively for rITG and VWFA. EBA also showed unique pattern of connectivity to body related areas. Our findings show that the ventral 'visual' stream specificities can emerge independently of sensory modality, and suggest that this might be mediated by cultural recycling of cortical circuits molded by distinct specializations and connectivity patterns.

These results show that SSDs have led to exciting experimental results. On the other hand, unfortunately, have yet to achieve their original goal of practical visual-rehabilitation, and are currently considered as reserved primarily for experiments in controlled settings. In the last part of the talk I will present recent evidence, much of which gathered utilizing SSDs, has changed our understanding of the neural mechanisms behind visual restoration. This evidence suggests that the brain is more than a pure sensory machine but rather is a highly flexible task-machine, i.e., brain regions can maintain or (regain their original function) in vision even with input from other senses. This is complemented by a recent set of new behavioral achievements using SSDs, and new SSD technologies and tools. These changes and advances suggest that the time has come to retry practical visual-rehabilitation with SSDs and we'll chart several key steps in this direction such as training protocols, online self-train tools and tailoring different combinations of SSDs for different scenarios.

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Cortical plasticity following sensory deprivation: Characterizing the patterns of thalamocortical and corticocortical projections in early- and late-deaf cats

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In the absence of one sensory modality, such as in deafness or blindness, compensatory advantages have been observed in the remaining sensory systems across a variety of species. These advantages are thought to reflect the recruitment of cortical areas that would normally be involved in processing stimuli from the missing modality. While such reorganization may confer supranormal acuity to the remaining senses, there are potentially critical functional implications for sensory restoration. Moreover, the nature of these changes appears to depend on the maturational state of the brain when sensory input is disrupted. Thus, we injected a retrograde neuronal tracer (BDA) into the auditory cortices of hearing cats, and cats that were ototoxically deafened shortly after birth (early-deaf) or in adulthood (late-deaf). Coronal sections were examined under a light microscope, and labelled neurons were counted and assigned to functional cortical and thalamic areas according to published criteria. Functionally relevant intramodal reorganization was observed between auditory cortical areas, while the balance of thalamocortical inputs also differed between groups. Additionally, crossmodal connections from visual and somatomotor cortices to auditory areas were altered following the onset of deafness. Importantly, many of these changes were dependent on the age at deafness onset. Understanding how anatomical connectivity differs as a result of deafness is critical to our understanding of crossmodal plasticity, and highlights an important consideration for hearing restoration.

Symposium

S14: Recent advances in basal ganglia research: action-selection, movement and pathologies

S14-1 Dynamics of basal ganglia circuits during movement initiation and suppression
Robert Schmidt

S14-2 Microcircuits Underlying Multisensory Integration in the Mouse Striatum
Gilad Silberberg

S14-3 Existence and control of Go/No-Go decision transition threshold in the striatum
Jyotika Bahuguna, Ad Aertsen, Arvind Kumar

S14-4 Pathological neuronal synchronisation in Parkinson's disease and its consequences
Peter Brown

S14-5 Optogenetic mapping of network dynamic in basal ganglia
Brice de la Crompe, Thomas Boraud, Nicolas Mallet

Dynamics of basal ganglia circuits during movement initiation and suppression

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Behavioral inhibition is a fundamental component of executive function. In a standard test of behavioral inhibition (the Stop-signal task) subjects are given Go cues to prompt movements, but on some trials a subsequent Stop cue indicates that movement preparation should be cancelled. We previously showed (Schmidt et al. 2013, Nat Neuro) that individual neurons in the rat subthalamic nucleus (STN) respond very quickly to Stop cues (~15ms latencies), and on successful Stop trials this appears to drive excitation of neurons in the substantia nigra pars reticulata (SNr, ~35ms latencies) that would otherwise pause to allow movement initiation. However, we also found evidence that this is an incomplete account of action cancellation. The STN- SNr response is fast enough to explain the speed of stopping, but is highly transient and thus by itself may only delay action initiation. An additional slower, more selective mechanism likely plays a key role by suppressing movement -related activity within striatum. We hypothesized that this additional mechanism involves the globus pallidus (GP). We therefore compared the timing and selectivity of GP single-unit cue responses to other basal ganglia regions. We report that although GP cells exhibit heterogeneous firing patterns, their overall responses to Stop signals are slower (~60-100ms) yet more selective than STN and SNr cells. Furthermore, the time course of GP Stop responses closely matches the time when movement-related activity in striatum is suppressed, in line with pallidostriatal inhibition. As pallidostriatal inhibition is primarily mediated by arkypallidal GP neurons (Mallet et al., 2012, Neuron), we categorized our GP neurons into putative arkypallidal and prototypical cells based on electrophysiological features during behavior and sleep. Preliminary analyses suggest that the slow, selective stop -signal responses are more pronounced in arkypallidal than in prototypical GP units. We conclude that movement cancellation involves multiple circuit-level mechanisms within the basal ganglia, with complementary temporal profiles and selectivity.

Microcircuits Underlying Multisensory Integration in the Mouse Striatum

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The striatal microcircuitry consists of the major projecting population, the medium spiny neurons (MSNs), and a diverse population of interneurons. Fast spiking (FS) interneurons provide robust and reliable feed-forward inhibition, targeting both direct and indirect pathway MSNs with high connection probability, and are considered important for the synchronization of their postsynaptic targets. Striatal cholinergic interneurons provide disynaptic inhibition to MSNs and under in vivo conditions display tonic and synchronized discharge. Whether and how FS interneurons control cholinergic interneurons and affect their synchronicity or regulate other striatal interneuron types remains unknown.
We have here combined multiple whole-cell recordings with optogenetics in order to directly characterize the target selectivity of feed-forward inhibition provided by striatal FS interneurons. Using transgenic and viral approaches we have specifically directed expression of ChR2 to FS interneurons to study their connectivity within the striatal microcircuit in acute brain slices. I will also present in-vivo work demonstrating bilateral and multimodal sensory integration by individual striatal projection neurons and interneurons.

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Existence and control of Go/No-Go decision transition threshold in the striatum

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A typical Go/No-Go decision is suggested to involve the activation of direct or indirect pathway respectively in the basal ganglia. Striatal Medium Spiny Neurons (MSNs) express D1 and D2 type dopamine receptors and show a functional dichotomy by projecting preferentially to direct and indirect pathway respectively. Thus far, in computational models of interaction between direct and indirect pathways, D1 and D2 MSNs have been considered as interchangeable inhibitory subpopulations. But recent in-vitro data has suggested that D2 MSNs preferentially inhibit D1 MSNs more than vice versa [Taverna et. al 2008, Planert et. al 2010]. There are conflicting views on whether fast-spiking interneurons (FSIs) innervate both MSNs equally [Planert et. al 2010] or innervate D1 MSNs more than D2 MSNs [Gittis et. al 2010]. Here we use both a reduced firing rate model and numerical simulations of spiking network model of the striatum to understand the dynamical consequence of the asymmetrical connectivity of the MSNs and FSIs.

Our model reveal following novel insights about the dynamics of D1 and D2 MSNs: (i) Due to higher total inhibitory input to the D1 MSNs as compared to D2 MSNs, for the same amount of cortical input to the striatum, the activity of D2 is always higher than D1thereby maintaining a default “No-Go” state in the striatum. We describe the stratum dynamics for two different mechanisms which can provide more input to D1 MSNs. (ii) In a regime with slightly higher cortical input to D1 MSNs, the striatal bias can be switched to “Go” (at lower cortical rates) or “No-Go” (at higher cortical rates) depending on input rate. We termed the cortical rate at which the striatal bias switches from “Go” to “No-Go” as decision transition threshold (DTT) because this represents the transition in striatal bias towards a Go or No-Go decision. (iii) FSIs play a crucial role in existence of DTT and influence DTT via their connectivity (asymmetric or symmetric) and their activity as compared to cortical activity. (iii) Correlations “within” and “between” the pools of presynaptic neurons of individual MSNs have a strong effect on the DTT..(iv) Pallidostriatal back projections [Mallet et. al 2012] can influence DTT by modulating the activity of FSIs.
Based on these insights, we propose that the asymmetrical connectivity of the striatal elements (D1 and D2 MSNs and FSIs) renders the striatum as a threshold device that indicates the change in cortical input and correlations by changing the striatal bias towards D1 MSN(“Go”) or D2 MSNs (“No-Go). The high level description of the striatum as a threshold function provides mechanistic explanations for several experimental and behavioral findings involving the basal ganglia.

Pathological neuronal synchronisation in Parkinson's disease and its consequences

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There is growing interest in how synchronised activity across populations of neurons might underlie impairment in some diseases and there is now a general consensus that exaggerated oscillatory synchrony occurs within and between the basal ganglia and cerebral cortex in patients with Parkinson's disease. In particular, activity in the beta frequency band (13-30 Hz) is prominent, and can be attenuated by treatment with dopaminergic drugs and by deep brain stimulation. The latter suggests that elevated beta activity may be a key disturbance in Parkinson's disease (PD). Here, I will consider both correlative evidence and tests of causality that support a mechanistic link between pathologically exaggerated beta activity and stiffness and slowness of movement in PD. Questions, however, remain with regards to the quantitative importance of beta and the role of this activity under physiological circumstances. In particular, recent studies suggest that the modulation of beta activity may play a part in both motor and cognitive domains. Finally, I will suggest how our new understanding of circuit-level disturbances in PD may provide a basis for the development of more sophisticated forms of therapeutic stimulation for Parkinson's disease.

Optogenetic mapping of network dynamic in basal ganglia

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The basal -ganglia (BG) form a complex loop with the cortex that is involved in action selection and decision-making. Synchronized oscillatory activities in BG neuronal circuits have been proposed to play a key role in coordinating information flow within and between neuronal networks. If synchronized oscillatory activities are important and necessary for normal motor function, their dysregulation in space and time can truly be pathological. Indeed, in Parkinson's disease (PD), many studies have reported an increase in the expression level of neuronal oscillations contain in the beta (ß) frequency range (15-30 Hz). These abnormal ß; oscillations have been correlated with two mains symptoms of PD: akinesia/bradykinesia. However, which BG neuronal circuits generate those abnormal ß; oscillations, and whether they play a causal role in PD motor dysfunction is not known. The subthalamic nucleus (STN) is a key nucleus in BG that receives converging inputs from the indirect (i.e. globus pallidus) and hyperdirect (i.e. cortex) pathways. Here, we used a clinically-relevant rat model of PD combined with electrophysiological recordings and optogenetic toolboxes to investigate how selective manipulation of STN inputs (globus pallidus/cortex) causally influence BG network dynamic and behavior. Our data provide new mechanistic information to help understand the generation and propagation of abnormal ß-oscillations as well as they role on motor behavior.

Symposium

S15: Is insect odor transduction primarily based upon an orco-dependent ionotropic mechanism or on metabotropic cascades?

S15-1 The contribution of metabotropic signal transduction cascades in insect olfaction
Robin Schumann, Thomas Schendzielorz, Andreas Nolte, Petra Gawalek, Monika Stengl

S15-2 The stimulatory Gas protein is involved in olfactory signal transduction in Drosophila
Eva M. Neuhaus

S15-3 Olfactory transduction in Drosophila melanogaster - the contribution of some G proteins
C Giovanni Galizia, Natalya Katanayeva, Vladimir L Katanaev, Jennifer SI Raja

S15-4 Function and regulation of insect odorant receptors
Dieter Wicher, Merid Getahun, Bill Hansson

S15-5 Rapidly responding olfactory receptor neurons in *Drosophila melanogaster*
Alpha Renner, Christoph J. Kleineidam, Paul Szyszka

The contribution of metabotropic signal transduction cascades in insect olfaction

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Female hawkmoths release a pheromone blend at night, which triggers pheromone-dependent upwind flight in their conspecific mates. *Manduca sexta* males detect the pheromones with olfactory receptor neurons (ORNs) which innervate long trichoid sensilla on their antenna. Pheromone transduction in insects is still under debate. In *M. sexta* so far we found no evidence for ionotropic signal transduction cascades in pheromone transduction, based upon a ligand-gated olfactory receptor (OR) coreceptor (Orco) ion channel complex. Activation of the conserved Orco ion channel with VUAA1 did not affect phasic pheromone responses and neither did Orco antagonist OLC15. In contrast, pheromone application in patch clamp experiments of *M. sexta* ORNs in vitro elicited a sequence of inward currents which is mimicked via activation of a phospholipase C (PLCß)-dependent signal transduction cascade. Also, PLC-antagonists and G-protein antagonists decreased pheromone responses recorded in tip recordings of long trichoid sensilla of intact hawkmoths. Furthermore, PKC-activity seems to modulate pheromone responses daytime-dependently. The PKC-agonist PDBu increased the pheromone response during the hawkmoths' resting phase. Accordingly, in the presence of PDBu pheromone significantly increased IP_3 levels in hawkmoth antennae during rest. Therefore, from biochemical, pharmacological and physiological experiments we follow that pheromone transduction in the hawkmoth does employ G-protein-dependent activation of a PLCß first activating IP_3 and DAG-dependent calcium channels which in turn activate directly and indirectly Ca^{2+}-dependent ion channels underlying pheromone-dependent action potential responses. In addition, apparently daytime-dependent changes in intracellular second messenger concentrations regulate changes in antennal second messenger-dependent ion channels which determine threshold and kinetics of the pheromone response in correlation with circadian changes in odor-driven behavior in different insect species. Currently, we challenge our hypothesis in ELISA experiments with hawkmoth and fruitfly antennae. [Supported by DFG SPP 1392,STE531/21-1,2 to MS]

The stimulatory Gas protein is involved in olfactory signal transduction in Drosophila

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Seven-transmembrane receptors typically mediate olfactory signal transduction by coupling to G-proteins. Although insect odorant receptors have seven transmembrane domains like G-protein coupled receptors, they have an inverted membrane topology, constituting a key difference between the olfactory systems of insects and other animals. While heteromeric insect ORs form ligand-activated non-selective cation channels in recombinant expression systems, the evidence for an involvement of cyclic nucleotides and G -proteins in odor reception is inconsistent. We addressed this question in vivo by analyzing the role of G-proteins in olfactory signaling using electrophysiological recordings. We found that Gas plays a crucial role for odorant induced signal transduction in OR83b expressing olfactory sensory neurons, but not in neurons expressing CO2 responsive proteins GR21a/GR63a. Moreover, signaling of Drosophila ORs involved Gas also in a heterologous expression system. In agreement with these observations was the finding that elevated levels of cAMP result in increased firing rates, demonstrating the existence of a cAMP dependent excitatory signaling pathway in the sensory neurons. Together, we provide evidence that Gas plays a role in the OR mediated signaling cascade in Drosophila.

Olfactory transduction in Drosophila melanogaster - the contribution of some G proteins

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Olfactory signal transduction in vertebrates and nematodes acts via G proteins. However, it still remains controversial to what extend G proteins contribute to transduction in insects. We investigated the role of the $G_{o/i}$ subgroup of G proteins in the olfactory signal transduction cascade for two olfactory receptors in *Drosophila melanogaster* by combing *in vivo* and *in vitro* methods. Flies with reduced Ga_o activity (by expression of pertussis toxin (PTX), which inhibits only Ga_o in *Drosophila*) in all the olfactory receptor neurons (ORNs) expressing Orco showed statistically significant olfactory behavioral deficits. *In vivo* calcium imaging of flies with reduced Ga_o activity (by expression of PTX) or downregulation of Ga_i expression (by expressing siRNA specific to Ga_i subunit) in the ORNs expressing Or22a showed lower response strength regardless of odor identity and intensity than the control flies. Importantly, both the fast (phasic) and the slow (tonic) response components are reduced when these two subunits had reduced activity. We moved to an in vitro approach for a detailed analysis. Drosophila ORs (dOr22a & dOr83b) were transiently expressed in human embryonic kidney cells (HEK293T). Stimulation with odorants elicited intracellular calcium increase in about 32% of cells, which is about 62% of transfected cells. Removal of external calcium completely abolished the response, indicating that calcium influx was generated by extracellular calcium. CICR (calcium induced calcium release) inhibitors reduced the response, indicating that calcium influx was amplified by CICR channels. Incubation with the $G_{o/i}$ inhibitor PTX greatly reduced the response. Further analysis showed that this effect was mediated by Ga_{i2}. In contrast, Ga_o subunit suppressed the response, possibly sequestering the Gßγ-subunits and arguing for a role of the Gßγ heterodimer in signal propagation in this system. In conclusion, our results suggest $G_{o/i}$ contributes to olfactory signaling in *Drosophila melanogaster*.

Function and regulation of insect odorant receptors

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Insects odorant receptors (ORs) consist of an odorant-specific protein OrX and a ubiquitous coreceptor (Orco) to form ligand-gated ion channels. Orco proteins expressed alone form also channels that are activated by cAMP. Enhanced cAMP production in insect olfactory sensory neurons leads to a stronger odor response. Repeated sub-threshold odor stimulation sensitizes the ORs. Interestingly, the glutamate receptor-like ionotropic receptors (IRs) do not sensitize. The sensitization of ORs can also be obtained by activation of Orco channels and prevented by inhibition of cAMP production. Flies expressing Orco mutants that do not respond to cAMP cannot be sensitized by sub-threshold odor stimuli. Taken together, Orco-mediated regulation of OR sensitivity is necessary to provide tunable receptors. This allows the animals to detect odors over a wide range of concentrations, and to respond fast and highly sensitive to brief stimulation as it happens during flight.

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Rapidly responding olfactory receptor neurons in *Drosophila melanogaster*

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Natural odor plumes often contain predictive information for insects about the quality and location of food sources or mating partners. Behavioral experiments demonstrate that insects are able to segregate concurrent odors based on few milliseconds difference in arrival time at the sensory organs. This implies fast and precise odor transduction at the level of the olfactory receptor neurons. However, previous studies suggest that insect olfactory receptor neurons have comparably slow response kinetics with minimum response latencies between 10-30 ms and a temporal resolution of up to 30 Hz.

This discrepancy between fast odor perception and reports on quite slow odor transduction motivated us to probe the temporal resolution of insect olfactory receptor neurons using single sensillum recordings. It has previously been shown that speed and temporal resolution of olfactory receptor neurons depend on temperature, odorant and receptor type. We aimed to quantify the maximal speed of sensory transduction and the temporal resolution under optimal conditions. We therefore developed an odor delivery system that allowed us highly accurate stimulus delivery with millisecond precision.

We show, that olfactory receptor neurons in the large basiconic sensilla of *Drosophila melanogaster* have shorter transduction times and a higher temporal resolution than hitherto reported. The shortest response onset latencies of single neurons, the time between odor arrival and spike generation, are below 3 ms and trains of action potentials can follow stimulus frequencies of up to 100 Hz.

The fast response kinetics enable olfactory receptor neurons to encode fast fluctuations in odor plumes, which might be important for odor-background segregation in a turbulent odor environment. Moreover, the fast odor transduction and high temporal resolution we described for olfactory receptor neurons have implications for current models of odor transduction and neuronal coding of odors.

Symposium

S16: Molecular, neuronal and behavioral effects of oxytocin: a translational approach

S16-1 Central oxytocinergic pathways and their involvement in sociality
Valery Grinevich

S16-2 Neuromodulation by Oxytocin in the central amygdala: an in vitro and in vivo optogenetic and electrophysiological dissection of the underlying circuitry.
Ron Stoop

S16-3 Intraneuronal signaling cascades mediating oxytocin on anxiety and stress regulation: effects of chronic treatment
Inga D. Neumann

S16-4 Oxytocin, Attachment and the Self in Relation to Other
Jennifer Adrienne Bartz

S16-5 Epigenetic adaptations of oxytocin system during social fear conditioning
Rohit Menon, Inga D. Neumann

S16-6 Fear activated-genetic targeting of oxytocin neurons and their behavioral effects.
Ferdinand Ludwig Althammer, Apar Jain, Miriam Kernert, Hilmar Bading, Rolf Sprengel, Peter H. Seeburg, Mazahir T. Hasan, Valery Grinevich

Central oxytocinergic pathways and their involvement in sociality

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Magnocellular hypothalamic nuclei and individual neuroendocrine neurons underwent tremendous morphological transformations during evolution of vertebrates. The single magnocellular nucleus, preoptic nucleus, in basal vertebrates was transformed to polycentric nuclear system in advanced vertebrates. In parallel, primitive unipolar neurons was transformed into sophisticatedly organized neurons with rich dendritic trees and specialized bifurcating axons. Using virus -based vectors we explored anatomical features of magnocellular oxytocin (OT) neurons in rodents, showing that these neurons are projecting to forebrain, especially to regions controlling social behavior and cognition, such as the anterior olfactory nucleus, septum, and cingulate and prefrontal cortex. Using the in vitro electrophysiology, we showed that neurons (preferentially interneurons) of all these regions are prominently responding to evoked axonal OT release. Importantly, the intensity of OT traffic was correlated with social challenges. Recently we developed genetic tools (named Genetic Activity-Induced Tagging, vGAIT) to express genes of interest in only those OT neurons, which have been exclusively activated by social stimuli. Taking advantage of this method, we are on the way to trace projections of “socially-challenged” OT neurons, especially in models of human psychosocial disorders. In conclusion, although evolutionarily determined axonal OT release seems to be critical for OT signaling in the mammalian brain, the developmental, physiological and pathological aspects of this pathway require further exploration.

Neuromodulation by Oxytocin in the central amygdala: an in vitro and in vivo optogenetic and electrophysiological dissection of the underlying circuitry.

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Oxytocin and vasopressin are sister nonapeptides that have emerged from a common ancestor peptide and that differ only in two amino acids. They have appeared early in evolution and in the rat brain, oxytocin and vasopressin receptors are expressed in separate regions with, in certain instances, remarkable complementary expression patterns. In the central amygdala, oxytocin and vasopressin receptors are adjacently expressed in resp. the lateral (CeL) and medial part (CeM). In the CeL, we have found that oxytocin excites interneurons with inhibitory projections onto neurons in the CeM that are, in turn, excited by vasopressin (Huber et al., 2005). To examine the neuronal projections from these nuclei, we injected their targets with fluorescent retrograde tracers and several days later assessed sensitivity of retrogradely labeled neurons in the amygdala to oxytocin. Interestingly, CeA neurons exhibited target-dependent oxytocin sensitivity (Viviani et al., 2011). To examine the upstream origins of endogenous oxytocin, we injected the hypothalamus with an adeno-associated virus expressing channelrhodopsin-2 and a fluorescent marker under the oxytocin promoter. This optogenetic approach revealed the presence of fluorescent fibers in the CeA affecting local circuits and associated behaviors when activated by blue light (Knobloch et al., 2012). We are currently examining, by combining this optogenetic approach with in vivo multi-electrode single unit recordings, the precise electrophysiological characteristics of the neuronal populations that are affected by endogenous oxytocin in the amygdala during fear learning and expression.

Intraneuronal signaling cascades mediating oxytocin on anxiety and stress regulation: effects of chronic treatment

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The neuropeptide OXT has received substantial interest due to its acute pro-social and anxiolytic properties, and the attenuation of stress responses. We have recently shown that OXT promotes social preference behaviour and prevents social phobia induced by social defeat stress in rats and mice[1]. Further, in a mouse paradigm for social fear conditioning[2], OXT specifically reversed social fear – an effect which was localized within the dorsolateral septum, where social fear was associated with reduced OXT receptor (OXTR) binding[3].

However, OXTR-mediated signaling cascades mediating these behavioral effects of OXT are largely unknown. We could reveal that central infusion of OXT activates the MAP kinase pathway within the hypothalamic paraventricular nucleus (PVN). This intracellular pathway was found to be crucial for the acute anxiolytic local effects of OXT[4]. Moreover, OXT also inhibits the expression of corticotropin releasing factor (CRF) in vivo and in vitro, in particular under stress conditions by inhibition of the translocation of the CREB co-factor CRTC3 into the cell nucleus as validated by siRNA and ChIP analyses. As CRF is anxiogenic and the important facilitator of the hormonal stress response, its inhibition by OXT is likely to contribute to the anxiolytic and stress-protective effects of brain OXT.

In contrast to its acute effects, chronic central OXT infusion (14 days) dose-dependently increased anxiety of male mice and reduced OXTR binding in the basolateral amygdala, nucleus raphe and dorsolateral septum. We are currently studying the consequences of chronic OXT or its highly specific agonist TGOT on OXTR-coupled intracellular signaling pathways.

Although the acute effects point towards the therapeutic use of OXT for anxiety-related disorders including social phobia more detailed behavioral and molecular studies are needed to reveal chronic effects. Supported by DFG.

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Oxytocin, Attachment and the Self in Relation to Other

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Background: Considerable evidence from pre-clinical models indicates that the neurohormone oxytocin (OXT) plays a critical role regulating attachment and other affiliative behaviors. While recent research has demonstrated some remarkable parallels in humans, careful inspection of the data reveals that the social effects of OXT in humans are inconsistent and often depend on the social context and/or person. For example, research indicates that intranasally administered OXT facilitates prosocial behavior, especially in those who are less socially engaged (e.g., avoidantly attached), but exacerbates interpersonal insecurities in those who are preoccupied with closeness (e.g., anxiously attached). Such variability in effects raises questions about the mechanism(s) by which OXT modulates human social behavior. Drawing upon research on OXT and the induction of other-directed (e.g., maternal) behavior in non-human animals, it is hypothesized that OXT may induce a similar motivational orientation to focus on, and be concerned with, others. This hypothesis could explain some of the person-specific effects of OXT since becoming more “other-oriented” should be helpful to those who are excessively focused on the self to the exclusion of others, but could be detrimental to those who are other-focused but have little sense of an agentic self because it could fuel feelings of vulnerability.
Methods: To test this hypothesis, a double-blind, randomized, placebo-controlled, crossover trial was conducted in which adult males received 24 IU intranasal OXT/placebo and then completed measures assessing communion (other orientation) and agency (self orientation). Individual differences in attachment were assessed at baseline.
Results: OXT significantly increased the endorsement of communal traits (e.g., “kind,” “gentle,” and “warm”) for the average person ($p=.05$). However, critically, this effect was moderated by attachment avoidance ($p<.05$), such that avoidant individuals (who are generally low in communion) showed the largest increase in communion following OXT. There was no main effect of OXT on agency; however, results revealed a significant OXT by attachment anxiety interaction predicting self-conceptions of agency ($p<.005$), such that anxious participants (who are generally low in agency) showed a selective decrease in agency following OXT—that is, reporting being even less "independent" and more "inferior".
Conclusion: These data shed light on the variability in extant research on the social effects of oxytocin in humans and help illuminate both the “pro-social” and occasional “anti-social” effects observed.

Epigenetic adaptations of oxytocin system during social fear conditioning

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Fear is an adaptive emotional response to threatening situations, whereas maladaptations of fear responses, in particular of social fear, are characteristic symptoms of psychopathologies such as social anxiety disorder (SAD). Treatment of SAD is rather unspecific and combines psychotherapy and pharmacotherapy (e.g., benzodiazepines or selective serotonin reuptake inhibitors). In order to reveal the molecular and neuronal underpinnings of SAD, and the contribution of oxytocin (OXT), we have established a model of social fear using a Social Fear Conditioning (SFC) paradigm in male mice which resembles SAD in humans (1). The neuropeptide OXT has been proposed as a potential therapeutic agent for SAD due to its pro -social, anxiolytic and stress attenuating effects (2) mediated via the brain oxytocin receptor (OXTR). Our previous results have shown that local infusion of OXT into the dorso-lateral septum (DLS) leads to rapid extinction of social fear. Further, SFC+ mice showed an increase in OXTR binding in the DLS which normalized after social fear extinction, while local OXT release in response to social stimuli was found to be blunted in SFC+ mice (3).

In order to analyze whether the changes observed at the protein level were also reflected upstream at the RNA level, we studied changes in the Oxt and Oxtr mRNA in the dorsal hippocampus, amygdala, DLS and PVN 2 hours after both fear acquisition and extinction. In line with the increase in OXTR binding we found an elevated Oxtr mRNA levels in the DLS, but not the dorsal hippocampus and amygdala. Further, the described changes in local OXT release were reflected by Oxt mRNA levels in the PVN. In detail, in response to social exploration during social fear extinction training Oxt mRNA was elevated in SFC-, but not in SFC+ mice. These results point towards the regulation of Oxtr and Oxt at DNA level possibly via epigenetic modifications.

Therefore, we studied the local expression of various transcription regulators including histone deacetylases (Hdac) as epigenetic markers in SFC+ versus SFC- mice. HDACs are key enzymes, which modify chromatin and, thus, contribute to the regulation of gene expression. Their activity can be blocked by HDAC inhibitors, which were shown to augment fear extinction and rescue cognitive deficits in animal models of impaired extinction (4). In our SFC model Hdac1 mRNA expression was found to be upregulated in the DLS, but not dorsal hippocampus or amygdala, 2 hours after social fear acquisition. Consistent with this result, local infusion of MS275, a potent HDAC1 inhibitor, into the DLS of SFC+ mice 60 min before social fear extinction training significantly improved extinction learning. These results point towards a DLS-specific role of HDAC1 in regulating social fear. Studies currently in progress are aimed to reveal the link between the SFC-induced alterations in the OXT system and the above mentioned alterations in HDAC activity, which may play a role in regulating social fear in mice and could lead to potential therapeutic targets for SAD treatment in humans. Fundedby Bayerische Forschungsstiftung and Deutsche Forschungsgemeinschaft.

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Fear activated-genetic targeting of oxytocin neurons and their behavioral effects.

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In our previous work (1) we reported that hypothalamic OXT neurons extensively project axons to several forebrain regions. However, it is unclear how the axonal OXT release is coordinated upon different demands. It was tempting to propose that there is a functional and anatomical specialization of OXT neurons, subsets of which orchestrate distinct types of behaviors. To address this question we developed an approach named “Genetic Activity-Induced Tagging (vGAIT) method”, which is based on the expression of a short promoter of the immediate early gene c-fos. This method consists of a recombinant adeno-associated virus (rAAV), driving the reverse tetracycline transactivator (rtTA) under the control of the c-fos promoter, which expression is controlled by doxycycline (Dox). In the presence of Dox, only a transient activation of c-fos leads to a permanent rtTA expression via autoregulated rtTA expression. To genetically target experience-activated OXT neurons, female Wistar rats received intrahypothalamic injections of a cocktail of three viruses: rAAV, expressing rtTA under the c-fos promoter (i); rAAV, expressing YC3.60 (yellow cameleon) and Cre recombination under the bidirectional CMV-TetO7 promoter-operator cassette (ii); 3) rAAV, containing the DIO (double floxed inverted open reading frame), expressing fluorescent marker mCherry (fused with channelrhodopsin 2) under the control of the OXT promoter (iii). Two weeks later, rats were subjected to a 3 day contextual fear conditioning protocol, comprising 2 shock sessions in 2 days and 3rd day with conditioning session. Fear expressing neurons were tagged by an i.p. injection of Dox 24h prior fear conditioning. 2 weeks later rats were subjected to either 1) single fear conditioning session to enhance lapse memory followed by a fear expression session on the second day or 2) new paradigm where animals were subjected to shock-free fear conditioning and expression sessions. Our new paradigm, which involves an irritable air puff, is an alternative approach to assess fear circuitry where overlapping of pain and fear circuit can be minimized, if not completely separated. In both the paradigms of fear conditioning, we analyzed behavioral effect of optogenetically stimulated oxytocin release by fear-experience tagged oxytocin neurons in CeA.

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Symposium

S17: Regeneration in the injured spinal cord - hopes and perspectives

S17-1 Neuronal regeneration in the spinal cord of adult zebrafish
Thomas Becker

S17-2 New perspectives for the treatment of spasticity and neuropathic pain after spinal cord injury
Laurent Vinay, Remi Bos, Pascale Boulenguez, Cecile Brocard, Frederic Brocard, Sylvie Liabeuf, Vanessa Plantier, Laetitia Stuhl-Gourmand, Annelise Viallat-Lieutaud, Florian Gackiere

S17-3 Serotoninergic control of locomotor hindlimb movements – prospective strategy for restoring locomotion after spinal cord injury
Urszula Slawinska

S17-4 Regeneration in the injured rodent cord induced by grafted stem cells: multiple mechanisms
Antal Nógrádi, Tamás Bellák, Krisztián Pajer

S17-5 Human iPS cells mediate tissue sparing with moderate functional improvement after spinal cord contusion injury in rats.
Krisztián Pajer, Tamás Bellák, Csilla Nemes, András Dinnyés, Antal Nógrádi

Neuronal regeneration in the spinal cord of adult zebrafish

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In contrast to mammals, adult zebrafish regenerate different neuronal cell types from local progenitor cells in the lesioned spinal cord. We have been studying the cellular and molecular key events following a lesion. Ependymo-radial glial progenitor cells, with a soma contacting the central canal and endfeet at the pial surface, are quiescent in the unlesioned spinal cord and start to proliferate after a lesion. Different domains of ependymo-radial glial cells then generate motor neurons and a range of interneuron cells types, re-deploying developmental signals, such as sonic hedgehog. Using drug screening strategies and expression profiling of highly purified progenitor cells in embryos, we have identified novel signals that promote motor neuron generation during development and adult regeneration. Some of these signals are derived from descending axons, such as dopamine (Reimer et al., Dev. Cell, 2013, 25, 478-91), which acts by augmenting hedgehog signalling. We find an unexpectedly large array of long-range and local signals interacting to promote development and regeneration of different neuronal cell types in the spinal cord and we will report on our recent progress on identifying these signals. We conclude that the zebrafish model is ideally suited to discover signalling pathways that are active during spinal cord differentiation and neuronal regeneration in a vertebrate, which may ultimately be translatable to mammals.

New perspectives for the treatment of spasticity and neuropathic pain after spinal cord injury

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Most spinal cord injury (SCI) patients suffer from spasticity and chronic pain. This is a major therapeutic challenge since medical therapies often are ineffective. It is now widely accepted that spasticity results from profound modifications of both the intraspinal inhibitory synaptic transmission and the electrical properties of neurons. We identified the mechanism responsible for a reduced inhibition within the networks below the lesion. Briefly, a low [Cl-] is a pre-requisite for inhibition mediated by GABA and glycine to occur. This concentration is maintained at low levels in healthy adult neurons by K-Cl co-transporters (KCC2), which extrude Cl- ions. The expression of KCC2 is down-regulated at the level of both motoneurons and laminae I-II neurons after SCI, thereby causing spasticity and pain, respectively. Concomitantly, the excitability of neurons is increased due to the upregulation of voltage-dependent persistent inward currents (PICs). Two important mechanisms characterize these PICs: they require long depolarizations to fully activate and they are inactivated by hyperpolarization of motoneurons. We showed that the down-regulation of KCC2 and the up-regulation of PICs play a synergistic role in the physiopathology of spasticity. Targeting KCC2 and sodium persistent currents open new avenues for the treatment of both spasticity and chronic pain after SCI.

Serotoninergic control of locomotor hindlimb movements – prospective strategy for restoring locomotion after spinal cord injury

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Serotoninergic neurons located in the brainstem and projecting to the spinal cord are engaged in initiation and control of the locomotor movements. This effect is exerted by actions on motoneurons as well as on the spinal cord neuronal network for locomotion, the Central Pattern Generator (CPG). It is known that the axons of serotonergic neurons terminate on specific target neurons in the spinal cord where the different types of serotonergic receptors allow the serotonergic system to play multiple roles in the control of locomotion. The role of different receptor populations in control of locomotion was confirmed in intact and paraplegic rodents as well as in rat brainstem-spinal cord in vitro preparations. Using defined serotonergic agonists and antagonists we also demonstrated in intact and in paraplegic adult rats that the 5-HT2 receptors control CPG activation as well as motoneuron output, while 5-HT7 receptors control mainly the activity of the locomotor CPG. Our findings show that the combined use of agonists of the 5-HT2 and 5-HT7 receptors in a low dose, that is not effective when applied by either drug alone, results in production of well-coordinated weight supported locomotion with a reduced need for exteroceptive stimulation. In another investigation we found that in adult paraplegic rats intraspinal grafting of different populations of 5-HT neurons dissected from embryonic brainstem can induce the spinal cord circuitry below the total transection to control recovery of plantar hindlimb stepping. After grafting various combinations of fetal serotonergic neurons destined to become specific defined populations of the descending serotonergic system we found that the capacity to reinnnervate crucial parts of the spinal cord locomotor system differed depending on the source of the grafted cells. Immunohistochemical verifications revealed that reinnervation of both motoneurons and CPG neurons was essential for recovery. In addition, we confirmed using defined serotonergic antagonists that the action of reestablished serotonergic innervation responsible locomotor recovery is mediated by 5-HT2 and 5-HT7 receptors, as it is in the normal condition of himdlimb locomotor control.
Our investigations demonstrate the marked potential of the remaining spinal cord circuitry below the total transection to enhance recovery of plantar hindlimb locomotor movements in paraplegic adult rats.

Regeneration in the injured rodent cord induced by grafted stem cells: multiple mechanisms

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Spinal cord contusion injury leads to severe loss of grey and white matter and subsequent deficit of motor and sensory functions below the lesion. The murine clonal embryonic neuroectodermal stem cell (NE-GFP-4C) line has been shown to prevent the loss of spinal motoneurons after avulsion injury. Here we studied the effect of these cells on the injured spinal cord following contusion injury. We grafted NE-GFP-4C cells intraspinally or applied them intravenously immediately or one week after thoracic (T11) spinal cord contusion injury. Control animals received physiological saline intraspinally or fibrin one week after injury. Stem cells applied either locally or intravenously induced significantly improved functional recovery accompanied by considerable morphological reorganization and regeneration compared with control animals. The stem cells integrated into the host tissue and differentiated to neurons, astrocytes and oligodendrocytes. In intraspinally grafted animals corticospinal tract axons regenerated along the ventral border of the cavity and have grown several millimeters below the lesion. The extent of regeneration and functional improvement was inversely related to the amounts of chondroitin sulphate and ephrin-B2 molecules found around the cavity and to the microglial and astrocytic reactions in the injured segment early after injury. Our data suggest that the grafted stem cells prevent the secondary damage, promote the regeneration of injured axons and are able to replace some of the lost cells.
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Human iPS cells mediate tissue sparing with moderate functional improvement after spinal cord contusion injury in rats.

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Spinal cord contusion injury leads to severe loss of gray and white matter and subsequent deficit of motor and sensory functions below the lesion. In this study we investigated whether application of human induced pluripotent stem cells (hiPS) can prevent the secondary spinal cord damage and induce functional recovery. Human induced pluripotent stem cells were grafted intraspinally or injected intravenously one week after a thoracic (T11) spinal cord contusion injury performed in Fisher 344 rats. Control animals received physiological saline one week after injury applied either intraspinally or intravenously. Locomotor analysis of the injured animals was performed by using open field tests (BBB) and detailed kinematic analysis of hind limbs movement to ascertain improvements in locomotor function. The retrograde tracer Fast Blue was injected into the distal spinal cord to determine the extent of propriospinal axonal sparing/regeneration at 9 weeks after the injury.
HiPS cells applied either locally or intravenously induced moderate functional recovery after contusion injury. Morphologically, the contusion cavity at the epicenter was significantly smaller in grafted animals than in controls. The amount of spared white matter was significantly greater in grafted cords, but no remarkable difference was found in the extent of gray matter rescue in grafted animals compared with controls. Retrograde tracing studies showed statistically significant increase in the number of propriospinal neurons projecting to the distal spinal cord in both intraspinally and intravenously treated rats. These data suggest that grafted hiPS cells prevent the secondary spinal cord damage and are able to induce moderate functional recovery in the acute phase of spinal cord injury.

Symposium

S18: Cellular adaptations for temporal precision in the auditory system

S18-1 Activity-dependent, homeostatic regulation of synaptic depression at the endbulb of Held
Matthew Alexander Xu-Friedman

S18-2 Dynamic Fidelity Control to the Central Auditory System: Synergistic glycine/GABAergic Inhibition in the Cochlear Nucleus
Ivan Milenkovic, Jana Nerlich, Thomas Künzel, Christian Keine, Michael Burger, Rudolf Rübsamen

S18-3 Cholinergic Signaling Influences Gerbils Spherical Bushy Cells Excitability in Vitro
David Goyer, Thomas Kuenzel

S18-4 The role of voltage gated ion channels in brainstem auditory processing
Ian Forsythe

S18-5 The role of inhibition for temporal precision in the lateral superior olive
Alexander U. Fischer, Eckhard Friauf

S18-6 Cellular mechanisms of context dependent signal processing in the lateral lemniscus
Ida Siveke, Julian Ammer, Sarah Berner, Benedikt Grothe, Christian Leibold, Felix Felmy

Activity-dependent, homeostatic regulation of synaptic depression at the endbulb of Held

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Auditory processing relies on precise timing information in sounds. This information is encoded by auditory nerve fibers in the timing of spikes and relayed via bushy cells in the cochlear nucleus to a number of brainstem targets. Auditory nerve synapses onto bushy cells are specialized to preserve and refine timing, by having many release sites (N), each with a high probability of neurotransmitter release (P), and large quantal size (Q). However, high P also causes strong depression during prolonged activity, which reduces the likelihood of postsynaptic spiking. This raises the question of how the auditory system deals with loud environments. We studied this by exposing mice to constant, non-damaging noise, and found that auditory nerve synapses changed to facilitating, reflecting low P. For mice returned to quiet, synapses recovered to normal depression. Noise-rearing had no effect on Q or average EPSC amplitude. However, immunolabelling for VGlut1 revealed that synapses from noise-reared animals were larger and had increased release site density, indicating that N increased to compensate for decreased P. These changes led to an increase in bushy cell spike fidelity in current-clamp recordings. Thus, auditory nerve synapses regulate excitability through a novel, activity-dependent, homeostatic mechanism. This appears to enhance temporal fidelity under different conditions.

Dynamic Fidelity Control to the Central Auditory System: Synergistic glycine/GABAergic Inhibition in the Cochlear Nucleus

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GABA and glycine are the major inhibitory transmitters that attune neuronal activity in the CNS of mammals. The respective transmitters are mostly spatially separated, i.e. synaptic inhibition in the forebrain areas is mediated by GABA, while glycine is predominantly utilized in the brainstem. Accordingly, inhibition in auditory brainstem circuits is largely mediated by glycine, but there are few auditory synapses employing both transmitters in maturity. Little is known about physiological advantages of such a two-transmitter inhibitory mechanism. We explored the benefit of engaging both glycine and GABA with inhibition at the endbulb of Held-spherical bushy cell synapse in the auditory brainstem of juvenile Mongolian gerbils. This model synapse enables selective in vivo activation of excitatory and inhibitory neuronal inputs through systemic sound stimulation and precise analysis of the input (endbulb of Held) - output (spherical bushy cell) function. The combination of in vivo and slice electrophysiology revealed that the dynamic AP inhibition in spherical bushy cells closely matches the inhibitory conductance profile determined by the glycine-R and GABAA-R. The slow and potent glycinergic component dominates the inhibitory conductance, thereby primarily accounting for its high-pass filter properties. GABAergic transmission enhances the inhibitory strength and shapes its duration in an activity-dependent manner, thus increasing the inhibitory potency to suppress the excitation through the endbulb of Held. Slow transmitter clearance from the synaptic cleft caused prolonged receptor binding and, in the case of glycine, spillover to nearby synaptic or extrasynaptic receptors. The GABAergic component appears not to derive from extrasynaptic receptors, but it rather prolonged the decay by contributing to the asynchronous vesicle release. Finally, in silico modelling provides a strong link between in vivo and slice data by simulating the interactions between the endbulb- and the synergistic glycine-GABA-conductances during in vivo-like spontaneous and sound evoked activities.

Cholinergic Signaling Influences Gerbils Spherical Bushy Cells Excitability in Vitro

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Low frequency spherical bushy cells (SBC) receive direct input from the auditory nerve via specialized axosomatic synapses, the Endbulbs of Held. SBCs form an integral part in the pathway of sound source localization in the azimuth by projecting bilaterally into the medial superior olive (MSO). Recent data have shown that inhibition changes SBC input-output function by tuning excitability in a stimulus-dependent manner, increasing temporal acuity of the SBC in the process. Furthermore, the existence of cholinergic projections into the cochlear nucleus has been shown before, formed by collaterals of the olivocochlear bundle as well as a projection from the tegmentum. We hypothesize that modulation through Acetycholine (ACh) plays an additional role in fine-tuning of SBC input-output function.
In parasagittal auditory brainstem slices of P14 -26 gerbils (Meriones unguiculatus), whole cell patch clamp recordings from SBC were obtained in the presence of Glycine and GABA receptor antagonists. 1mM ACh or 500µM Carbachol (both in ACSF) was locally applied onto the SBC with a Picospritzer to investigate the principal effect of ACh on the cell. At the same time we electrically stimulated auditory nerve fibers to evoke Endbulb inputs onto SBC. Besides normal pulse trains ranging from 50 to 300Hz, in-vivo-like (IVL) stimuli were used as the principal stimulation method. IVL stimuli mimic the response of the auditory nerve to tonal stimulation and were obtained from earlier in-vivo recordings of the Gerbil.
The principal effect of ACh application was a transient depolarization of 3.23 mV (+/- 1.7 mV), relaxing back to rest with a weighted-tau of 282 ms (+/- 311ms, n=7). Additionally, repeated ACh applications (10sec interval) tonically shifted the RMP towards depolarized values from **RMP ± std** to **RMP+1.34** mV (+/- 1.66mV, n=18), lasting minutes. The combined electrical and pharmacological stimulation revealed an increase in SBC's firing probability to pulse trains while under muscarinic modulation. For IVL stimuli, the cells showed increased firing rate under tonal stimulation and an increased firing probability to spontaneous auditory nerve activity. Cholinergic modulation also had an effect on the temporal accuracy of the SBCs.
This work shows that SBCs can be modulated by Acetylcholine, impacting their membrane potential, firing probability and temporal acuity. The results also indicate that SBCs can be modulated on two different time scales. This could mean that SBCs excitability and temporal precision could be altered by top-down, non-stimulus dependent modulation.

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The role of voltage gated ion channels in brainstem auditory processing

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There are more than 80 genes specifying potassium channel subunits that are associated into 12 gene families. Most potassium channels are composed of 4 alpha subunits, so that even with structural limitations to assembly, there are many possible native channel subunit configurations. We would like to understand the general rules governing potassium channel utilization in neuronal function and start to explore subunit specific roles in auditory processing. We have focused on voltage-gated potassium channels (kcna-kcnd; Kv1-Kv4) in the brainstem auditory circuits underlying sound-source localization – namely the superior olivary complex (SOC). In this pathway excitatory synaptic transmission is associated with giant synapses (endbulb and calyx of Held) that generate large synaptic currents, to maintain the temporal precision of transmission. However the magnitude of these EPSCs is such that the target neuron excitability must also be tightly controlled, so as to repolarize action potentials (APs) and enable recovery of voltage-gated sodium channels from inactivation. Kv1 channels are associated with regulating AP firing threshold, while Kv2 and Kv3 channels are responsible for AP repolarization. In the medial nucleus of the trapezoid body (MNTB) that receives the calyx of Held synapse, AP repolarization can be mediated by either Kv3 or Kv2 channels. Activity-dependent modulation allows MNTB neurons to switch the basis of their AP repolarization between these two different potassium channel families. This switch is controlled by neuronal nitric oxide synthase (nNOS). Additionally we note that each of the nuclei across the SOC expresses both Kv3.1 and Kv3.3 subunits, but there is a gradient of expression that has functional consequences. We conclude that each family of voltage-gated potassium channel plays specific roles in regulating auditory brainstem excitability and postulate that both genetic and activity-dependent processes tune channel activity to the physiological role of each nucleus.

The role of inhibition for temporal precision in the lateral superior olive

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Due to the sound shadow of the head, lateralized sound sources are louder on the near side ear than on the far side ear. In mammals, the resulting interaural level differences are integrated by neurons of the lateral superior olive (LSO). Ipsilateral excitatory and contralateral inhibitory inputs, encoding for the respective monoaural sound levels, are compared and consequently translated into a specific discharge rate. During this very precise and highly sensitive process (Tollin et al., 2008), LSO neurons face about 10 excitatory and 8 inhibitory inputs, with average transmission rates of 200 Hz and 100 Hz each (Bures & Marsalek, 2013). Integration of these theoretical 2800 inputs/s can result in average discharge rates of 200 spikes/s with onset rates of up to 1000 spikes/s. Given the probabilistic nature of synaptic transmission and action potential generation, cellular adaptations are crucial to guaranty temporal precision in a time scale where such random processes become limiting. Here we focus on the role of inhibition in shaping temporal precision of LSO neurons and their synaptic inputs.

It is well known that glycine but not GABA is utilized during phasic synaptic transmission in the LSO. Surprisingly we found that puff application of GABA (10 µM) in acute brain slices caused a huge decline in membrane resistance (21 %), accompanied by only weak hyperpolarization (1.9 mV). From this, we concluded that extrasynaptic $GABA_A$Rs render powerful shunting inhibition of LSO neurons. In line with this finding, excitability of LSO neurons due to current injections was also significantly decreased. Notably, spike thresholds remained unchanged. In addition to these extrasynaptic $GABA_A$R-mediated effects, we found that activation of presynaptic $GABA_B$Rs in the LSO reduces calcium influx into inhibitory terminals during synaptic activity. As the decrease of presynaptic calcium is considered to be a key feature of temporally precise synchronous transmitter release, we argue that tonic $GABA_A$Rs activation renders sustained precision during ongoing high-frequency activity, whereas imprecise asynchronous release will be diminished.

In summary, we found tonic GABA-receptor-mediated modulation of synaptic and extrasynaptic mechanisms that are known to affect temporal integration or precision. As tonic GABA sharpens coincidence detection in many systems, we hypothesize that it also affects temporal precision in the mouse LSO. Therefore, we focus on the impact of tonic GABAergic inhibition on temporal synaptic precision, spike generation and the balance of synchronous and asynchronous transmitter release.

Cellular mechanisms of context dependent signal processing in the lateral lemniscus

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NMDA receptor mediated excitation is known to play a fundamental role during mechanism involved in learning especially during development. More recent studies verified the presence and role of NMDA receptors also in adult animals. Throughout different sensory systems and brain structures it has been shown that NMDA receptor mediated excitation increases and prolongs stimulus induced neuronal responses. In vitro studies in the dorsal nucleus of the lateral lemniscus showed such presence and increase responsibility by NMDA receptor mediated excitation in the early auditory system involved in processing of binaural cues. However the function of this increased excitation remains unclear. Recent in vivo studies compared the binaural responses in the medial superior olive, which is one of the major nuclei that first process binaural cues, and the DNLL, which gets direct inputs from the medial superior olive. This study shows an increase of the neuronal information between these sequent neuronal stages. Using a combined in vitro and in vivo approach we now could show, that NMDA receptor mediation excitation increases the neuronal responses in the DNLL. In contrast to AMPA receptors induced excitation, NMDA receptors have a substantial role for the ongoing component of the stimulus-induced responses. Furthermore by blocking NMDA receptors pharmacologically we found, that the neuronal information is increased by NMDA receptor mediation excitation. In line with in vitro and immunohistological findings our data show that within the DNLL different subtypes of the NMDA receptors contribute to a increase of the neuronal information.

Symposium

S19: Novel mechanisms influencing synaptic plasticity at GABAergic synapses

S19-1 Mitochondrial reactive oxygen species couples cellular metabolism to neuronal communication
Derek Bowie

S19-2 BDNF Regulates Synapse Maintenance After Oxygen-Glucose Deprivation in the Hippocampus
Anne McKinney

S19-3 Adaptations at GABAergic postsynapses is facilitated by gephyrin posttranslational modifications.
Shiva Kumar Tyagarajan, Carmen Flores, Irina Nikonenko, Pablo Mendez, Dominique Muller

S19-4 KCC2 and CA7: Neuronal ion-regulatory proteins with a morphogenic function
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Florian Walker, Jochen Staiger, Mirko Witte

Mitochondrial reactive oxygen species couples cellular metabolism to neuronal communication

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Neuronal communication imposes a heavy metabolic burden in maintaining ionic gradients essential for action potential firing and synaptic signalling. Given its high energy requirements and limited energy reserves, the evolving brain has developed metabolically -efficient wiring and signalling strategies to transmit information. How energy consumption is distributed among different cell types has yet to be established. In this presentation, I will discuss recent work highlighting the bioenergetics of inhibitory GABAergic synapses and the signalling role of reactive oxygen species (mROS) derived from the mitochondria. Emerging data suggest that mROS may act as a homeostatic signalling molecule coupling cellular metabolism to the strength of inhibitory transmission.

BDNF Regulates Synapse Maintenance After Oxygen-Glucose Deprivation in the Hippocampus

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In the aftermath of ischemic stroke, patients can experience mild to severe cognitive deficits, caused by a disruption in network connectivity. Animal models of stroke have shown morphological changes in dendritic spines, the postsynaptic compartment of excitatory synapses, and also a disturbance in inhibitory synapses. However, the cellular mechanisms that underlie this disruption are poorly understood. Previously, it has been shown that high levels of the neurotrophin BDNF can induce dendritic spine retraction. Moreover, signaling molecules downstream of BDNF, such as ERK and GSK3ß;, can regulate the plasticity of GABAergic synapses. Though BDNF is upregulated after stroke, we do not know if it plays a role in synapse reorganization after ischemia. Therefore, **we hypothesized that ischemia induced BDNF production and activation of downstream signaling can destabilize GABAergic synapses and induce dendritic spine retraction.**

To test our hypothesis, we treated organotypic hippocampal slice preparations with oxygen-glucose deprivation (OGD), a model of ischemia that is associated with excitotoxic structural remodeling of dendritic spines. We found a significant reduction of gephyrin, a postsynaptic scaffolding protein at GABAergic synapses, in area CA1 ($p<0.001$), accompanied by a significant reduction in the number of dendritic spines on CA1 pyramidal neurons 90 minutes after OGD ($p<0.05$). This led to functional deficits of the CA1 neurons, as we found a reduction in the amplitude and frequency of AMPA-mediated miniature excitatory postsynaptic currents (mEPSCs) and a reduction in the frequency of GABAA-mediated miniature inhibitory postsynaptic currents (mIPSCs). This conclusion is further supported by the observation that OGD results in a significant reduction of a1- and a2-GABAA receptor subunit expression ($p<0.05$), specifically in CA1 tertiary dendrites.

In order to determine whether BDNF was involved in this process, we treated hippocampal slices with a BDNF blocker, TrkB-Fc, and found that we could prevent the observed reduction in gephyrin clusters and dendritic spines. This suggests that ischemia-induced loss of gephyrin and changes in dendritic spine morphology are BDNF-dependent. We found that using TrkB-Fc we could also rescue the functional deficits in mEPSCs and are currently investigating the effect on mIPSCs. Lastly, we found that treatment with ERK and GSK3ß inhibitors can prevent gephyrin clustering loss after OGD, suggesting BDNF activation of ERK and GSK3ß downregulate GABAergic transmission via gephyrin cleavage.
Findings from this work contributed to a better understanding of how BDNF can induce network reorganization after injury and suggest new therapeutic targets for intervention or prevention of cognitive deficits following stroke.

Adaptations at GABAergic postsynapses is facilitated by gephyrin posttranslational modifications.

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Mechanisms of synaptic homeostasis have been studied extensively at excitatory synapses. Little is known, however, about their counterparts at inhibitory synapses. Our recent work identified phosphorylation residues on gephyrin and characterized their regulation of gephyrin scaffold formation and their functional consequences on GABAergic transmission. Here, focusing on novel signaling cascades that converge on gephyrin, we report how alterations in neuronal activity facilitate homeostatic adaptations at GABAergic postsynapse via gephyrin scaffolding changes. By identifying the signal transduction pathway and also the regulatory residues on gephyrin we demonstrate at a single molecule resolution how long term adaptation at GABAergic postsynapse shape neuronal homeostasis.

KCC2 and CA7: Neuronal ion-regulatory proteins with a morphogenic function

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GABAergic signaling is subject to a variety of plasticity mechanisms, which operate over a wide range of timescales. These mechanisms have traditionally been associated with modulation of the expression patterns and properties of distinct types of $GABA_A$ receptors ($GABA_A$Rs). In addition, $GABA_A$R-mediated signaling is subject to ionic plasticity, which is based on changes in the driving forces for Cl^- and $HCO3^-$. The transmembrane gradients for these anions are under dynamic control by ion-regulatory proteins (IRPs), such as cation-chloride co-transporters and carbonic anhydrases (CAs), which set the nature (inhibition vs. excitation) and strength of GABAergic signaling.

So far, research on IRPs has mainly focused on their direct influence on ion regulation at the cellular and subcellular level. We have previously shown that the neuron-specific KCC2 is, in addition to its well-known role in neuronal Cl^- extrusion, an important structural factor in the maturation of dendritic spines and, thereby, in the development of glutamatergic transmission in cortical neurons. This morphogenic function is independent of ion transport, and requires the interaction of KCC2 with the cytoskeleton.

Recently, we have focused on the neuron-specific carbonic anhydrase isoform VII (CA7). In rodents, expression of CA7 starts at around postnatal day 10, and it is critically involved in $HCO3^-$-driven GABAergic excitation during intense activation of $GABA_A$Rs. This contributes, for example, to the facilitation of experimental febrile seizures in infant rodents. Our unpublished data now show that CA7 localizes to somatic and dendritic compartments, with high levels close to the plasma membrane and, strikingly, also within dendritic spines. Several lines of evidence indicate that CA7 directly interacts with actin. This interaction is in line with a structural role for CA7 in dendritic spine formation, a conclusion supported by preliminary analyses of spines in CA7 KO and WT neurons. Thus, accumulating evidence indicates that, like KCC2, CA7 is a multifunctional neuronal IRP.

Coordination of innate behaviours by GABAergic cells in lateral hypothalamus

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Lateral hypothalamus (LH) is crucial for the regulation of innate behaviors, including food intake and sleep-wake cycle yet temporal coordination of hypothalamic neuronal populations remains elusive. Here we used combination of high-density electrophysiological recordings and optogenetics in behaving mice to study function of GABAergic cells in LH. Excitatory (ChETA) or inhibitory (halorhodopsin, eNpHR3.0) opsins were expressed in LH of VGAT-Cre mice to ensure selective targeting of GABAergic cells. Recordings of neuronal activity and optostimulation were performed in various behavioral paradigms assessing innate behaviours, including a "free-will" environment where an animal could choose between compartments with food, water, enriched environment and home-cage like enclosure. We found that optogenetic stimulation of GABAergic LH cells at various frequencies as well as stimulation of projections of these neurons changed transitions between innate behaviors. Activation of GABAergic neurons in LH increased food intake in satiated mice whereas optogenetic inhibition of LH GABA cells decreased feeding even despite food deprivation. Furthermore, neuronal activity of LH neurons was behavior- and state-dependent. We now characterize selective roles of various projections of LH GABA cells in innate behaviors.

Integration of Martinotti cells into dis-/inhibitory cortical circuits

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GABA releasing interneurons play a crucial role in information processing within the neocortex and are directly integrated in the circuitry of the primary somatosensory cortex, also known as the barrel cortex. Martinotti cells, a well-defined subclass of GABAergic interneurons, modulate the dendritic excitation of local pyramidal cells, thereby having direct influence on the barrel cortex output structures. One feature of these cells is that they receive excitatory inputs from pyramidal cells. This makes them suitable for feedback and lateral inhibition, proposed core mechanisms for the regulation of cortical activity. In addition, in vivo data showed that Martinotti cells also receive inhibition during active whisking. The neocortical origin of inhibitory inputs to Martinotti cells, presumably vasoactive intestinal polypeptide (VIP)-expressing interneurons, is in focus of several research groups. In our study, a combination of whole-cell patch clamp and local photolysis of caged glutamate in acute brain slices has been used to identify local neocortical areas, at a layer- and column-specific resolution, responsible for inhibition of Martinotti cells of layer (L) II/III and LV. Martinotti cells of LII/III obtain very focused (>50%) inhibitory inputs from the same but also other layers of their home column. In contrast, Martinotti cells of LV receive extensive inhibitory inputs from LVa and LVb and a less prominent, but very conspicuous, portion from LII/III of the home column. By crossing PV (parvalbumin)cre- or VIPcre-expressing lines with GIN-mice, and stereotaxic injection of floxed ChR2-mCherry viral vectors we could show that the population of VIP - and PV -expressing interneurons projects to LII/III and LV Martinotti cells. To verify our optogenetic approach, we perform paired patch-clamp recordings from Martinotti cells and their upstream connected inhibitory interneuron in triple transgenic mice e.g. tdTomato -labeled PVcre - and VIPcre-expressing lines crossed with GIN -mice. Based on their electrophysiological characterization and morphological appearance we could clearly identify the connection between VIP - or PV -expressing interneurons and Martinotti cells in the barrel Cortex. This novel disinhibitory cortical circuit (PV-MC) motif will lead to a reconsideration of the contribution of GABAergic interneurons to processing of sensory information, and the function of state dependent inhibition of Martinotti cells.

Symposium

S20: Actin cytoskeleton in neuronal morphogenesis and plasticity

S20-1 The Arp2/3 complex is required for de novo formation of dendritic branches
Anastasia Tatarnikova, Jan Mueller, Yun Zhang, Barbara Schaffran, Maria Nemethova, Sven Bogdan, John Victor Small, Gaia Tavosanis

S20-2 Actin nucleation and membrane remodelling in neuromorphogenesis and synaptic plasticity
Britta Qualmann

S20-3 Regulation of actin-dynamics in processes of neuronal plasticity,
memory formation and synapse stabilization
Martin Korte

S20-4 PTEN regulates dendritic spine function by targeting the actin binding protein Drebrin
Britta Eickholt, Eugenia Rojas-Puente, Till Mack, Patricia Kreis

S20-5 Fast Nogo-A signaling acutely modulates neuronal structure and function in the mature mouse hippocampus
Niklas Lonnemann, Steffen Fricke, Yves Kellner, Martin E. Schwab, Martin Korte, Marta Zagrebelsky

S20-6 Unconventional Myosin affects presynaptic assembly
Torsten Götz, Martin Alemndinger, Torsten Götz, Stephan J Sigrist

The Arp2/3 complex is required for de novo formation of dendritic branches

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The processes underlying the formation of neuronal dendrite branches are fundamental to our understanding of the development, wiring and function of the nervous system. Here, we identified the Arp2/3 complex as the major actin-nucleating factor involved in dendrite collateral branching. Cell-autonomous RNAi-mediated depletion of Arp2/3 complex, including Arp2, Arp3 or Arpc1, as well as loss-of-function mutations led to a strong reduction in the number of terminal branchlets in all classes of Drosophila larvae sensory da neurons. The reduction in the number of terminal branchlets upon loss of Arp2/3 function was largely due to impairment of initiation of dendrite branching as revealed by in vivo time-lapse analysis of differentiating da neurons. Consistently, the localization of Arp3GFP predicted the site of branch formation. WASP (Wiskott-Aldrich Syndrome) family proteins activate the Arp2/3 complex. Among the WASPs encoded in the fly genome, only loss of WAVE function phenocopied Arp2/3 depletion and WAVE mutants genetically interacted with Arp3 loss of function alleles, indicating that WAVE is an important activator of Arp2/3 complex in the process of dendrite formation. Using modified constructs, we showed that recruitment of WAVE and its regulatory protein Sra1 / CYFIP to the cell membrane promotes the induction of dendritic branching. Further, local photoactivation of the small GTPase Rac1 induced ectopic branching, which was suppressed in a WAVE knock-down background, suggesting that Rac1 initiates WAVE complex activation to promote branch formation. Performing electron tomography of primary cultures of fly larval neurons, we found patches of branched actin filaments at the base of collateral dendrite processes, supporting a local role for the Arp2/3 complex in dendrite branch formation. Taken together, local activation and membrane recruitment of Arp2/3 through Rac1 and WAVE promotes the nucleation of patches of branched actin, which initiate collateral dendrite branches in different classes of Drosophila sensory neurons. Thus, we have uncovered a general mechanism of dendrite branch formation via actin remodeling.

Actin nucleation and membrane remodelling in neuromorphogenesis and synaptic plasticity

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The complex architecture of neuronal networks and the extreme connectivity of cells within these networks is a basis for the function and abilities of vertebrate brains. Spatial control of cortical actin nucleation is indispensable for proper establishment and plasticity of cell morphology. With Cobl, we have identified a novel and very powerful actin nucleator indispensable for proper neuromorphogenesis that belongs to the new class of WH2 domain-based nucleators. As excessive, uncoordinated actin nucleation would interfere with a huge variety of cellular processes relying on a proper organization of the actin cytoskeleton, it is obvious that the Cobl-mediated actin nucleation activity needs to be carefully regulated. Actin filament formation at defined sites at the cell cortex is a source for the forces required for induction and elaboration of axonal and dendritic morphologies formed by neurons. Such active role of the actin cytoskeleton in membrane remodeling requires targeting of actin nucleation machineries to membranes and their specific activation at distinct membrane areas. Our examinations shed light on how neuronal cells use actin nucleators to steer the complex cytoskeletal reorganizations underlying neuronal shape modulation and the formation of neuronal networks – prerequisites for proper brain development and neuronal cell-cell communication. Cobl, which plays an important role in the proper development of early neuronal morphology, is targeted to cortical membranes through complex formation with the F-BAR domain containing protein syndapin I that can discern the curvature of membranes by offering curved, crescent-shaped membrane interaction surfaces. Our studies revealed important molecular mechanisms for the spatial control of the actin nucleator Cobl in neuronal cells and furthermore demonstrated how it is interconnected to N-WASP/Arp2/3 complex functions in neuromorphogenesis. The defects observed upon syndapin I deficiency in synapse formation and function likely reflect the crucial importance of syndapin I interactions with factors promoting actin filament formation, such as Cobl and the Arp2/3 complex activator N-WASP. Functional studies and ultra-structural studies based on combining freeze-fracturing of the plasma membrane of neuronal cells with quantitative immunogold labeling procedures and electron microscopy revealed that syndapin I enriches in membrane nanodomains and is involved in shaping regular cylindrical dendritic membrane areas into local curved membrane topologies giving rise to dendritic branches and synaptic compartments. Consistently, *syndapin I* gene knockout and RNAi in individual neurons caused significant reductions of dendritic arborization as well as of synapse and dendritic spine densities and thereby reveal that syndapin I is a crucial postsynaptic coordinator in formation of excitatory synapses. These studies suggest that membrane-associated syndapin I provides cortical anchor points for its interactions partners and molecular organizing platforms that are critical for shaping distinct neuronal membrane areas into morphological structures that are important for the proper formation and function of neuronal networks.

Regulation of actin-dynamics in processes of neuronal plasticity, memory formation and synapse stabilization

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In the mature CNS a tightly regulated balance between plasticity and stability of the neuronal network allows the acquisition, storage and clearance of memories in a dynamic and highly controlled manner. While much has been described about molecules promoting plasticity, very little is known regarding molecules and mechanisms maintaining the stability of the mature neuronal circuitry. In this context, temporal control of actin dynamics is of vital importance for both synaptic function and morphology. Among the proteins involved in regulating actin polymerization and organization in neurons, Cortactin (cttn) might play a central role as it is not only able to bind actin but provides a substrate for several kinases. Here, we will present data about the influence of Cttn both on neuronal function and structure in the mouse hippocampus in vitro and in vivo by taking advantage of a cttn KO mouse. Therefore, hippocampal neurons were transfected with farnesylated eGFP for detailed morphological analysis. Moreover, we investigated synaptic plasticity in the form of long-term potentiation (LTP) in acute hippocampal slices and spatial memory formation in the Morris Water Maze in cttn-/- mice. Our results indicate that indeed, both neuronal function and structure in the hippocampus are compromised in cttn-/- animals. In particular we found a significant reduction in LTP in cttn-/- mice. Most notably, spatial memory formation in the MWM was in addition altered in the absence of Cttn. Taken together our results point out a crucial role for Cttn in hippocampal neurons and specifically in processes of memory formation. In addition, the talk will cover results from a series of recent studies which indicate that in the intact CNS the myelin-associated neurite growth inhibitor Nogo-A is involved in negatively regulating activity-dependent synaptic plasticity, in stabilizing dendritic architecture as well as regulating the morphology of dendritic spines of mature hippocampal pyramidal neurons. In this context, we study whether Nogo-A acutely controls dendritic spines number and morphology by altering actin dynamics in the mature, uninjured hippocampus. We apply different loss- or a gain-of-function approaches to influence Nogo-A signaling and use time-lapse confocal microscopy to examine the dynamics of dendritic spines and fluorescence recovery after photobleaching (FRAP) of actin-GFP in order to analyze actin turnover at single spines of principal neurons in the mouse hippocampus. A loss-of-function approach using acutely applied function blocking antibodies for Nogo-A results in a fast and progressive increase in dendritic spine number. Moreover, while upon Nogo-A neutralization spines length and mobility significantly increase over time they are transiently decreased by a Nogo-A gain-of-function approach. Interestingly, also the turnover-time of the actin treadmilling within single dendritic spines is significantly altered under these conditions. Moreover, neutralizing the function of the Nogo receptor (NgR1) and blocking its downstream signaling by inhibiting the Rho-associated protein kinase (ROCK) reproduces the changes in terms of spine density observed upon blocking Nogo-A but mimicks only partially the increase in spine length. Taken together our results so far point to a critical role of Nogo-A signaling in the mature hippocampus in acutely modulating actin dynamics to regulate dendritic spine number and morphology on a fast time scale.

PTEN regulates dendritic spine function by targeting the actin binding protein Drebrin

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PTEN is highly expressed in neurons and plays fundamental roles in controlling a wide range of neuronal function. Human germline PTEN mutations or conditional deletions of PTEN in mice have been associated with neurological disorders such as macrocephaly, ataxia, seizures, mental retardation and autism. PTEN-loss in neurons leads to several abnormal morphological features, including neuron hypertrophy, ectopic dendrites and aberrant axonal projections, as well as increased dendritic spine density. Reduction in PTEN also decreases synaptic transmission, and impairs long term potentiation (LTP) and long term depression (LTD). At the synapse, PTEN is recruited to the post synaptic membrane following NMDA stimulation and its activity is required for NMDA receptor dependent LTD. Conversely, inhibition of PTEN promotes AMPA receptor trafficking to increase synaptic strength. Whilst most, if not all, of the characterized neuronal responses can be credited to PTEN's role in the regulation of membranous PIP3 and PI3K signaling, PTEN has other potential mechanisms of action. The physiological significances of these PIP3 -independent roles, especially in neurons, remain largely unclear.

We have recently identified the interaction of PTEN with Drebrin (DBN), which negatively regulates a phosphorylation site present in the DBN C-terminus, S647, independently of PI3K. Neuronal DBN accumulates in regions highly enriched in F-actin, such as dendritic spines, and DBN regulates several important postsynaptic signalling proteins. For example, it regulates the synaptic targeting of NMDA receptors, it interacts with the scaffolding protein Homer and it induces the accumulation of PSD95 in dendritic spines. Interestingly, neuronal loss of the DBN has been linked to Mild Cognitive Impairment (MCI), a classification that identifies people in a transitional state between healthy aging and mild dementia.

Here, we investigated the consequences of PTEN targeted S647 -DBN phosphorylation and identify unique PTEN functions during dendritic spine plasticity through spatial control of DBN protein abundance and turnover.

Fast Nogo-A signaling acutely modulates neuronal structure and function in the mature mouse hippocampus

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The myelin-associated neurite growth inhibitor Nogo-A has been initially identified as an inhibitor of axonal regeneration upon a lesion in the central nervous system. Interestingly, in the mature healthy brain Nogo-A and its receptors NgR1 and S1PR2 are expressed both pre- and post-synaptically and have been shown to negatively modulate neuronal architecture and to control synaptic plasticity, by restricting long-term potentiation (LTP) on a fast time scale both in the hippocampus and in the cortex. Moreover, NgR1 expression has been shown to restrict in vivo the experience-driven turnover of synaptic structures within the somatosensory cortex. However, what are the cellular and molecular mechanisms mediating the fast action of Nogo-A on activity-dependent synaptic plasticity and especially whether Nogo-A regulates structural changes at spines at a fast time scale is still largely unknown. Here we addressed this question by combining time-lapse confocal imaging, fluorescence recovery after photobleaching (FRAP) and calcium imaging with a series of loss- or gain-of-function approaches for Nogo-A and its receptors in mature hippocampal cultures. Our results show that Nogo-A signalling acutely modulates the spine actin cytoskeleton dynamics to control structural plasticity at dendritic spines within seconds to minutes. Indeed, Nogo-A signalling loss-of-function experiments transiently increase F-actin stability within minutes and results in an increase in dendritic spine density and length. In addition, Nogo-A acutely restricts AMPA receptor (AMPAR) insertion and formation of new AMPAR clusters. Accordingly, calcium imaging reveals a role for Nogo-A in negatively modulating synaptic strength and the number of functionally active synapses at spines. To assess the in vivo relevance of the Nogo-A effects on synaptic plasticity, spatial learning and memory acquisition were assessed using the Morris water maze. The behavioural analysis shows a faster acquisition of memory in Nogo-A knockout when compared to wild type mice. Our data provide a cellular and molecular mechanism mediating the role of Nogo-A signalling in controlling activity-dependent synaptic plasticity thereby maintaining the balance between the plasticity and stability of the neuronal circuitry in the mature central nervous system. Furthermore, in vivo experiments indicate a role of Nogo-A in controlling hippocampus-dependent learning processes.

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Unconventional Myosin affects presynaptic assembly

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Unconventional myosins are motor proteins transporting diverse cargos along the filamentous actin network. They are implied in almost all basic cellular processes requiring a short range transport, often towards the membrane, to locally enrich protein amounts or tether membrane proteins at a specific site. Furthermore, several myosins (i.e. MyoVI, MyoV) are acting in the presynaptic synaptic vesicle (SV) cycle controlling exo-and subsequent endocytosis processes.
Here we describe in the Drosophila model system a novel function of a hitherto scarcely investigated unconventional myosin. By use of the glutamatergic model synapse at the neuromuscular junction (NMJ) we were able to identify defects in synapse formation upon myosin knock down by an RNAi approach. Presynapses are reduced in size and seem unable to extend over a certain threshold size, while overall synapse numbers are increased. Interesting, glutamate receptor (GluR) fields are enlarged, indicating a defect in presynaptic release counterbalanced by a plasticity regulated upregulation of GluRs. However, evoked presynaptic release is not affected upon a single stimulus, while the paired pulse ration shows a slight facilitation. In addition is the frequency, but not the amplitude, of the spontaneous release significantly increased. The myosin localizes in ring shaped structures with a diameter of 200 nm in the presynapse, observed by STED (stimulated emission depletion microscopy), as well as to the soma of motoneurons in the cortex of the ventral nerve cord (VNC) in a stereotyped three-digit branch form.
We suggest that the unconventional myosin is implied in the actin based short range transport of presynaptic scaffolding or SV proteins. Firstly in the soma by charging cargo on to microtubules (MT) for axon long range transport and secondly at the NMJ discharging this cargo from the MT on to the actin network for local distribution to the sites of synapse formation and growth. Absence of this myosin causes a synaptic undergrowth phenotype with synapses not able to grow over a certain size.

Symposium

S21: Neuronal mechanisms of behavioral timing

S21-1 Neuronal mechanisms of behavioural timing
Valter Tucci

S21-2 Circadian rhythms in second messengers and biogenic amines set olfactory thresholds in insect antennae
Monika Stengl, Thomas Schendzielorz

S21-3 Sensory systems and molecular mechanisms involved in synchronizing the *Drosophila* circadian clock
Ralf Stanewsky

S21-4 Timing of the peptide-orchestrated eclosion behaviour in *Drosophila*
Mareike Selcho, Franziska Ruf, Jiangtian Chen, Benedikt Hofbauer, Kouji Yasuyama, Christian Wegener

S21-5 Chronological Interactions between Antennal Lobe and Mushroom Body: Extracting the Behaviorally Relevant Stimulus
Martin Fritz Strube-Bloss

S21-6 Variable event timing in visual conditioning leads to memories with opposite valence in *Drosophila*
Katrin Vogt, Hiromu Tanimoto

S21-7 The Hofbauer-Buchner-eyelet signals to the ventro-lateral neurons and thereby mediates siesta and phase-shifts in *Drosophila*.
Matthias Schlichting, Katie Lelito, Orie Shafer, Charlotte Helfrich-Förster

Neuronal mechanisms of behavioural timing

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An integrated understanding of neurobiological timing in mammals

How the circadian clock and sleep influence behavior and cognition is not yet fully understood. In our laboratory we studied several mouse mutants, who express circadian and sleep defects and we observed that timed behavior are affected. Often, mutants fail to show the ability to anticipate events in time, both at hour and seconds-to-minutes scale. The mutations alter the regulation of other genes according to the brain regions where they are expressed. For istance, we identified a novel role of the complex Per2/Cry1 circadian elements in a brain region that governs cognitive timing and temporal decision-making. In this presentation I will present in-depth analyses to define the specific contribution of circadian clock and sleep process in the modulation of complex behaviors through the 24 h. Circadian and sleep can be considered as predictors of behavioral performance and they are in antiphase with each other.

Circadian rhythms in second messengers and biogenic amines set olfactory thresholds in insect antennae

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Pheromone-dependent reproduction is under strict circadian control in several insect species such as the crepuscular hawkmoth *Manduca sexta*. Female hawkmoths release pulses of a sex-pheromone blend at night to attract their conspecific mates. Male hawkmoths depend on the intermittent pheromone trail to find their mates during the night and early morning, while they are resting during the day. Male hawkmoths detect the pheromones with long trichoid sensilla on their antennae. Each of the sensilla is innervated by two olfactory receptor neurons (ORNs) one of which always responding to bombykal the main sex-pheromone component. Extracellular tip-recordings of trichoid sensilla revealed previously that sensitivity and kinetics of bombykal responses are more sensitive and faster at night, during the hawkmoths activity phase as compared to the resting phase during the day. In addition, perfusion of the stress hormone octopamine (OA) via the tip -recording pipette increased the sensitivity of bombykal responses and rendered responses more phasic during rest, but not during the activity phase, while OA receptor antagonists prevented any pheromone response during the resting phase. Furthermore, perfusion of a membrane-permeable cAMP analog in tip-recordings partly mimicked OA-dependent sensitization of bombykal responses.

In ELISAs we tested whether daytime-dependent changes in antennal OA levels might be responsible for the daytime-dependency of OA effects. In addition, we examined OA signaling in the hawkmoth antenna. Indeed, antennal OA levels were minimal during the resting phase of the hawkmoth, while they were maximal at night, during maximal pheromone-dependent flight activity. Furthermore, we showed that OA elevates cAMP - and IP3 levels in hawkmoth antennae, reminiscent of a-adrenergic-type OA receptors. Accordingly, also cAMP- and IP3-levels varied daytime-dependently with minima during rest and maxima during activity phases, in correlation with daytime -dependent changes in OA concentrations. Thus, we hypothesize that circadian control of OA levels in the antenna sensitize ORNs allowing for pheromone detection during the activity phase of hawkmoths cAMP- and IP3-dependently. In contrast, in the presence of low levels of OA during the day ORNs remain adapted and thus, allow hawkmoths to rest.

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Sensory systems and molecular mechanisms involved in synchronizing the *Drosophila* circadian clock

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Circadian clocks are synchronized by the natural daily fluctuations of light and temperature. Previous work suggests that in Drosophila melanogaster peripheral mechanosensory organs, otherwise known to function as stretch receptors (chordotonal organs; ChO), also function as temperature sensors. The temperature receptors mediating this synchronization have not been identified. Transient Receptor Potential (TRP) channels function as thermoreceptors in animals and we have previously shown that the Pyrexia TRP channel mediates temperature synchronization in the lower range (16°C:20°C Temperature Cycles; TC). Here, we isolated proteins interacting with the Nocte protein, known to play a role in temperature entrainment and ChO function. A member of the Ionotropic Receptor (IR) family, a new class of chemosensory receptors in flies, physically interacts with Nocte and is also expressed in ChO. IR loss-of-function mutants fail to synchronize their behaviour to TC in the higher range (25:27°C) and clock protein oscillations in subsets of the clock neurons are blunted and abolished in peripheral clocks. Interestingly, mechanical stimulation of the ChO using (12h:12h) cycles of vibration and silence also results in synchronization of the circadian clock. Our results suggest that ChO form part of the temperature-sensing or -signaling apparatus from peripheral sensory organs to the clock neurons in the brain covering different intervals of the physiological relevant TC fruit flies are normally exposed to in nature. In addition we propose that proprioceptive feedback from ChO to the brain clock may help an animal to keep its circadian clock in sync with its own, stimulus-induced activities.

Timing of the peptide-orchestrated eclosion behaviour in *Drosophila*

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Neuroendocrine peptide release is often rhythmic, potentially coupled to circadian oscillators in the brain. The neuronal mechanisms underlying clock-controlled release of neuropeptides is however only little understood. Eclosion in the fruit fly *Drosophila melanogaster* is a classic example for both: peptide-orchestrated and circadian-timed behaviour. It was shown that PDF-positive clock neurons are essentially for the rhythmic eclosion of flies, while the ecdysis behaviour itself is regulated via peptide hormone signalling. Therefore a yet unknown connection between the circadian pacemaker cells and the gated release of peptide hormones involved in eclosion must exist.

In a behavioural screen we identified a new neuropeptide involved in eclosion timing: prothoracicotropic hormone (PTTH). PTTH was so far known to regulate ecdysone production. Ablating and electrical silencing of PTTH -positive neurons leads to arrhythmic eclosion under constant darkness. Down-regulation of Torso, the PTTH receptor, specifically in the prothoracic gland but not in the nervous system leads to an arrhythmic eclosion pattern. Anatomical and imaging data suggest that PTTH signalling is independent of PDF clock output, at least during the time of eclosion. Our results suggest that PTTH is a key signal linking the central clock with a peripheral clock gating eclosion.

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Chronological Interactions between Antennal Lobe and Mushroom Body: Extracting the Behaviorally Relevant Stimulus

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There are mainly two categories of animal behaviors. One is innate, meaning if confronted with a specific key stimulus, animals respond with a stereotypical behavior. The other one is learned, and manifestation of a behavior depends on experience. The latter is supposed to be flexible, whereas there is a broad consent that innate behaviors are hard wired and therefore inflexible. However, both trigger the behavior of an animal, and the initiating stimulus, in both cases, needs to be separated from irrelevant environmental information by neural networks acting at different processing levels.

We focus on the olfactory system of Hymenoptera to compare odor representation at mainly two processing stages, the antennal lobe (AL), which is the first order olfactory neuropil receiving input by olfactory sensory neurons located on the antennae, and the mushroom body (MB), a higher order multimodal integration center. Olfactory information into the MB is provided by projection neurons of the AL. Comparing the ensemble response between both processing levels after simultaneous recordings in honeybees revealed that a neutral olfactory stimulus evoked a rapid population response in MB neurons which settles back to baseline activity before the AL network has reached maximal stimulus separation. At this time point it seems that the MB is not “waiting” for the AL’s computational output. After classical conditioning computation of the reward associated stimulus at the MB level is prolonged and drastically increased. Its maximal separation of the behaviorally relevant stimulus is now chronologically synchronized to the AL computation with both reaching their maximal odor separation 200 ms after odor onset. The reward associated behavior is exhibited 300 -400 ms later. Thus the MB extracts the behaviorally relevant stimulus after classical conditioning and may provide that information to downstream networks to initialize the animals’ response.

In a specific case, we detected strong and prolonged ensemble responses reflecting the separation of a behaviorally relevant stimulus already at the level of the AL. We tested different sesquiterpenes, one of them was farnesol, and recorded the AL activity in alive bumblebees (Bombus terrestris). Farnesol has been identified as a component of the recruitment pheromone in bumblebees, which is used by successful foragers to activate or recruit unemployed nestmates by triggering an innate behavior. The neural representation of farnesol was unique compared to all other tested odors. Its spatio-temporal activity pattern, therefore, provides a reliable separation of farnesol from other odor stimuli. This indicates that the AL has the capacity to separate a behaviorally highly relevant stimulus and may then provide that information directly to downstream networks. To which extent this information is hard wired and how the MB may affect processing farnesol before and after learning, we plan to investigate in the future.

Variable event timing in visual conditioning leads to memories with opposite valence in *Drosophila*

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In order to optimally adapt our behavior for future events we must associate temporally coinciding environmental cues as well as cues which happen at different time points. The latter kind of non-overlapping associative memory has been studied in *Drosophila* with two olfactory conditioning tasks: trace and relief conditioning, where an odor either precedes or follows an electric shock punishment, respectively. In order to detach memory performance from sensory specificity and to precisely dissect these conditioning tasks we have used a newly established visual conditioning paradigm. We apply electric shock as a reinforcer and flies can walk freely, hence our assay allows experiments comparable to classical olfactory conditioning. We have obtained very similar results as in olfactory conditioning: Flies are able to form visual memories of opposite valence (punishment/relief learning) and they are able to associate a vanished visual stimulus with punishment (trace conditioning). We suggest that formation of associative memories with non-overlapping stimuli is a general cognitive skill used across sensory modalities.

The Hofbauer-Buchner-eyelet signals to the ventro-lateral neurons and thereby mediates siesta and phase-shifts in *Drosophila*.

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Light influences the physiology of animals in many ways with vision being the most important function. Besides that, light is used by circadian clocks as a zeitgeber to synchronize the animals´ behavior and physiology to the changes of day and night. For example during the course of the day *D. melanogaster* wild-type flies show a bimodal locomotor activity with a siesta in the middle of the day. This lower midday activity is thought to be of adaptive significance for the fly to avoid harmful high temperatures.
To perceive light, the fruit fly possesses the blue light photoreceptor CRYPTOCHROME (CRY) as well as several eye structures: ocelli, compound eyes and Hofbauer-Buchner (H-B) eyelets. The latter organs consist of only 4 Rhodopsin 6 (Rh6) expressing neurons per brain hemisphere. The H-B eyelet tracts directly innervate the accessory medulla which is known to be the location of the main pacemaker neurons of the *Drosophila* clock. Given this anatomical architecture, we hypothesize a direct interaction between visual and circadian systems. Using the GRASP technique we show that the fibers of the lateral neurons and the axon of the H-B eyelets are in close vicinity and probably share synapses. By activation of the eyelet and imaging of the lateral neurons using the P2X2 system we observe an increase in Ca2+ especially in the small ventro-lateral neurons indicating a physiological contact that we suppose is mediated by acetylcholine. To confirm the relevance of this connection we looked for a role of the H-B eyelet in phase shifting the clock and on the entrainment. We found that the wild-type like siesta is mediated by the H-B eyelet. Moreover, we showed that activation of the H-B eyelet seems to exclusively phase advance the locomotor activity rhythm most likely because of its direct connection to the lateral clock neurons.

Symposium

S22: Recognition molecule-associated glycans in synaptic plasticity and regeneration after trauma

S22-1 Recognition molecule-associated glycans in synaptic plasticity and regeneration after trauma
Melitta Schachner

S22-2 NCAM-associated polysialic acid regulates hippocampal and cortical synaptic plasticity
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S22-6 Nuclear import of polysialic acid carrying fragments of the neural cell adhesion molecule NCAM
Nina Westphal, Thomas Theis, Ralf Kleene, Melitta Schachner

Recognition molecule-associated glycans in synaptic plasticity and regeneration after trauma

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Carbohydrate chains attached to neural adhesion molecules of the immunoglobulin superfamily and of the extracellular matrix have increasingly been implicated not only in developmental mechanisms, but also in synaptic plasticity and regeneration after trauma.
The human natural killer cell (HNK-1) carbohydrate was the first to be recognized to be a functionally decisive glycan in that it interacts with laminin, the GABA-B receptor, and the high mobility group box 1 protein (HMGB1, also called amphoterin), which is a ligand of RAGE, the receptor for advanced glycation end-products. Carried predominantly by the extracellular matrix glycoprotein tenascin-R in the adult mouse brain, HNK-1 localizes to perineural nets surrounding the cell bodies and apical dendrites of certain types of neurons. HNK-1 expressed in the motor, but not sensory branch of the femoral nerve, enhances preferential motor reinnervation of muscles in mice and monkeys, after the nerve is severed before bifurcation into the two branches, and when HNK-1 is applied as glycomimetic peptide. HNK-1 glycomimetic peptides were screened for with HNK-1 monoclonal antibody in peptide phage display libraries using a competition ELISA. In adult zebrafish, HNK-1 contributes to regeneration after complete transection of the adult spinal cord.
Another unusual glycan is polysialic acid which is also implicated in regeneration after central and peripheral nervous system injury as well as in synaptic plasticity by its influence on the extrasynaptic NMDA-type glutamate receptor GluN2B. Other receptors have been identified to be involved in polysialic acid-dependent neurite outgrowth by using an anti-idiotype immunization approach. One of these receptors is histone 1A, which is not only present in the cell nucleus, but is found by others and us also in the cytoplasm and in the extracellular space. Another receptor for polysialic acid is MARCKS (myristoylated alanine-rich protein kinase C substrate). MARCKS and polysialic acid bind to each other at the cell surface plasma membrane, with polysialic acid penetrating the plasma membrane to interact with cytoplasmic MARCKS.

These observations contribute to the concept that glycans can be considered as functional fine-tuners in conjunction with their protein backbones.

NCAM-associated polysialic acid regulates hippocampal and cortical synaptic plasticity

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The neural cell adhesion molecule NCAM -associated polysialic acid (PolySia) is required for NMDA receptor-dependent synaptic plasticity in rodents. A deficit in PolySia expression is associated with schizophrenia. Remarkably, PolySia specifically inhibits activation of GluN2B-containing NMDA receptors by low micromolar (extrasynaptic) concentrations of glutamate. Thus, PolySia carried by NCAM restrains extrasynaptic GluN2B - Ras-GRF1 - p38 signaling pathway and regulates hippocampal synaptic plasticity and contextual fear conditioning by properly balancing the transmission through synaptic versus extrasynaptic NMDA receptors (Kochlamazashvili et al., J. Neuroscience, 2010, 2012). Our recent patch clamp study indicates that enzymatic removal of PolySia by endosialidase (endoNF) leads to increased transmission through GluN2B -containing NMDA receptors and impaired LTP in the medial prefrontal cortex (mPFC). The latter could be rescued by a low concentration of GluN2B antagonist Ro25-6981 and by stimulation of the glycine modulatory sites on the NMDA receptor by blocking glycine transporter-1 with sarcosine (Varbanov et al., Abstr. German Soc. Neuroscience, 2015), a drug used for treatment in schizophrenia. Similarly, a deficit in mPFC LTP and its full rescue by sarcosine were found in NCAM-deficient mice and in mice deficient in PolySia -synthetizing enzyme ST8SiaIV, but not in ST8SiaII-deficient mice. These results suggest a key role of PolySia carried by NCAM in regulation of hippocampal and cortical synaptic plasticity through control of NMDA receptor-mediated transmission.

Complex glycans and their carrier proteins in the neural stem cell niche

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The stem cell niche is characterized by a combination of factors i) delivered by the cerebrospinal fluid, ii) originating from the blood or endothelial cells and iii) produced by cells that reside within or adjacent to the niche. The laboratory studies the implications of this concept in detail and focuses on the extracellular matrix (ECM) of the stem cell niche. Special emphasis is given to glycoproteins, e.g. tenascin-C and chondroitinsulfate proteoglycans (CSPGs) of the niche. The significance of these components for the differentiation of glial progenitors, namely astrocytes and oligodendrocyte precursors and their roles for myelination during development and in conditions of disease are also themes of the laboratory. Another aspect of the work concerns the expression of these ECM molecules in glial tumors and their potential effects on tumor stem cells of the nervous system. Finally, the response of the stem cell niche to lesions and the potential use of stem cells for regeneration and repair of the CNS are topics of the laboratory. Studies of the past years suggest that specialized chondroitinsulfates and particular variants of the LewisX-glycan, as well as the corresponding carbohydrate presenting proteins contribute to the regulation of proliferation and differentiation of neural stem cells. CSPGs are expressed in free-floating neurospheres (NSPHs), a culture model of NSPCs. When NSPCs are treated with the bacterial enzyme chondroitinase ABC (ChABC) that specifically degrades the chondroitinsulfate (CS) carbohydrate chains, the proliferation rate is reduced. The neurospheres settle down on the culture dish substrate, which may reflect a shift in the relationships of adhesive versus repulsive interactions. Beyond proliferation, lineage decisions are modified in that neurogenesis is reduced and gliogenesis is enhanced. Recent studies revealed that this may result from a preferential reduction of FGF2-related signalling caused by ChABC treatment, which primarily affects neurogenesis. By comparison, the EGFR-related signalling pathway that favors gliogenesis seemed less affected. Chondroitinsulfates in NSPHs are exposed amongst others by receptor protein tyrosine phosphatase (RPTP)-ß/ζ, a transmembrane based tyrosinephosphatase receptor. This receptor also expresses LewisX-glycan epitopes, as shown by use of the monoclonal antibodies Mab487 ^{LeX} and 5750 ^{LeX}. These Mabs also revealed microheterogeneity of Lex-carbohydrate structures and demonstrated a strong enrichment of the 5750^{LeX}-epitope on NSPHs. In order to study the functional relevance of this glycan tag, expressing proteins were identified by immunoaffinity chromatography and subsequent proteome analysis. The lipoprotein-receptor related protein-1 (LRP1) could be identified a novel LeX-carrying glycoprotein. Conditional ablation of LRP1 from NSPHs in culture using a membrane-premeant CRE-recombinase fusion protein revealed a functional role of this receptor in several neural lineages. These results warrant further studies using genetic studies in vivo.

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Polysialic acid on NCAM: Regulator of cortical development with relevance to schizophrenia

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The neural cell adhesion molecule NCAM is a cell-surface glycoprotein that is uniquely modified by the carbohydrate polysialic acid (polySia). PolySia is added to NCAM by two polysialyltransferase enzymes, ST8SIA2 and ST8SIA4. Abnormal levels of NCAM or polySia as well as polymorphisms in NCAM and ST8SIA2 have been repeatedly related to schizophrenia. A complete loss of polySia by simultaneous deletion of both polysialyltransferases causes a severe malformation of major brain axon tracts. Even a minor reduction of polySia during embryonic brain development of ST8SIA2-deficient mice results in enlarged lateral ventricles and a size reduction of the thalamus accompanied by a smaller internal capsule and a highly disorganized pattern of fibers connecting thalamus and cortex. Since polySia is also present during interneuron migration and maturation, we comparatively analyzed the composition of selected interneuron populations in the prefrontal cortex of the different polysialyltransferase-deficient mouse lines. Immunofluorescence and co-localization studies of major interneuron markers revealed pronounced alterations of different GABAergic interneuron subtypes in the prefrontal cortex of mice with reduced polySia levels. Developmental studies including live imaging of genetically labeled interneurons in organotypic slice cultures suggest that the observed loss of interneurons in polySia-deficient mice is at least in part explained by compromised tangential migration. These findings will be discussed in relation to working memory deficits observed in specifically the ST8SIA2-deficient mice, which are exacerbated by a “second hit”, as well as in the context of new data on altered polySia levels correlating with symptom severity in patients with schizophrenia.

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Low density lipoprotein receptor-related protein 1 (LRP1) – a novel modulator of the neural stem cells' proliferation, differentiation and survival.

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In the developing and adult CNS multipotent neural stem cells reside in distinct niches. Specific glycoproteins are expressed in these niche microenvironments that are important regulators of cell fate determination and stem cell maintenance. In previous studies we described LewisX (LeX) glycan as a marker of the cortical neural stem precursor cells (NSPCs). Subsequently we have identified a novel LeX carrier protein – Low-Density Lipoprotein Receptor Related Protein 1 (LRP1). We have also identified that LeX-glycosilatated LRP1 is expressed in the stem cell compartment of the developing spinal cord.
LRP1 has classically been associated with the modulation of lipoprotein metabolism, however modern studies indicate a diversity of roles for this receptors in various aspects of cellular activities, including cell proliferation, migration, differentiation and survival.
LRP1 is essential for the normal neuronal function in adult CNS, whereas the role of LRP1 in development is still unclear.
In the current study we investigated the basic properties of LRP1 conditional knock-out NSPCs (neural stem precursor cells) from crotex and spinal cord, created by means of Cre-loxp mediated recombination. The elimination of LRP1 in vitro was induced by the addition of cell permeable Cre-recombinase to NSPCs derived from embryonic cortex and spinal cord of LRP1lox/lox mice. The functional status of LRP1-deficient cells was studied using proliferation, differentiation and apoptosis assays.
LRP1 knock-out cells can be maintained as neurospheres retaining the ability to migrate and differentiate. However, their differentiation capability is significantly altered. LRP1 ko NSPCs from cortex generated much less OPCs and neurons in comparison to wt cells, more of the ko cells remained undifferentiated. In the spinal cord OPCs were also negatively effected, but there were no major changes in neuronal differentiation. Moreover, LRP1-deficient spanal cord-derived NSPCs generated significantly more astrocytes. In addition, the LRP1 deletion affected proliferation and survival of NSPCs from both parts of the CNS. These findings suggest LRP1 as a new regulator of basic NSPCs properties.
The preliminary observations propose that LRP1 facilitates NSPCs differentiation via interaction with APOE, and the downstream effect could be dependent on cholesterol-uptake and on the activation of the downstream MAPK signaling cascade.

Nuclear import of polysialic acid carrying fragments of the neural cell adhesion molecule NCAM

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In the mammalian nervous system, the neural cell adhesion molecule NCAM is the major carrier of the glycan polymer polysialic acid (PSA) which plays important roles in development, regeneration after trauma and synaptic plasticity. I present evidence for a nuclear localization of PSA-carrying NCAM fragments after stimulation of cultured murine neurons with function-triggering NCAM antibodies or the recombinant extracellular domain of NCAM. PSA-carrying NCAM fragments are generated by the matrix metalloproteases-2 and -9 and are transported via the late endosomal compartment to the nucleus. The nuclear import of PSA-carrying NCAM fragments is associated with an increase in neurite outgrowth as well as with alterations in dimethylation of histone 3 and expression of clock-related genes. Interestingly, levels of PSA on NCAM at the cell surface, nuclear PSA localization and association of PSA with chromatin in adult murine brain tissue depend on the circadian rhythm that animals live under. The functional consequences of the nuclear import of a NCAM fragment carrying PSA will be important to investigate.

Symposium

S23: Breaking News II

S23-1 Representation of visual information in the archerfish Mauthner-cell
Benedikt Bausewein, Peter Machnik, Stefan Schuster

S23-2 Temporal dynamics and genome-wide target regions of cytosine methylation and hydroxymethylation during long-term memory formation in honeybees.
Stephanie D. Biergans, C. Giovanni Galizia, Judith Reinhard, Charles Claudianos

S23-3 Developmental changes of striatal interneurons in an animal model of paroxysmal dystonia
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S23-10 Learning of arbitrary visual associations in the corvid endbrain
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Representation of visual information in the archerfish Mauthner-cell

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Hunting archerfish use their so-called predictive start to secure downed prey. The predictive start is an adapted form of the teleost escape C-start response. It depends heavily on visual information and is initiated by the two reticulospinal Mauthner (M)-cells. The M-cells integrate incoming sensory information and make the final decision whether to initiate a C-start or not. Behavioural experiments show that the predictive start is always executed accurately. Because an action potential in the M-cell always triggers a C-start either the M-cell itself decides whether to launch a start or not, or it receives a command from other brain areas. Here we analyse the degree of decision-relevant visual information that is available at the level of the M-cell. Using in vivo intracellular recording of M-cell PSPs during stimulation with natural stimuli we show that the cell has a rich representation of visual information and would be capable of deciding on its own to elicit a C-start. In our experiments motion stimuli were presented on a screen placed above an anaesthetised archerfish, in the region where falling motion of prey would occur. Besides looming stimuli and brightness changes we addressed the question whether the PSPs of each of the two M-cells would contain information about movement direction. We recorded strong PSPs in response to moving circles and even small flies. Both amplitude and time-course of these PSPs – and their difference between the two M-cells – are clearly directionally-dependent.

Temporal dynamics and genome-wide target regions of cytosine methylation and hydroxymethylation during long-term memory formation in honeybees.

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Long-term memory formation involves changes in neuron morphology, synapse development and also gene expression. Epigenetic marks (e.g. cytosine modifications), among other mechanisms, regulate gene expression. In honeybees, DNA methyltransferase (DNMT) dependent cytosine methylation is necessary for stimulus specific long-term memory, that is, the ability to form a memory of one specific stimulus (e.g. an odorant), without generalising it to similar stimuli. The counterplayer of DNMT, the dioxygenase TET, oxidises methyl-cytosine to hydroxy-methylcytosine. It has not yet been analysed in this context. Thus, it is unknown what the specific roles of DNMTs and cytosine modifications during memory formation are. Here we ask: (1) How does DNMT inhibition affect target gene expression? (2) When are DNMTs and TET expressed during memory formation? (3) And what genomic regions are then targeted by DNMTs and TET?
(1) We treated bees with two functionally different DNMT inhibitors (RG108 and zebularine) after appetitive olfactory training. During training, bees receive an odor (CS) and then a sugar reward (US), which causes the proboscis extension reflex (PER). After learning bees respond with a PER to the odorant alone. We searched for DNMT dependent memory-associated genes using qPCR. We found that, among others, expression of actin and synaptotagmin 24 hours after training was upregulated in the DNMT inhibition group. (2) We assessed the temporal expression of DNMTs and TET itself during memory formation and found DNMT1b to be upregulated as early as 1 hour after training, whereas DNMT3 was upregulated after 5 hours. TET was upregulated 1 hour after training. (3) We examined cytosine methylation and hydroxymethylation marks genome wide using MEDIP sequencing and Sequenome technology. Memory formation was associated with changes in cytosine methylation and hydroxymethylation patterns 24 hours after training in a number of genomic regions.
Our study provides the first indications on how, when and at which genomic regions DNMTs and TET regulate gene expression during memory formation in the honeybee.

Developmental changes of striatal interneurons in an animal model of paroxysmal dystonia

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Dystonia is a common movement disorder characterized by prolonged or intermittent muscle-contractions leading to abnormal movements and painfully distorted body postures. Symptoms of the young onset forms of dystonia often manifest during childhood or adolescence with spontaneous remission in adulthood in some cases. Investigations in animal models and reports in patients support an important role of the basal ganglia in the pathophysiology of dystonia. The insufficient knowledge of pathogenesis and underlying molecular and cellular substrates hamper the development of rational therapy for dystonia. The dtsz mutant hamster is the best characterized phenotypic animal model for this disorder. In this model stress induced paroxysmal dystonic attacks occur at about 16 days of age with full remission at about 90 days of age. At 33 days (P33) but not 90 days of age a decrease in GABAergic interneurons (e.g. parvalbumine, parv) in the main basal ganglia input structure, the striatum, was described. The concomitant reduction of the GABAergic tone in the striatum is likely a key component in the pathogenesis of dystonia in this model. Here we thought to investigate if the reduction in GABAergic interneurons is the result of delayed migration and/or maturation of these neurons.

We used antibodies against nkx 2.1 to immunohistochemically label and quantify striatal interneurons migrated from the medial ganglionic eminence to the striatum in dtsz hamsters and controls at age of maximum dystonia (P33). Striatal mRNA of nkx 2.1 and parv was quantified via qPCR. Expression of the neurotrophine “brain derived neurotophic factor” (BDNF), which is important for the development of GABAergic interneurons, was quantified with ELISA, immunohistochemistry and qPCR in the cortex and striatum of dtsz- and control-hamsters. In order to study the maturation of the striatal GABAergic system the mRNA expression of carboanhydrase isotyp VII (CarVII) and calcium -chloride-cotransporter 2 (Slc12a5) was quantified via qPCR.

We found that the expression of parv mRNA was lower in the striata of mutant hamsters compared to controls, which is in line with the reduced density of parv-positive striatal interneurons shown previously. However, expression of striatal nkx2.1 mRNA, which is present in striatal interneurons was unaltered in the dtsz hamsters. Lack of differences in nkx 2.1 positive cell density in the mutant versus control hamsters further support that the striatal interneurons have fully migrated into the striatum at this age (P33). This suggests a delayed maturation of GABAergic interneurons at P33 in this model of dystonia. Importantly, there were no differences in expression of CarVII and Slc12a5 which indicates that there is no general delay in maturation of the GABAergic system. Immunohistochemical investigations showed a slight increase of BDNF expression in cortex and striatum of dtsz hamsters at P33, which may indicate an ongoing delayed maturation of interneurons. In summary, our results support complete migration of striatl interneurons at P33 but a delay in specific maturation of GABAergic interneurons. Ongoing studies will reveal the mechanism for delayed maturation and further describe the time course of interneuron migration and development from embryonic to early postnatal ages. This could lead to discovery of novel

targets for the treatment of dystonia and other neurological disorders with aberrant development of interneurons.

Rett syndrome provokes redox imbalance already in neonatal neurons, affecting the cytosol and the mitochondria

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Rett syndrome is a neurodevelopmental disorder which is associated with mitochondrial impairment, chronic oxidant challenge, and aberrant cellular redox homeostasis. Since mitochondria are partly uncoupled and show increased respiratory turnover rates, they may underlie the oxidative burden in MeCP2-deficient (*Mecp2*$^{-/y}$) brain tissue. Since these alterations manifest early in life, they may facilitate disease progression in Rett syndrome. Earlier we reported exaggerated responses of *Mecp2*$^{-/y}$ hippocampus to redox challenge and mitochondrial inhibition, and showed that extramitochondrial ROS production, e.g., by various cellular oxidases, is not intensified. To decipher the molecular causes of redox imbalance in more detail, we took advantage of viral constructs expressing the genetically-encoded optical redox sensor roGFP1 (reduction-oxidation-sensitive green fluorescent protein 1) specifically in neurons. For subcellular, compartment specific analyses in hippocampal cell and slice cultures, roGFP1 was expressed under the control of the synapsin I promoter and targeted to either the cytosol or the mitochondrial matrix. To enable quantitative recordings, the ratiometric response ranges of mito-roGFP1 and cyto -roGFP1 were calibrated to full oxidation and reduction. In organotypic hippocampal slice culture, clear genotypic differences became evident: both the mitochondrial and the cytosolic compartment showed slightly more oxidized redox baselines in *Mecp2*$^{-/y}$ than in wildtype neurons. In addition, the redox balance of both compartments was more vulnerable in *Mecp2*$^{-/y}$ hippocampus. Severe hypoxia caused more pronounced reducing shifts and acute oxidant challenge by H_2O_2 (200 µM) elicited more intense oxidizing transients in *Mecp2*$^{-/y}$ neurons. Block of superoxide dismutase by DEDTC (5 mM) evoked less intense oxidizing responses in *Mecp2*$^{-/y}$ cytosol and mitochondria, suggesting a reduced activity of this scavenging enzyme in *Mecp2*$^{-/y}$ hippocampal neurons. Overnight treatment of slice cultures with the radical scavenger Trolox (1 mM, dissolved in DMSO) somewhat improved cellular redox balance. In the cytosol of *Mecp2*$^{-/y}$ neurons, the responses to acute H_2O_2 challenge were dampened. In the mitochondrial compartment, however, Trolox treatment seemed less beneficial. To fully rate the potential pharmacotherapeutic potential of radical scavengers in Rett syndrome, the merit of chronic systemic Trolox treatment is currently being evaluated. In conclusion, mito-roGFP1 and cyto-roGFP1 respond reliably to oxidation and reduction, thereby allowing analyses of subcompartmental redox dynamics. Genotypic differences among wildtype and *Mecp2*$^{-/y}$ mice are evident not only in the cytosolic but also in the mitochondrial compartment, and they already manifest at the neonatal developmental stage. Since mitochondria are the primary cellular source of reactive oxygen species, this supports our hypothesis that it is especially the mitochondrial dysfunction which underlies the oxidative burden in Rett syndrome. In view of the early onset of redox alterations, radical scavenger treatment may be of potential benefit in Rett syndrome.

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Input spike trains suppress chaos in balanced neural circuits

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A longstanding hypothesis claims that spatio-temporally structured input in neural circuits enhances precision and reliability of spiking responses. While studies in single neurons well support this hypothesis both experimentally and theoretically (e.g. [1-3]) the impact of input structure on the dynamics of recurrent networks is not well understood. The high dimensional and often chaotic dynamics of large recurrent networks requires a type of analysis that is sensitive to recurrent interactions and characterizes the networks collective dynamics [4-11].

To address this challenge, we here demonstrate how the analysis of dynamical stability and entropy production can be generalized for examining balanced networks driven by a stream of input spike trains. Previous studies of the dynamic stability of the balanced state used a constant external input [4-9] or white noise [10-11]. An analytical expression for the Jacobian of the flow enables us to calculate the full Lyapunov spectrum. Using a single neuron model in which action potential onset rapidness can be adjusted we solved the dynamics in numerically exact event-based simulations and calculated Lyapunov spectra, dynamical entropy production rate and attractor dimension. We examined different scenarios to study the transition from constant to stochastic input, varying the input spike rate and/or coupling strength, while keeping the firing rate of the driven population fixed.

We find a suppression of chaos by input spike trains. This finding holds both for variations of input rate and coupling strength and is robust to deviations from Poisson statistics. We also find that both independent bursty input spike trains and common input more strongly reduces chaos in spiking networks. Our study extends studies of chaotic rate models to spiking neuron models [12-13] and opens a novel avenue to study the role of sensory streams in shaping the dynamics of large networks.

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A Young Pilocarpine Model for Epilepsy

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Epilepsy is a neurological disorder that is characterized by involuntary spontaneous seizures. Temporal lobe epilepsy (TLE) is a complex partial epilepsy in which patients experience recurrent epileptic seizures emerging mainly from the hippocampus and amygdala of the uni- and/or bi- lateral temporal lobes of the brain.

The pilocarpine animal model mimics both the underlying aetiology and symptoms of TLE. This includes: i) seizure foci in the hippocampus; ii) a latent period as a seizure-free time before phenotype manifestation iii) hippocampal sclerosis. The model is very common in adult rats and induces chronic epileptic seizures in an already consolidated brain.

Our study goal is to electrophysiologically and pharmacologically shed light on why epileptic children have a high tolerance to bromide and why adults generally have severe side effects. Therefore, we developed a juvenile rat model which reconstructs epilepsy in children.

Epilepsy was induced in rat pups at different ages, with different pilocarpine doses, variable durations of treatment and diazepam doses. Based on survival rates and seizures, a protocol was selected for further validation. EEG was recorded at 30, 60 and 120 days after treatment in the hippocampal CA1 region and motorcortex for 3-7 days and seizures were analyzed with a Seizure Detection Module (NeuroScore, DSI).

Furthermore, literature suggests that voltage activated Ca^{2+} channels are likely to be predominant candidates for calcium elevation during most epileptiform activity. Therefore, we studied molecular changes in the juvenile epileptic rat hippocampal system using quantitative Real Time PCR analysis.

In conclusion, our model is easy to use and yields the desired outcome. Mortality rates are as low as 10-20% while 70-90% of the survival animals present an epileptic phenotype. It presents seizures in the hippocampus and motorcortex on a reproducible manner and proofs the distinct role of voltage-gated calcium channels in epileptogenesis.

The model can be used to mimic epilepsy in children and for testing antiepileptic drugs such as bromide.

Effects of Vagus Nerve Stimulation on Hippocampal Neurophysiology in Freely Moving Rats

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Background: Vagus Nerve Stimulation (VNS) is an adjunctive treatment for pharmacoresistant epilepsy and depression. Clinically, VNS is known to suppress seizures and improve mood and cognitive functions, such as memory. However, the underlying neurophysiological mechanisms remain to be fully understood. In the present study, effects of VNS on both spontaneous and evoked hippocampal activity were examined.

Methods: Sixteen male Sprague Dawley rats (300-350 gram) were implanted with a custom made cuff electrode around the left vagus nerve. A depth electrode was implanted in the left dentate gyrus of the hippocampus for EEG recording and a stimulation electrode was implanted in the left dorsomedial perforant path to evoke dentate field potentials. After six weeks of recovery, effects of VNS on spontaneous and evoked hippocampal activity were assessed in freely moving animals. VNS was applied with a rapid cycle (7 seconds on, 18 seconds off), a frequency of 30 Hz, a pulse width of 250 µs, and a stimulation intensity of either 0 (SHAM), 250 or 1000 µA . There were at least 24 hours between each intensity test session and session order was randomized. Each session consisted of a one hour baseline condition, a two hour VNS or SHAM condition and a one hour post VNS condition. During the off phase of each VNS duty cycle, two perforant path evoked dentate field potentials (EPs) and a ten second sweep of spontaneous EEG was acquired. For each EP, the excitatory postsynaptic potential (EPSP) slope coefficient and the population spike amplitude and latency were calculated. All EEG sweeps transformed to the frequency domain using the Fast Fourier algorithm and the 1-100 Hz power spectrum was extracted. All outcome measures were averaged into 20-minute epochs and processed in Two Way Repeated Measures ANOVAs, with the factors time and condition.

Results: Application of VNS at 1000 µA significantly decreased the EPSP slope coefficient ($p<0.05$), increased the population spike amplitude ($p<0.05$) and increased the latency of the population spike ($p<0.05$) compared to baseline and SHAM condition. Application of VNS at 250 µA significantly decreased the EPSP slope coefficient ($p<0.05$) and increased the latency of the population spike ($p<0.05$), while the tendency for an increase in population spike amplitude did not reach statistical significance. VNS-mediated effects on EPs reached statistical significance in the second 20 minute epoch of VNS, increased towards the end of the two-hour VNS period and slowly returned towards baseline outlasting the stimulation period by up to one hour. In parallel with observed EP changes, VNS at both 1000 and 250 µA suppressed spontaneous EEG power across the full 1-100 Hz spectrum ($p<0.05$), though the most pronounced suppression was seen in a 6-9 Hz theta band ($p<0.05$). Onset of effects was more immediate and full effect was reached within the first hour of VNS. EEG power returned to baseline within the post VNS acquisition period. For all parameters, no significant differences were

observed between the 1000 μA and 250 μA conditions.

Conclusion: VNS facilitates evoked activity, but suppresses spontaneous activity in dentate gyrus. The observed effects could provide an explanation of how VNS suppresses spontaneous seizure activity while simultaneously facilitating cognitive functions, such as memory.

Integration of sky compass cues in the brain of the desert locust

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Like other migratory insects, the desert locust likely uses a sky compass mechanism for spatial orientation. In the sky, several cues including direct sunlight, the polarization pattern of the sky and the chromatic and intensity gradient can be exploited for compass orientation. Previous work showed that the central complex in the locust brain holds a polarotopic internal representation of celestial *E*-vectors and may therefore act as an internal sky compass (Heinze and Homberg 2007, Science 315:995). To explore whether other celestial cues contribute to this internal compass, we examined whether polarization-sensitive (POL) neurons of the central complex receive additional input from the chromatic gradient of the sky. The intensity gradient of long wavelengths (green light) and the uniform distribution of short wavelengths (UV-light) across the sky lead to a chromatic gradient with highest intensity difference between long and short wavelengths near the sun and smallest difference in the antisolar hemisphere. We tested the responses of central complex-neurons to zenithal polarized light and a green and UV-light spot rotating at an elevation of 45° around the head of the locust. All POL-neurons were sensitive to the azimuth of the rotating unpolarized stimuli. In many neurons the azimuthal tunings to both light spots were in the same position or close to each other, favoring the intensity gradient of green light as the prominent unpolarized compass cue. The preferred position of the green light spot and the preferred *E*-vector orientation were strongly correlated in input- and output-neurons suggesting a robust input- and output-compass signal. In addition the signal seems to be shaped by certain neuronal states, characterized by phasic or tonic responses to a stationary green light spot. Taken together our results show that unpolarized light serves as an important compass cue.

Dynamic coding patterns in single units of the forebrain across three stages of learning

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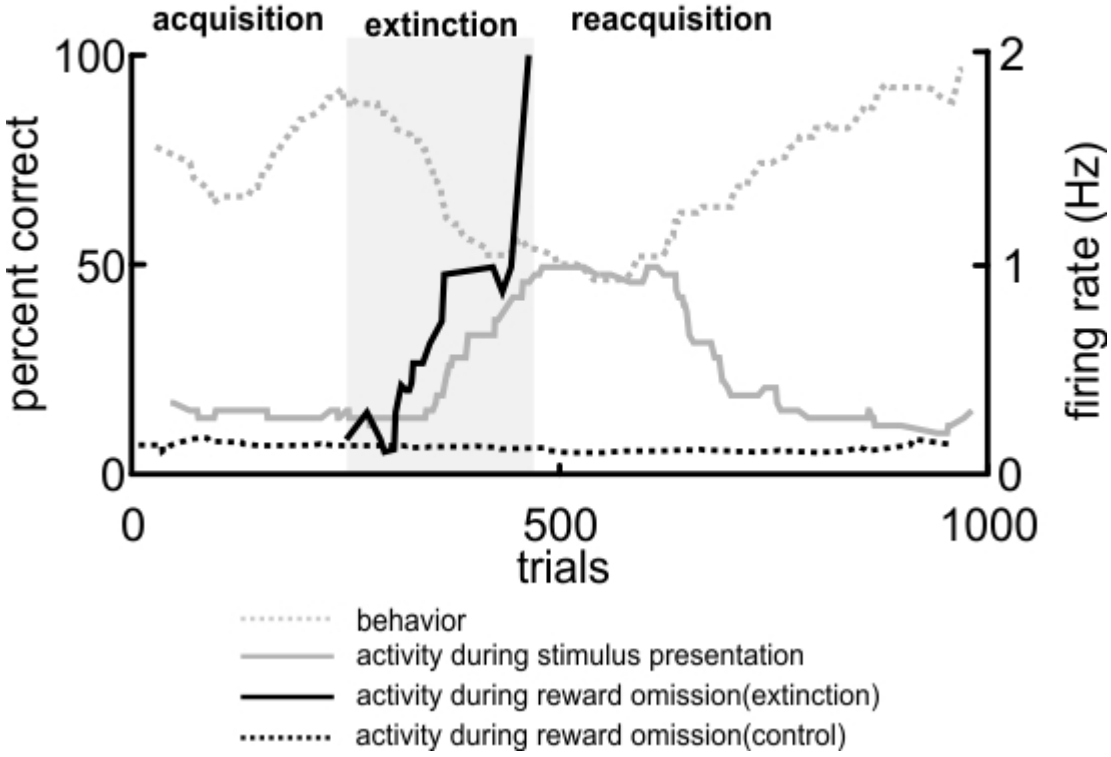
Flexibly adapting behavior in a changing environment entails learning to stop responding following the repeated omission of an expected reinforcement - extinction. Neuronal mechanisms underlying extinction learning have been well characterized employing fear conditioning in rodents. By contrast, neural processes underlying the extinction of appetitive, operantly conditioned responses are largely unknown. This is quite remarkable, considering that most human and animal behavior is operant in nature. Knowledge about extinction in this setting may prove useful for the behavioral treatment of maladaptive conditioned responses that are maintained by positive reinforcement, such as drug seeking.
To investigate the time course of neuronal plasticity during acquisition, extinction, and reacquisition in an operant conditioning paradigm with positive reinforcement, we established a behavioral learning task enabling us to monitor single-neuron activity during these phases in a single experimental session lasting >1,000 trials. We subjected pigeons to a visual discrimination task with two stimulus pairs. One pair was familiar to the animals; thus, they knew how to respond to these stimuli. This condition served as a control and correct responses were rewarded in 70% of the cases. The other pair of stimuli consisted of pictures which the animals had never seen before. Thus, in each behavioral session, animals had to learn how to respond to either of two stimuli. After reaching a pre-defined performance criterion, responding to one of the novel stimuli was no longer reinforced. Once responding to this stimulus had decreased (i.e., had been extinguished), the previously extinguished response was reinforced again and the animals reacquired it. While animals were performing this task, we repeatedly recorded extracellular unit activity in the avian nidopallium caudolaterale (NCL), an associative forebrain structure thought to be functionally analogous to mammalian prefrontal cortex.
We found that neurons flexibly adapted their response patterns during presentation of the critical novel sample stimulus to the altered reward contingencies during acquisition (reward), extinction (no reward) and reacquisition (reward). Similar changes in responses patterns were observed during choice outcomes. The figure displays one example unit and the corresponding behavioral performance. While behavioral performance (grey dotted line) increases during acquisition, decreases during extinction and increases again during reacquisition, neuronal activity patterns show a different dynamic. During sample presentation (grey solid line) activity is stable in the acquisition phase and increases during extinction, while it decreases during reacquisition again. When a reward is omitted, activity increases during the course of the extinction phase for the critical stimulus (black solid line), while it stays constant for control stimuli (black dotted line). The subpopulation of forebrain neurons which were especially active during reward omission leading to extinction (but not in the control condition) could provide a teaching signal for behavioral modification during extinction learning. We propose that neurons in the NCL provide a rapidly adapting representation of the associative value of specific stimuli, combined with coding of outcome value. It is precisely this kind of signal which allows for rapid and highly flexible adaptation of behavior in non-stationary environments.

Activity of an example unit and behavioral performance over time



Learning of arbitrary visual associations in the corvid endbrain

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The ability to form arbitrary associations between sensory stimuli is fundamental for many learned behaviors. We trained crows (*Corvus corone*) to perform a visual delayed paired association learning task and investigated neuronal activity in the nidopallium caudolaterale (NCL) . The NCL is a multimodal integration area in the avian endbrain involved in high-level cognition in corvids.
The crows were presented with a sample image, followed by a brief delay. At the end of the delay, the birds chose one of two simultaneously presented test images on a touchscreen monitor. In each session, blocks with familiar associations (familiar association block) were alternated with blocks requiring the learning of new associations (novel association block). In each novel association block, crows learned to associate two arbitrary new sample images to two familiar test images by trial and error. The two test images were kept constant, while the sample images were exchanged for each new pair of associations. In the familar association blocks, crows responded to two familiar sample images with well -trained associations to the same two test images.
We used a state-space model of dynamic learning (Smith, Wirth, Suzuki, Brown, 2007) to determine the trial when the animal started performing reliably above chance. This learning criterion could be determined for 88% of all presented associations and was typically reached after about 50 trials (correct and error trials). Learning was also reflected in a change of reaction times, which decreased significantly by approximately 25% after the learning criterion was met.
We recorded the activity of single neurons in NCL. The block structure of the task allowed us to compare neuronal responses for familiar and novel sample items mapping onto the same test items. A large fraction of neurons discriminated between the two familiar sample images during the delay in the well-trained blocks. In novel blocks these neurons tended to show selectivity for the sample items associated with the same test item. Some of these neurons changed their activity during the learning process. Thus, selective delay activity in NCL does not simply reflect working memory related to the sample item, but actively processes information for the upcoming behavioral choice.
These data provide new insights into the nature of working memory representations in birds, and how these representations are formed during learning. They suggest NCL plays a role in forming arbitrary associations between sensory stimuli, a cornerstone of corvids' remarkable behavioral flexibility and adaptability.

Symposium

S24: The emerging etiopathogenic role of infections and inflammation in chronic CNS diseases

S24-1 Emerging Virus Induced CNS Diseases
Albert Osterhaus

S24-2 Role of autoantibodies in neuropsychiatric diseases
Christian Hammer, Hannelore Ehrenreich

S24-3 Role of lymphocyte invasion in CNS diseases
Alexander Flügel

S24-4 Role of CNS infections for neurodegenerative diseases
Kristin Michaelsen-Preusse, Martin Korte

S24-5 Long-term influences of an immune stimulation on neuronal structure and plasticity
Marianna Weller

S24-6 Validity of a “two-hit” developmental model of schizophrenia (prenatal Poly I:C and neonatal PCP)
Christin Schifani

Emerging Virus Induced CNS Diseases

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Infectious diseases as well as neurodegenerative and inflammatory disorders of the central nervous system (CNS) represent major medical challenges of the health care systems in the coming decades. Numerous CNS diseases are triggered directly or indirectly by infections or a misdirected immune response against their causative agents. Additionally, some emerging diseases, many of them arising from zoonotic pathogens, like spongiform encephalopathy
(new variant of Creutzfeldt-Jacob disease in humans), influenza, tick-borne encephalitis, and West Nile disease, are neurotropic to a varying degree. Moreover, several neuro-degenerative diseases including Alzheimer's disease and multiple sclerosis (MS) are suspected to be caused or aggravated by infections. To develop new strategies for diagnosis, prevention, and treatment of these and other CNS disorders, the complex interactions between CNS and pathogens urgently require a more profound understanding. This is not limited to a better understanding of the pathogenesis of neurological and psychiatric disorders and associated infections, but should also encompass their epidemiology by studying routes of transmission within and between species. The chairs and speakers of this symposium are principal investigators of the novel research network N-RENNT (Niedersachsen-Research Network on Neuroinfectiology), which brings together a unique consortium of experts and institutions in the integrated fields of neuroscience and infectious diseases in Lower-Saxony. The talks give examples of the N-RENNT research, which is funded by the Ministry of Science and Culture of Lower Saxony and the Volkswagenstiftung in Germany.

Role of autoantibodies in neuropsychiatric diseases

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Circulating autoantibodies (AB) occur in classical autoimmune diseases and paraneoplastic syndromes. However, recent studies reported the presence of serum AB directed against brain antigens in up to 90% of healthy subjects. These findings raise the question under which circumstances, and dependent on which factors, AB induce or contribute to pathology, as suspected in the case of anti-NMDAR encephalitis.

Using the GRAS (Göttingen Research Association for Schizophrenia) sample of neuropsychiatrically ill patients and healthy controls, we tested >2,800 sera for presence of anti-NMDA receptor AB. Unexpectedly, we found AB of IgA, IgM, or IgG isotype, directed against the NR1 subunit, in 10.5% of all subjects. There was no difference between patients and controls with regard to seroprevalence or titer. All NMDAR1-AB tested were shown to be functional by inducing NMDAR1 endocytosis in primary neurons. We hypothesized that a compromised blood-brain barrier (BBB) might contribute to their pathophysiological significance. To investigate this, we employed ApoE knockout mice (with known BBB leakage) for an intravenous injection of extracted serum from human NMDAR -AB carriers. When compared to their wildtype littermates, the ApoE KO mice showed alterations in spontaneous open-field activity and in the response to MK-801. In the human (GRAS) study cohort, seropositive individuals with a history of neurotrauma or birth complications had more neurological abnormalities than seronegative patients.

Since *APOE4/ApoE4* carrier status is associated with BBB leakage in mouse and man, we performed *APOE* genotyping in a total of 2,492 patients and controls. While *APOE4*carrier status was not different in neuropsychiatric disease groups when compared to controls, the interaction of *APOE4* carrier status and NMDAR -AB seropositivity was significantly associated with schizoaffective disorder (P=0.001, OR=6.109). Delusions of grandiosity and mania are common psychiatric symptoms in anti-NMDAR encephalitis, and thus NMDAR1 -AB may cause or boost these symptoms in neuropsychiatrically ill *APOE4* carriers, which may then more likely be diagnosed schizoaffective. We also investigated possible factors implicated in the formation of NMDAR-AB, and found that individuals carrying antibodies against Influenza A or B are more likely to be NMDAR-AB seropositive.

We next wondered whether similar results would be obtained for 24 other brain antigen-directed AB connected with pathological conditions, using an expanded sample of >4,200 individuals. Again, seroprevalence of all screened AB was comparable in healthy and ill individuals. None of them, however, reached the abundance of NMDAR-AB (again ~10%). Appreciable frequency was noted for AB against amphiphysin (2.0%), ARHGAP26 (1.3%), and CASPR2 (0.9%), with titers and Ig class distribution similar among groups. Interestingly, the predominant Ig class depended on antigen location, with intracellular epitopes predisposing to IgG ($p=2.8x10^{-48}$). While our results challenge an unambiguous causal relationship of NMDAR-AB with neuropsychiatric disease, they may point to the existence of a thus far underexplored physiological autoimmunity modulating brain functions.

Role of lymphocyte invasion in CNS diseases

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Autoimmune diseases, for example multiple sclerosis, are initiated when autoreactive T cells enter their target organ and become re-activated upon encountering their cognate self-antigens. The central nervous system (CNS) is shielded from the periphery by the blood–brain barrier (BBB) that prevents uncontrolled access of cells and molecules. How autoreactive T cells transmigrate through an intact blood–brain barrier and which molecular cues guide them in the different CNS milieus remains an open question. By combining two-photon imaging and functional characterization in a Lewis rat model of EAE we found that just after transfer blast T cells have to undergo a complex reprogramming of their gene expression profile in order to enter the CNS: starting in the lung effector T cells changed their motility properties and their functional profile by down-regulating their activation and proliferation program and up-regulating their cellular locomotion molecules and their adhesion and chemokine receptors. This newly acquired migratory phenotype allowed the effector T cells to to enter the CNS tissue. We detected that CXCR3 ligands mediated the firm arrest of T cells arriving at the meningeal vessels and their subsequent crawling and orientation on the inner-vascular walls. After entry into the meningeal milieu CXCR3 and CCR5 strengthened T cell contacts with meningeal and perivascular phagocytes and stabilized the T cells at the interface between the parenchyma and cerebrospinal fluid (CSF).
We therefore propose that 1) the migratory mode is a prerequisite for T cell entry into the CNS and for autoimmune disease induction; and 2) chemokines crucially determine the motility characteristic of effector T cells both in the intravascular and extra-vascular phases of T cell infiltration into the CNS.

Role of CNS infections for neurodegenerative diseases

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Up to now many of the essential processes resulting in the development of neurodegenerative diseases remain elusive. In addition to many other risk factors, inflammatory incidents in the central nervous system (CNS) sustained early in life are suspected to play an important role for the onset and progression of neurodegenerative processes.
The CNS is vulnerable to different inflammatory occasions: Peripheral infections caused by various pathogens provoke inflammatory responses first in the periphery, however, they can also affect the otherwise immune privileged CNS. In contrast, other pathogens are able to enter the CNS resulting in acute and chronic brain infections. To gain a better insight into cellular mechanisms and consequences of CNS infection and inflammation we used different experimental approaches (immunstimulation with bacterical endotoxins, viral infection and infection with the protozoan *Toxoplasma gondii*) and analyzed long-term effects on neuronal function and structure with a special focus on the hippocampus, a brain region involved in learning and memory formation.

Previously, it has been shown that the peripheral administration of bacterial endotoxins resulted in acute cerebral dysfunction and in the activation of microglia, the immune cells of the CNS. To analyze the long-term effects of a peripheral immune stimulation, young and aged mice were treated with cell wall components of Gram positive and negative bacteria, respectively. Three month after the stimulation, the analysis of dendritic morphology and spine density revealed distinct effects on hippocampal neurons of old mice for both types of stimulating agents whereas no prominent neuronal changes were observed in young animals. Furthermore, immune challenged old mice showed a reduction of synaptic function as indicated by a reduced long-term potentiation and did perform less precisely in the Morris Water Maze indicating a long lasting cognitive impairment due to immune stimulation.
Influenza viruses represent another model for peripheral infections, however, some strains are neurotropic and can in addition infect the CNS. Intranasal infection with different influenza virus subtypes was used in young mice and the long-term consequences for hippocampal neuronal morphology were analyzed one month after infection. Our data indeed show viral subtype specific effects on dendritic spine density indicating a longer lasting effect on cognitive function. In addition, we asked the question whether factors otherwise involved in mediating the immune response in the brain would also play a role under basal conditions in the healthy brain and therefore analyzed neuronal morphology and function in type-1 interferon receptor (IFNAR) KO animals.
T. gondii is an obligate intracellular protozoan infecting warm-blooded animals and causes chronic cerebral infections. Following an infection with *T. gondii* cortical neurons revealed alterations in dendrite morphology which might be linked to behavioral changes described in humans like mood and schizophrenia disorders associated with *T. gondii* infections.
Taken together, our data provide evidence that peripheral and cerebral immune challenges can result in long-lasting changes in neuronal morphology and function which might compromise CNS function and therefore promote neurodegenerative processes.

Long-term influences of an immune stimulation on neuronal structure and plasticity

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The innate immunity provides rapid and strong protection against invading pathogens and clears pathogen-associated as well as host-derived material. Inflammation, the response of the innate immune system to danger signals, can not only occur in the periphery but also in the central nervous system. Here, processes and molecules associated with the immune response can have protective as well as detrimental effects. These adverse effects can be monitored in a situation of an acute systemic infection exclusive of brain invasion or blood brain barrier brake down. An acute inflammation in the periphery can negatively affect neuronal morphology, plasticity and learning behavior in rodents. However, the influences of the innate immunity on neuronal structure and plasticity long after an acute immune stimulation are largely unexplored.
The aim of the present study is to elucidate changes in neuronal morphology, function and learning behavior of immune stimulated mice after recovery from an acute peripheral inflammation.
Therefore, the immune system of old wildtype mice (16 month old C57Bl6/J) was challenged via peripheral administration of different types of lipopolysaccharide, an endotoxic component of the gram negative bacterial cell wall. The exposure to 0.4 µg per g bodyweight of LPS from *Salmonella enterica serogroup typhimurium* leads to dramatic reduction of dendritic complexity and spine density in different brain areas 3 months after incubation, which was analyzed using the Diolistic technique. Mice stimulated with the same amount of LPS of *E.coli* 0127:B8 show dendritic and spine phenotypes comparable to control neurons. Compatibly, the long term potentiation (LTP), which as an enduring enhanced synaptic transmission reflects the
cellular correlate of learning and memory, is impaired long after the exposure to LPS of *S.typhimurium*. The LTP of LPS *E.coli* stimulated mice is on control level. An equivalent outcome is also visible for the learning behavior in the Morris water maze task. Here, all mice managed to remember the position of the hidden platform, but the proportion of the search strategies used is different within the immune stimulation treatment. While the LPS *E.coli* treated mice used more hippocampus-dependent search strategies, the LPS *S.typhimurium* treated ones show a higher proportion of hippocampus-independent learning. To explain the differences between the two types of LPS treatment, pro-inflammatory as well as astrocyte and microglia specific markers were quantified.
Taken together, the stimulation of the peripheral immune system can have long-lasting effects on neuronal structure and function depending on the immune stimulatory component.

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Validity of a “two-hit” developmental model of schizophrenia (prenatal Poly I:C and neonatal PCP)

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Different genes and environmental factors are evident to be at risk for the development of schizophrenia. Those factors at risk (‘hits’) are used for neurodevelopmental animal models changing genes or environment in early animals’ development. One widely used example is an immune activating agent called Polyinosinic-polycytidylic acid (Poly I:C) mimicking maternal, viral infection during pregnancy. One disadvantage however, is the problem of result reproducibility between laboratories and the limited validity. Due to the hypothesis which describes the disease onset as an interplay of at least two hits during development, we decided to combine the Poly I:C model (infusion on gestation day 15) with an early life insult (neonatal exposure of offsprings to glutamatergic insult by repeated treatment with the NMDA receptor antagonist phencyclidine on postnatal days 7, 9 and 11). Goal of the project is to establish a model showing stronger effects which help to increase reproducibility and validity.

There are three symptom classes which form the individual symptoms of patients with schizophrenia in a varying extend including positive, negative and cognitive symptoms. To assess if our two-hit model can represent a good face validity model for schizophrenia, different behavioral tests were assessed. Indeed, differences in all three domains could be shown in double treated animals including for instance changes in locomotor activity and social behavior.

Beside from behavior, neuroanatomical parameters known to be often changed in schizophrenia patients were assessed, too. Schizophrenia is discussed as at least partial neuroinflammatory disease during adolescent stage. To test this hypothesis, neuroinflamatory state of our animals was examined. Thereby, an enhanced number of microglia was found in the treated animals. In addition, this effect was found to be ameliorated by sub-chronic, juvenile treatment with Minocycline and Pregnenolone, two substances shown some effects for the treatment of schizophrenia symptoms. This shows that the two-hit model also fulfill predictive validity.

In addition to anatomical changes, imaging studies show changes in metabolic brain activity in patients suffering from schizophrenia. Similarly, we found changes in our double treated animals. Therefore, the two-hit model not only show face and predictive but also construct validity.

One disadvantage of the model is that with regard to behavioral read-outs the variance in the Wistar rat groups (treated vs. non-treated) is quite high. To assess if our two-hit treatment induces less variance in an already more homogenous group with the same genetic background, another group of animals was generated using the inbred Lewis rat strain. Less variance could be shown for instance in juvenile and adult spontaneous locomotor activity.

To conclude, double treatment combining viral, maternal infection and neonatal glutamatergic insult lead to behavioral, neuroanatomical and metabolic changes showing similarities to those of patients suffering from schizophrenia which can be even reversed by substances showing some efficacy in treating schizophrenia. Effects could be even improved by using inbred Lewis rats with a genetically identical background instead of outbred Wistar rats.

Symposium

S25: Regulation of normal and impaired sleep

S25-1 The molecular mechanisms of sleep homeostasis
Tarja Stenberg

S25-2 The multiple facets of wakefulness, control by histamine and orexins
Jian-Sheng LIN

S25-3 The role of CRH in stress-induced sleep impairment
Mayumi Kimura

S25-4 Sleep-related neuroplasticity in healthy subjects and psychiatric patients
Martin Dresler

S25-5 Targeting the serotonergic and noradrenergic brain system to treat narcolepsy in a mouse model
Christian Schmidt, Judith Leibiger, Markus Fendt

S25-6 High-density electrophysiological characterization of the hippocampal and cortical network activity in the awake and sleeping mouse.
Nikolaos Karalis, Eduardo Blanco-Hernández, Anton Sirota

The molecular mechanisms of sleep homeostasis

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Brain responds to prolongation of a waking period by producing more and deeper sleep, a phenomenon called sleep homeostasis. Regulation of sleep homeostasis has been modeled from EEG data collected from several species and formulated as the two process model of sleep regulation. The model accurately predicts the duration of sleep not only in humans but also in other mammalian species. In spite of the accurate model, the molecular mechanisms of sleep homeostasis are presently not well understood.
One explanation for sleep homeostasis is provided by the energy depletion hypothesis: during waking the neuronal activity consumes energy, and the longer the waking period the more energy is consumed, resulting (local) energy depletion in active brain areas. Increasing adenosine concentration is a marker of energy depletion, and simultaneously adenosine can, through its A1 receptors decrease neuronal activity, forming an ultra -short negative feed -back regulation loop. Experimental evidence supports this view: adenosine concentration increases locally in basal forebrain in the course of wakefulness and decreases during sleep. Moreover, nitric oxide (NO) concentration increases at the same area through activation of inducible nitric oxide synthase (iNOS) coupling the adenosine increase also to immunological activation. The effects of these molecules on sleep homeostasis in the BF are connected to cortically projecting cholinergic neurons in this area, since inactivation of these cells abolishes sleep homeostasis.

The multiple facets of wakefulness, control by histamine and orexins

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Despite the presence of variable behavioural and cognitive activities during wakefulness (W), this vigilance state has long been regarded as relatively homogeneous on the basis of the EEG phenomenology. Recent studies have shown, however, that cortical oscillation and neuronal properties undergo major changes from quiet to active W. We hypothesize that each brain arousal-promoting system exerts a distinct control during different behavioural contexts of W. To test this hypothesis, we have investigated the role of histamine (HA) and orexins (Ox), two widespread-projecting W-promoting systems originated from the posterior hypothalamus.

Wild-type (WT) and KO mice lacking HA, or Ox or both were prepared for chronic EEG and sleep-wake monitoring under baseline conditions and after pharmacological dosing or behavioural tests, including locomotion, exploration, motivation, anticipation and sexual arousal.

We found that 1) Ox KO but not HA-deficient mice showed impaired W when they were subjected to a voluntary wheel test, indicating that Ox but not HA promotes W by enhancing locomotion. 2) HA-deficient mice were unable to remain awake in a new environment while Ox KO mice could preserve an enhancement of W. 3) In a test of motivation, WT and Ox KO mice were motivated enough to catch difficult-to-reach palatable food and maintained highly awake, while HA deficient mice, though interested by the food, made no apparent effort to catch it and, as a result, slept as usual. The two last tests show that as compared to Ox, HA is more involved in the cognitive aspects of W. 4) When WT mice were fed with a predictable restricted schedule (11 am-17 pm) instead of ad libitum, they displayed an anticipatory W of 70±9 min before the meal time. This anticipatory W was significantly reduced in both Ox KO or HA-deficient mice. Moreover, Ox KO, but not HA-deficient mice exhibited W deficit during the feeding period. Finally, the anticipatory W disappeared completely in double KO mice lacking both HA and Ox; 5) In a sexual arousal test, defined as increased W in a male mouse facing a female, acute inhibition of HA synthesis or antagonism of Ox1-receptor abolished sexual arousal while this function was intact in Ox KO or HA-deficient mice, indicating a compensation under long term loss of HA or Ox. This compensation became ineffective when both HA and Ox were deficient as sexual arousal was severely impaired in KO mice lacking both HA and Ox.

These data support our hypothesis according to which, wakefulness is a heterogeneous state with multiple behavioural facets. Each arousal system contributes complementarily and synergistically to the maintenance of cortical activation during wakefulness, while in different behavioural and cognitive contexts, their individual participation and specific functional role are fundamentally distinct.

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The role of CRH in stress-induced sleep impairment

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Stress affects sleep. A key chemical modulator initiating humoral and behavioral stress responses is corticotropin-releasing hormone (CRH). Considering the critical role of CRH in depression, brain-site specific effects of CRH on sleep have been studied in Cre-expressing mouse models. Our earlier study demonstrated that forebrain/limbic-specific (Cam) conditional CRH-overexpressing mice (CRH-COE) display enhanced REM sleep. Another previous study also reported that elevated hypothalamic-pituitary-adrenocortex activity does not contribute to the effects of central CRH on sleep alteration. During the last years, therefore, we verified a mechanism of how CRH affects sleep, especially the relation of limbic CRH and REM sleep. Since monoaminergic systems in CRH-COE mice seem to be normal, we focused on the cholinergic system. As suspected, CRH-COE mice showed hyper-cholinergic sensitivity, and microinjected atropine, a muscarinic antagonist, into the amygdala suppressed REM sleep. In response to sleep deprivation, CRH-COE-Cam showed a prolonged rebound of REM sleep, which corresponded with greater stimulation of cholinergic neurons locating in the brainstem, crucial for REM sleep generation. According to the recent results, enhanced REM sleep in CRH-COE mice would be interpreted to originate from the action of limbic CRH through the cholinergic activation. Amygdaloid CRH is likely involved in stress-driven REM sleep enhancement.

Sleep-related neuroplasticity in healthy subjects and psychiatric patients

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Background: Compelling evidence supports a major role of sleep in neuroplasticity processes. However, disturbed sleep is a major symptom of many psychiatric disorders, suggesting that sleep-related neuroplasticity might be impaired in these conditions.

Methods: In a series of studies, we investigated sleep-related neuroplasticity in depressed patients, patients with schizophrenia, and healthy controls. Subjects performed procedural and declarative memory tasks before and after a night of sleep with polysomnography. Sleep EEG data were analyzed with an automatic sleep spindle detecting algorithm. In a control study, we studied the effects of high doses of corticosteroids in patients and healthy controls undergoing the same procedures. Neural mechanisms underlying disease-specific changes in sleep-related neuroplasticity were tested with fMRI.

Results: We observed a double dissociation of memory impairments in depressed patients: During acute depression, sleep-related memory consolidation is decreased for procedural, but not declarative tasks. During wakefulness, the same tasks show a reversed pattern, with declarative but not procedural memory being impaired. Sleep-related memory impairments were strongly modulated by age, being most pronounced in patients above the age of 30 years and thereby coinciding with the age threshold above which depression-related sleep disturbances are most pronounced. However, neither sleep disturbances nor pharmacological REM sleep suppression turned out to underlie these memory impairments, and the relationship between sleep spindles and sleep memory consolidation seems to be undisturbed in depression. In contrast, high-dose application of corticosteroids led to depression-like impairments of procedural memory consolidation. Neuroimaging data demonstrated that sleep-related memory impairments were related to abnormal prefrontal-hippocampal connectivity as compared to healthy controls.

Conclusions: Psychiatric patients show a specific pattern of sleep-related neuroplasticity impairments. Underlying mechanisms include disturbed medial prefrontal-hippocampal connectivity and stress hormone dysfunction rather than sleep per se.

Targeting the serotonergic and noradrenergic brain system to treat narcolepsy in a mouse model

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Narcolepsy is a neurodegenerative disease which is characterized by the lack of orexin neurons in the brain. The main symptoms are exaggerated daytime sleepiness and cataplectic episodes (immediate loss of muscle tone). Mice lacking the gene for orexin express narcoleptic episodes and are therefore used as an animal model of narcolepsy.

The aim of the present study was to investigate whether pharmacological modulation of the serotonergic and noradrenergic brain system is able to reduce narcoleptic episodes in orexin-deficient mice. Therefore, orexin-deficient mice were treated with different serotonergic and adrenergic agents at the beginning of the lights-off period. Then, they were placed into a box with enriched environment. The behavior of the animals during the next four hours was video-taped and the occurrence and length of narcoleptic episodes were analyzed afterwards.

In general, we observed an increase of the narcoleptic episodes in the time course of the single experimental trials, as well as in the life time of the mice. Both application of the selective serotonin reuptake inhibitor (SSRI) escitalopram and of the norepinephrine reuptake inhibitor (NRI) reboxetine significantly prevented narcoleptic episodes. Interestingly, reboxitine was much more effective than escitalopram. Prazosin, an alpha -1 adrenergic receptor antagonist, had no effects on narcoleptic episodes. Cirazoline, an alpha1-adrenergic receptor agonist and alpha-2 adrenergic receptor antagonist, reduced the number of the narcoleptic episodes but also strongly decreased locomotor activity.

These findings demonstrated that both the serotonin and norepinephrine systems affect narcoleptic episodes in a mouse model of narcolepsy. This suggests that the central serotonergic and noradrenergic systems could be targets to treat symptoms of narcolepsy.

High-density electrophysiological characterization of the hippocampal and cortical network activity in the awake and sleeping mouse.

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A long-standing goal of neuroscience is the elucidation of the mechanisms of learning and memory. There has been accumulative evidence that sleep and the underlying activity of the brain is involved in the consolidation and reinforcement of the memory trace.
It has been suggested that, during sleep, the communication between hippocampus and the neocortex, two structures involved in memory consolidation, is facilitated by means of slow oscillations dominating the cortical structures and fast transient hippocampal events termed sharp-wave/ripple complexes, that allow a bidirectional information flow.

However, the specifics as well as the functional importance of these interactions remain elusive, while all the evidence have been so far correlational. The advent of optogenetics and the extended genetic toolbox available for the mouse presents the opportunity for a causal dissection of the circuit and the unraveling of the mechanisms that support memory formation.
Importantly, the mouse sleep network patterns of activity and the inter-regional interactions have not been so far characterized with high spatial resolution, due to technical and size limitations. The detailed characterization of the network activity in this species is necessary before attempting the causal investigation of the involvement of this system in memory mechanisms.

To this end, we attempt the simultaneous detailed characterization of activity across multiple structures in the mouse brain.
In particular, we set to investigate the functional connectivity between the hippocampus, medial prefrontal cortex and entorhinal cortex. To do so, we employ multi-shank high-density silicon probes to record the laminar profiles of local field potentials (LFPs) as well as the activity of large neuronal populations simultaneously in these structures. This data allows for the analysis of network and cell ensemble dynamics in multiple interconnected structures, which enables the investigation of the mechanisms of information flow between and within brain structures.

To overcome the limitations of chronic recordings in freely moving mice, while maximizing the complexity and number of experiments possible on a given animal, we designed a paradigm that allows head-fixed mice to run freely on an air-cushioned ball and sleep at will. In parallel, we monitored a range of physiological parameters, such as respiratory activity, heart rate and ocular movements, to better characterize the behavioral state of the mice.

The combination of multidimensional behavioral monitoring, paired with the detailed electrophysiological recordings, provide us with an unprecedented perspective of the mouse sleep network activity patterns that will provide the backdrop to the insightful probing of the memory circuit mechanisms using modern perturbation techniques.

Symposium

S26: Nanostructure and function of presynaptic active zones

S26-1 Ultrastructural determination of dynamic vesicle pools at inner hair cell ribbon synapses
Carolin Wichmann, Susann Michanski, Christian Vogl, Rituparna Chakrabarti, SangYong Jung, Tanja Maritzen, Volker Haucke, Tobias Moser

S26-2 Nanoarchitecture of Active Zones at the Calyx of Held
Thomas Kuner

S26-3 "The Morphological and Molecular Nature of Synaptic Vesicle Priming at Presynaptic Active Zones."
Benjamin H. Cooper, Cordelia Imig, Sang-Won Min, Stefanie Krinner, Marife Arancillo, Christian Rosenmund, Thomas C. Südhof, Jeong-Seop Rhee, Nils Brose

S26-4 Munc13-3 superprimes synaptic vesicles at granule cell-to-basket cell synapses in the mouse cerebellum
Jens Eilers, Hartmut Schmidt, Benjamin Cooper, Nils Brose, Shimpei Ishiyma

S26-5 Activity dependent nanostructure of inner hair cell ribbon synapses
Rituparna Chakrabarti, Sarah Grant , Sangyong Jung, Tobias Moser , Ellen Reisinger, Carolin Wichmann

S26-6 Role of Piccolo in high frequency transmission at central auditory synapse
Tanvi Butola, Tobias Moser

Ultrastructural determination of dynamic vesicle pools at inner hair cell ribbon synapses

Carolin Wichmann[1,2], Susann Michanski[1,2], Christian Vogl[3], Rituparna Chakrabarti[1,2], SangYong Jung[3], Tanja Maritzen[4], Volker Haucke[4,5], Tobias Moser[2,3,6,7]

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Encoding auditory signals into a neuronal code during the process of hearing, in mammals is mediated through the ribbon synapses of the inner hair cells (IHCs) and spiral ganglion neurons. Acoustic information is transmitted with high temporal precision over long periods of time requiring high rates of transient and sustained release. These specialized synapses comprise a synaptic ribbon capable of tethering dozens of synaptic vesicles and a presynaptic density that anchors the ribbon to the membrane. Morphologically, synaptic vesicles are also tethered to the active zone membrane, which, functionally, might represent the readily releasable vesicle pool. Neither the proteins mediating vesicle tethering to the ribbon nor to the active zone membrane at IHC ribbon synapses are identified so far. Moreover, an understanding of the mechanisms underlying exocytosis and endocytosis in IHCs in order to maintain the high release rates is just starting to emerge.
We investigate, which proteins contribute to the release machinery to coordinate synaptic vesicle release and recycling. To approach this, we study mutants of active zone proteins that show impairment in function, taking advantage of recent advances in electron microscopy technology such as high-pressure freezing/freeze-substitution (HPF/FS) and electron-tomography.
In my presentation I will focus on two proteins: (i) otoferlin, a C2-domain protein critical in vesicle fusion (Roux et al., 2006) and vesicle replenishment (Pangrsic et al., 2010) and (ii) the clathrin adapter protein AP-2 that in IHCs promotes efficient vesicle replenishment and interacts with otoferlin (Duncker et al., 2013). I will present detailed analyses of vesicle pool changes upon activity, vesicle reformation and also tethering of vesicles on the ultrastructural level in otoferlin and AP-2μ KOs.

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Nanoarchitecture of Active Zones at the Calyx of Held

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The molecular nanoarchitecture of active zones defines the functional properties of presynaptic function. While the identity of most proteins involved in presynaptic function is known, we lack fundamental insights into their three-dimensional arrangement. The work of the Sigrist laboratory on the Drosophila neuromuscular junction provides a flavour of the intricate architecture of the active zone protein network with Drosophila RIM-binding protein forming a ring around Ca channels and Bruchpilot positioned within the ring like spokes in a wheel. Almost certainly, many more nanostructural designs of protein arrangement will be found in the future and wait to be correlated with specific functional synaptic properties they mediate. Super-resolution microscopy, now endowed with a nobel prize, has revealed entirely new perspectives on nanoarchitecture. Importantly, 3D protein localization needs to be connected to our traditional electron microscopic view of active zone ultrastructure to fully comprehend nano- and ultrastructural dimensions.

The talk will explore the nanoarchitecture of active zones in the rat calyx of Held giant presynaptic terminal using stimulated emission depletion (STED) microscopy, stochastic optical reconstruction microscopy (STROM), novel resin-free scanning-electron-microscopy (RF-SEM) and electron-tomography (RF-ET) of chemically fixed resin-free sections. The molecular focus will be on one of the main determinants of the cytomatrix, actin, as well as on the large matrix proteins Piccolo and Bassoon.

Using confocal whole-calyx 3D immunohistochemistry analysis we found that actin is predominantly located within the compartment close to the active zones. Dual-color STED microscopy revealed that actin strands pervade the synaptic vesicle cluster and interconnect neighboring active zones. A dense actin network is present at the active zone and strands of actin project into the cytoplasm surrounding vesicle clusters. The filamentous architecture of the active zone and surrounding presynaptic space could be visualized using RF-SEM. This revealed a dense network of cytomatrix surrounding and directly contacting the synaptic vesicles. The molecular identity of actin strands could be identified with immuno-gold labeling on resin-free samples using RF-SEM and RF-ET. These experiments suggest that actin directly contacts synaptic vesicles and thereby structures the synaptic vesicle cluster. Furthermore, actin strands may provide a means of synaptic vesicle exchange between neighboring active zones.

Finally, we followed up on our observation that calyx of Held active zones segregate into three populations with regard to the expression of Piccolo and Bassoon: Piccolo-only, Bassoon-only and mixed. Using 3D dual color STORM microscopy we found that also within mixed active zones, this segregation was maintained.

"The Morphological and Molecular Nature of Synaptic Vesicle Priming at Presynaptic Active Zones."

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Synaptic vesicle docking, priming, and fusion at active zones are orchestrated by a complex molecular machinery. We employed hippocampal organotypic slice cultures from mice lacking key presynaptic proteins, cryo-fixation, and three-dimensional electron tomography to study the mechanism of synaptic vesicle docking in the same experimental setting, with high precision, and in a near-native state. We dissected previously indistinguishable, sequential steps in synaptic vesicle active zone recruitment (tethering) and membrane-attachment (docking), and found that vesicle docking requires Munc13/CAPS family priming proteins and all three neuronal SNAREs, but not Synaptotagmin-1 or Complexins. Our data indicate that membrane-attached vesicles comprise the readily-releasable pool of fusion-competent vesicles, and that synaptic vesicle docking, priming, and trans-SNARE complex assembly are the respective morphological, functional, and molecular manifestations of the same process, which operates downstream of vesicle tethering by active zone components.

Munc13-3 superprimes synaptic vesicles at granule cell-to-basket cell synapses in the mouse cerebellum

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Munc13-3 is a presynaptic protein implicated in vesicle priming that is strongly expressed in cerebellar granule cells (GCs). Mice deficient of Munc13-3 (Munc13-3$^{-/-}$) show an increased paired-pulse ratio, which led to the hypothesis that Munc13-3 increases the release probability (p_r) of vesicles. In the present study, we analyzed unitary synaptic connections between GCs and basket cells in acute cerebellar slices from wild-type and Munc13-3$^{-/-}$ mice. Unitary EPSCs recorded from Munc13-3$^{-/-}$ GCs showed normal kinetics and synaptic latency, but a significantly increased paired-pulse ratio and fraction of synaptic failures. A quantal analysis revealed that neither the charge of single quanta nor the binominal parameter N were affected by loss of Munc13-3, but that pr was almost halved in Munc13-3$^{-/-}$. Presynaptic Ca^{2+} influx was not affected by deletion of Munc13-3, as well as replenishment of the readily releasable vesicle pool. However, high concentration of EGTA led to a reduction in EPSCs that was significantly stronger in Munc13-3$^{-/-}$. We conclude that Munc13-3 is responsible for an additional step of molecular and/or positional “superpriming” that substantially increases the efficacy of Ca^{2+}-triggered release.

Activity dependent nanostructure of inner hair cell ribbon synapses

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Sensory synapses, involved in vision, hearing or balance are specialized structural and functional entities. The synapses of the cochlear inner hair cells (IHCs) are molecularly and morphologically distinct to transmit acoustic information at hundreds of Hz with sub-millisecond precision over long periods of time. They exhibit a striking presynaptic electron dense projection called ribbon that tethers synaptic vesicles (SVs). Disruption of this synaptic structure could lead to impaired vesicle release and synaptopathy. Presently, we lack a clear understanding of the mechanism(s) underlying the SV release at the presynaptic active zones in IHCs. To address this question, we have studied (i) alterations of synaptic vesicle pools and (ii) the tethering of vesicles in wild type and mutant mice in different activity states. To do so we have applied different durations of stimulations using high extracellular K^+ solutions and determined number, size and volume of vesicles in two morphological distinct pools: the ribbon-associated and membrane -proximal SVs. Additionally, we investigated the number, length and localization of tethers at IHC ribbon synapses.

Here we present our results for the temperature sensitive mutant of the multi C2 domain protein otoferlin (*Otof* $^{I515T/I515T}$) and the rab-interactin-molecule 2a (*RIM2a*) KOs (*RIM2a*$^{-/-}$). Otoferlin plays a striking role for human and mice hearing (Roux et al., 2006; Pangršic, Reisinger and Moser, 2012). IHC exocytosis is nearly abolished In *Otof*$^{-/-}$ animals (Roux et al., 2006) and strongly impaired in *Otof* $^{I515T/I515T}$ mutants. *RIM2a*$^{-/-}$ IHCs exhibit defective sustained exocytosis and reduced synaptic Ca^{2+} influx. We employ a combination of conventional and advanced electron microscopic techniques such as high pressure freezing/freeze substitution and electron tomography. This enables us to analyze the ultrastructure with great precision and highest resolution in order to correlate structural to functional defects.

We found that the ribbon size and shape remained unaltered in both mutants, but significant differences were observed in terms of SV size and fraction of tethered vesicles. Therefore, these two proteins seem to play a crucial role in the release mechanism in IHCs.

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Role of Piccolo in high frequency transmission at central auditory synapse

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Piccolo is a cytomatrix of the active zone (CAZ) protein involved in scaffolding and regulating neurotransmitter release at neuronal active zones. Here, we used Piccolo mutant mice to study central auditory synapses of the cochlear nucleus, which are specialized in high throughput synaptic transmission, capable of sustaining signaling frequencies of several hundreds of Hertz. We argue that even subtle deficits in the regulation of vesicle dynamics and neurotransmitter release will be revealed at synapses with such high functional demands. Moreover, the signal this synapse receives is unbiased by the mutation due to the presence of an unaffected, short isoform of Piccolo (i.e. 'Piccolino') upstream at the ribbon synapses of cochlear inner hair cells. Hence, this site of investigation provides a unique opportunity to study the implications of Piccolo deficiency on neuronal synaptic transmission. At the endbulb of Held synapse, we observed faster rise of miniature excitatory postsynaptic currents (mEPSCs) in the mutants, while mEPSC amplitude and frequency were unchanged. Likewise, we found a faster rise of evoked EPSCs in the mutants. Moreover, when stimulated with high frequency train stimulation, the mutant responses showed faster kinetics of depression and an increased probability of vesicle release, while estimates of pool size and vesicle replenishment remained unchanged. Interestingly, a subset of mutant responses showed significantly increased EPSC amplitudes and readily releasable pool sizes. This finding might be due to target-cell specificity within the bushy cell population itself or, alternatively and less likely, an effect that can be attributed to loss of Piccolo from synapses that solely employed Piccolo as scaffold, unsupplemented by Bassoon (a CAZ protein closely homologous to Piccolo). Current experiments aim to dissect the precise presynaptic mechanism by a combination of presynaptic patch-clamp recordings and electron microscopy observation of synaptic vesicle pools. In addition, we address changes in the abundance of other CAZ proteins at the Piccolo-deficient endbulb AZs and study the consequences of altered synaptic transmission for auditory signal transmission using in vivo single neuron electrophysiology.

Symposium

S27: Brain tumors strongly interact with different cell types in the CNS: biological mechanisms and therapeutic impact

S27-1 Generation of neuronal progenitor cells in response to tumors in the human brain
Stefan Momma, Jadranka Macas, Min-Chi Ku, Christian Nern, Helmut Bühler, Marc Remke, Michael Synowitz, Kea Franz, Volker Seifert, Karl H. Plate, Helmut Kettenmann, Rainer Glass

S27-2 Site matters - immunotherapy of malignant brain tumors using pro-inflammatory cytokines and systemic immunostimulation
Johannes vom Berg

S27-3 Dissecting the Role of Apelin Signaling in Gliomagenesis
Roland Eugen Kälin

S27-4 The dual role of neural precursor cells (NPCs) in tumorigenesis: NPCs are the point of origin for gliomas and also constitute a first line of defence against brain tumors
Rainer Glass, Michael Synowitz

Generation of neuronal progenitor cells in response to tumors in the human brain

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Data from transgenic mouse models show that neural precursor cells (NPCs) can migrate towards experimental brain tumors and modulate the course of pathology. However, the pathways attracting NPCs to CNS neoplasms were unknown and it was unexplored if NPCs migrate towards brain tumours (high-grade astrocytomas) in humans. We analyzed the tumor-parenchyma interface of neurosurgical resections for the presence of (NPCs) and distinguished these physiological cells from the tumour mass. We observed that PSA-NCAM-positive NPCs accumulate at the border of high-grade astrocytomas and display a marker profile consistent with immature migratory neuronal progenitor cells. Importantly, these high-grade astrocytoma associated NPCs do not carry genetic aberrations that are indicative of the tumor. Additionally, we observed NPCs accumulating at CNS metastases. These metastatic tumors are distinguished from neural cells by defined sets of markers. Transplanting murine glioma cells embedded in a cell impermeable hollow fiber capsule into the brains of nestin-gfp reporter mice shows that diffusible factors are sufficient to induce a neurogenic reaction. In vitro, vascular endothelial growth factor (VEGF) secreted from glioma cells increases the migratory and proliferative behavior of adult human brain derived neural stem cells via stimulation of VEGF receptor-2 (VEGFR2). Overall, our data reveal a mechanism for NPC attraction CNS tumours and suggest that NPCs accumulate in human high-grade astrocytomas.

Site matters - immunotherapy of malignant brain tumors using pro-inflammatory cytokines and systemic immunostimulation

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Gliomas are immunosuppressive. Regulatory T cells (Treg) are one of the most prominent local cell populations mediating this effect. Proinflammatory cytokines such as Interleukin-12 can counteract Treg expansion and induce an effector T cell (Teff) driven anti-glioma immuneresponse. Not only T cells but also microglia and monocyte derived cells within the brain tumor contribute to this immune response. In experimental settings blockade of co-inhibitory T-cell receptors boosts this effect. Intratumoral delivery of Interleukin-12 and systemic blockade of Cytotoxic T -Lymphocyte Antigen -4 (CTLA -4) leads to an increase of Teffs at the expense of Tregs and to an efficient anti-tumor immune response. In a therapeutic setting this approach is highly effective even in advanced stages when monotherapy with either of the respective agents fails. Local immunostimulation at the tumor site might also synergize with other systemically applied (immuno-)therapeutic appraoches and represents a promising new avenue for the clinic.

Dissecting the Role of Apelin Signaling in Gliomagenesis

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Glioma are the most frequent malignant primary tumors of the brain. Glioma survival and growth is determined by its interaction with brain parenchyma including intense tumor angiogenesis. This process is supported by glioma-invading myeloid (GIM) cells, namely brain resident microglia or brain-invading macrophages. Depletion of GIM cells leads to a significant decrease in glioma volume. We have previously established the novel neuroendocrine hormone apelin as an angiogenic factor and described its upregulation in human glioblastoma multiforme (GBM).

Our aim is thus to study the functional role of apelin signaling in tumor angiogenesis and its effect on brain monocytes for the regulation of glioma growth. Intracerebral implantation of the human glioma cell line U87, expressing lentiviral control or apelin shRNA, allows us to study the specific contribution of glioma-derived apelin to brain tumor formation. To further dissect a putative immune function of apelin, we orthotopically implanted isogenic GL261 mouse glioma cells into immunocompetent mice lacking (Apelin-KO) or overexpressing apelin (Apelin-Tg).

Loss-of-apelin expression in U87 xenografts led to an almost complete blockage of glioma angiogenesis and a significant reduction of GIM cells while intracerebral infusion of the bioactive apelin-13 peptide was able to rescue this phenotype. Also GL261 implants in Apelin-KO mice reproduced the blockage of glioma vascularization. Interestingly, expression analysis showed an upregulation of the apelin receptor in microglia making them competent to respond to glioma-derived apelin peptide. While blockage of tumor angiogenesis by apelin depletion led to a significant decrease in subcutaneous U87 tumor volume, intracerebral glioma expansion was able to recover suggesting it to occur independently of tumor angiogenesis.

Together, our findings demonstrate that apelin is required and sufficient for the formation of a complex tumor vasculature. We show that both, glioma cell-derived apelin and apelin expressed by the tumor neovasculature are contributing to tumor angiogenesis, GIM invasion and tumor expansion. We anticipate that our study will provide insights whether apelin signaling may serve as a future novel target for anti-angiogenic tumor therapy.

The dual role of neural precursor cells (NPCs) in tumorigenesis: NPCs are the point of origin for gliomas and also constitute a first line of defence against brain tumors

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Data from mouse models suggest that gliomas origin from somatic mutant neural stem and precursor cells (NPCs). We found that endogenous NPCs also exert strong anti-tumorigenic effects against malignant brain tumors. Here, I describe the signal transduction mechanism of NPC-mediated tumor suppression and present data indicating that glioma cell-death is specifically initiated by NPCs and not by other progenitor cells. We observed that NPCs migrate from the subventricular zone to gliomas, reduce glioma expansion and prolong survival by releasing a group of fatty acid ethanolamides (vanilloids) that have agonistic activity on the vanilloid receptor (transient receptor potential vanilloid subfamily member-1; TRPV1). TRPV1 expression is much higher in gliomas than in tumor-free brain and TRPV1 stimulation is cytotoxic for glioma cells. NPC-mediated tumor suppression can be mimicked in the adult brain by systemic administration of synthetic vanilloids and phytovanilloids (which hold clinical potential) suggesting that TRPV1 agonists can serve as new glioma therapeutics. Furthermore, our data reveal that brain tumour suppression is an intrinsic feature of NPCs, but not other stem and precursor cells. Factors secreted from induced pluripotent stem cells (iPS cells) did not trigger glioma cell death, but when iPS cells were differentiated into NPCs they acquired anti-tumourigeneicity. Overall, our data show that NPCs have a specific role in suppressing HG-gliomas and that NPC-released anti-tumorigenic factors can be used as pre-clinical HG-glioma therapeutics.

Symposium

S28: Processing of temporal stimulus cues in the insect olfactory system

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Co-localization of insect olfactory sensory cells improves the discrimination of closely separated odour sources

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Olfaction is crucial for survival and reproduction of most animals, enabling them to find food, mates, and egg laying sites and to stay away from harmful places. Hence, odour molecules are detected by very sensitive and specific olfactory sensory neurons (OSNs). In insects, the OSNs are stereotypically grouped into sensilla located on the chemosensory organs. The functional significance of OSN co-localization is poorly understood, but it has been suggested that it allows for coincidence detection of odour filaments, improving discrimination of closely separated odour sources. We conducted the first experimental test of this hypothesis, using an insect in its natural environment. We manipulated the distance between odour sources of an attractive pheromone and either of two host-derived attraction antagonists (1,8-cineole and verbenone) and investigated the effect on trap catches of the spruce bark beetle, Ips typographus (Coleoptera: Scolytinae). 1,8-Cineole is detected by an OSN co-localized with an OSN for one of the pheromone components, while verbenone is detected by OSNs in other sensilla, not co-localized with pheromone OSNs. Consistent with the hypothesis, trap catch increased with distance between odour sources more for 1,8-cineole than for verbenone. The strongest effect was found among the males, that is the sex that first locates and attacks the host tree. Our results provide direct experimental support for the hypothesis that co-localization of OSNs in sensilla improves the discrimination of closely separated odour sources. Thus, selection for improved odour source discrimination could be one of the factors explaining the strict co-localization of OSNs that is seen across the Insecta.

Temporal processing of odor stimuli from Olfactory Receptor Neurons to Projection Neurons

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Olfactory Receptor Neurons (ORNs) respond to odor stimuli with temporal patterns of activity that depend on odor identity, odor concentration and receptor type. We performed measurements of the activity of single ORNs in *Drosophila* and analyzed these temporal patterns taking in account a precise quantification of the time dependent stimulus. We found that the dynamics of the odor stimulus itself depends on odor identity and concentration and that it strongly modulates the ORN response. We were able to assign a single response function to a single ORN and to predict the response of this ORN to two different odors solely from measurements of the stimulus. We also found that ORN adaptation capabilities maintain response dynamics remarkably similar across a large range of stimulus and background intensities (Martelli, 2013).

These results suggest that ORN response properties enable the olfactory system to capture information about stimulus independently from its intensity. In order to investigate this hypothesis we developed a computational model of the *Drosophila* Antennal Lobe (AL), the first synaptic processing center of olfactory information. Our simulation includes three neuron populations: ORNs, inhibitory Local Neurons (LN) and output Projection Neurons (PNs). The model reproduces known static properties of the AL (the divisive normalization, Olsen 2010) while providing temporal resolution on the activity of the single neurons. We investigate how dynamical properties of the input ORN activity affect the encoding of single odorants and odor mixtures in the population of PNs.

High speed smelling and odor object segregation in insects

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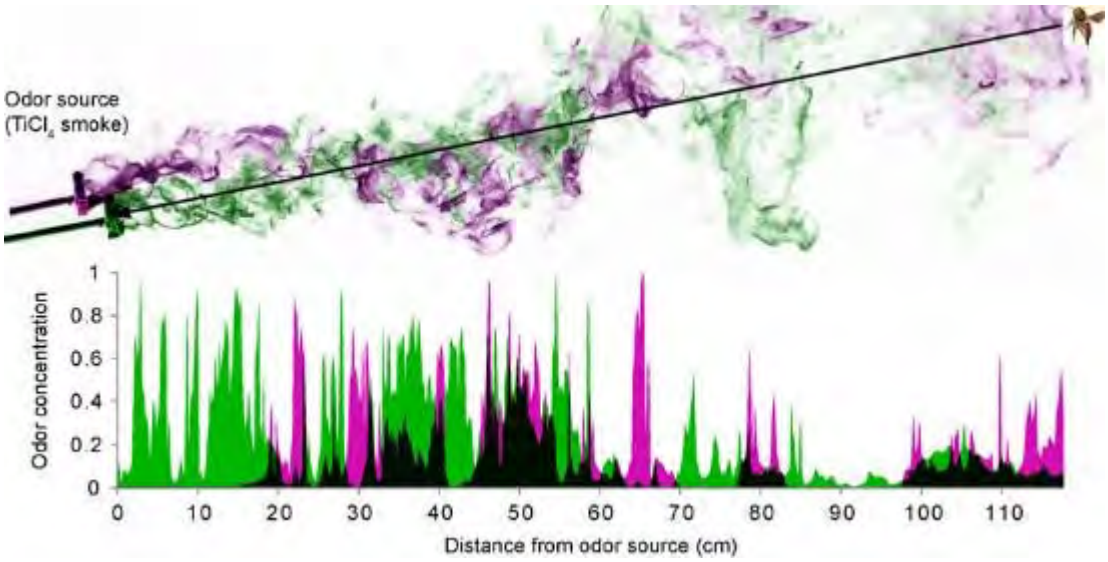
Animals as diverse as insects, fish and mammals rely on olfaction to find resources such as food, mates or hosts. Finding an odor source poses difficult challenges. Target odors occur in turbulent plumes and quickly break into thin filaments, such that an animal moving upwind might only be exposed to the odor for a few brief milliseconds at a time, and target odors intermingle with background odors. In order to understand how insects process fast olfactory information, we investigated their ability to detect temporal olfactory stimulus cues and to use those for segregating concurrent odors.
In insects, odor detection begins in olfactory receptor neurons on the antenna and continues in the antennal lobe in the brain. We probed the temporal resolution in insect olfactory receptor neurons and found that transduction times and pulse tracking capabilities are at least one order of magnitude faster than previously thought. We used behavioral experiments to show that honey bees can exploit millisecond short stimulus-asynchrony for odor object segregation, and we used in vivo calcium imaging to show that output neurons of the antennal lobe can resolve millisecond short stimulus-asynchrony between odor stimuli.
Our data suggest that olfactory object recognition shares a common principle with visual and auditory object recognition: Temporal synchrony between features is used to bind these features into unitary object representations, whereas asynchrony is used to segregate them into different object representations.

Figure: Odor plumes (monitored with smoke) break up into thin filaments. Odor filaments from different odor sources (green and magenta) intermingle. The area chart shows the flucuations of odor concentrations that would be encountert by an insect which flies along the black line.



Drosophila Kenyon cell responses to temporally complex odor mixtures generated with a novel high-bandwidth olfactory stimulator

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Natural odor plumes distribute in spatially complex filaments, and thereby contain much temporal structure when they reach a nose. For example, this temporal structure contains information about the number of odor sources, as odorants from the same sources fluctuate synchronously, while odorants from spatially separated sources fluctuate asynchronously. Behavioral experiments have shown that insects can use rapid temporal cues for odor-background segregation.

However, it is not known yet how the brain processes and extracts information from these temporal stimulus cues. In order to address this question we developed an olfactory stimulus delivery device that is able to generate temporally complex odor mixture stimuli with high temporal precision and high reproducibility. We characterize the device with a photoionization -detector and demonstrate that it is capable of simulating high bandwidth- fluctuations of natural odors.

We then used this odor delivery device to investigate how synchronous and asynchronous mixtures are represented in the mushroom body of Drosophila. Employing 2-photon imaging to acquire calcium signals from Kenyon cells with single-cell resolution, we found different forms of mixture interactions. We found mixture responses of individual Kenyon cells which were weaker or stronger than expected from the components, and in some Kenyon cells these mixture effects differed between the synchronous and asynchronous mixtures. As the mushroom body is thought to serve as an odor identification and association center, such time sensitive mixture effects could serve as a neural basis for the analytic extraction of individual mixture components as observed in other insects behavior.

Exploring neural mechanisms of odor object-segregation in computational models

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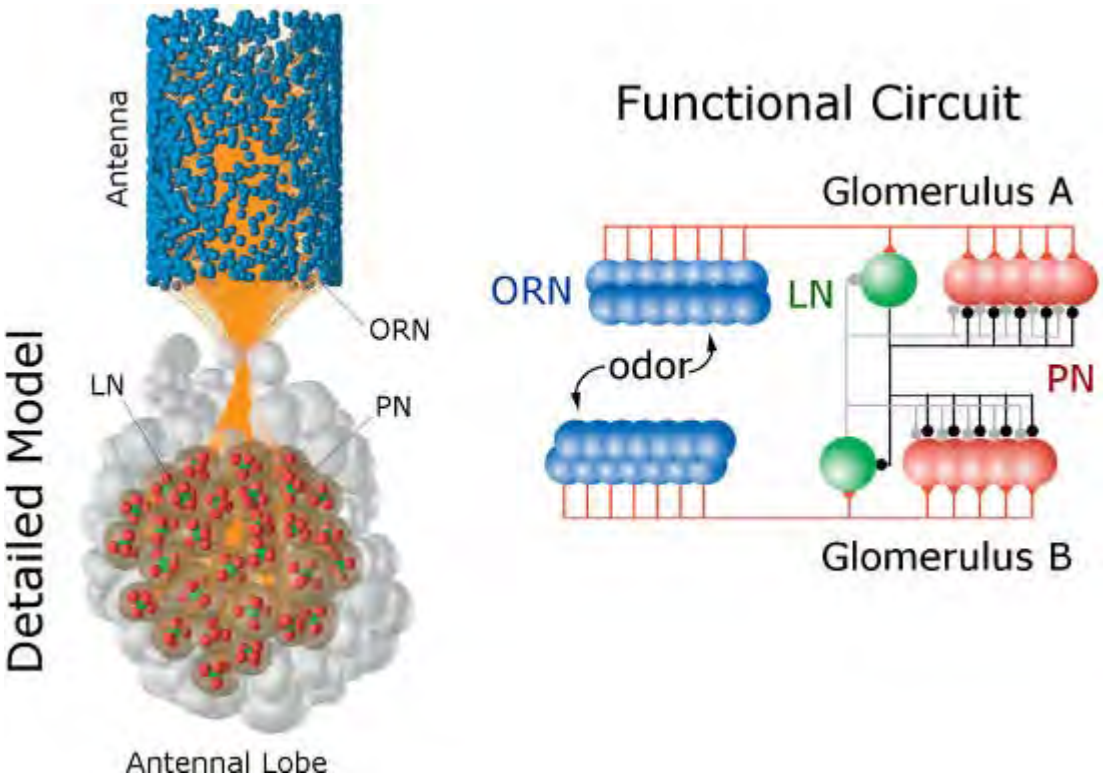
Insects have a remarkable ability to identify and localise odour sources in multi-odour environments. Behavioural experiments have shown that this ability appears to rely on detecting millisecond stimulus asynchronies between odourants that originate from different sources. Honeybees, Apis mellifera, are able to distinguish mixtures where both odourants arrive at the same time(synchronous mixtures)from those in which odourants arrive with a time difference as small as 6 ms (asynchronous mixtures).

In this talk I will present computational work that seeks to find an explanation how the early brain areas of olfactory information processing, the olfactory receptors and the antennal lobe, may be able to distinguish stimuli that differ on such a rapid timescale. We hypothesize that a winner-take-all inhibitory network of local neurons in the antennal lobe has a symmetry breaking effect, such that the response pattern in projection neurons to anasynchronous mixtures is different from the response pattern to the corresponding synchronous mixture, even though the antennal lobe operates on a much larger timescale than the difference in odour onset. The winner-take-all circuit would so form a temporary memory of which odour arrived first or whether they indeed arrived together.

I will present a detailed data-driven model of the bee olfactory receptors and antennal lobe that reproduces a large data set of experimentally observed physiological odour responses, successfully implements the hypothesized symmetry-breaking mechanism and so demonstrates that this mechanism is consistent with our current knowledge of the olfactory circuits in the bee brain.

Towards the end I will also discuss to what extent the model predictions do, or interestingly do not, match with subsequently performed physiological measurements in the bee antennal lobe.

The figure illustrates the model and the hypothesised functional circuit in the antennal lobe.


Detailed Model
Antenna
ORN
LN
PN
Antennal Lobe
Functional Circuit
Glomerulus A
ORN
LN
odor
PN
Glomerulus B

Symposium

S28/2: Role of glial heterogeneity in brain function

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Leda Dimou

S28/2-2 Developmental changes in synaptic communication between axons and oligodendrocyte precursor cells in corpus callosum
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S28/2-7 Role of protein translation and protein turnover for astrocyte heterogeneity in the hippocampus, striatum and prefrontal cortex
Daniela Christiane Dieterich

NG2$^+$glia: A journey through their diversity in the adult brain

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The adult central nervous system consists of neurons and glial cells. An important glial cell type resembles the oligodendrocytes, which are building the myelin, a structure that is responsible for the fast, saltatory nerve conduction. Myelination was thought to take place only during development and should be completed in young adolescence. Interestingly, the cell type that gives raise to mature oligodendrocytes during development was identified some years ago also in the adult brain and spinal cord. These oligodendrocyte progenitor cells (OPCs) that in the adult they are also named NG2-glia (as they express the proteoglycan NG2) are the only proliferating cells in the adult brain outside the neurogenic niches. However, despite their high number (around 5% of the neural cells), their role and potential in the adult brain is still unknown. Although further functions have been speculated, NG2-glia have been shown to differentiate into mature myelinating oligodendrocytes in a region dependent manner and with recent transplantation experiments, we could show that NG2 -glia in distinct regions are intrinsically different, pointing to a heterogeneous population. This concept became more complex when we observed subsets amongst these cells localizing in the same cortical areas that transiently express the membrane G-protein coupled receptor GPR17. Genetic fate mapping by a newly generated BAC-transgenic mouse line, the GPR17-iCreERT2 line, indicated that this subpopulation of NG2-glia in the adult but also developing brain differentiates into mature oligodendrocytes with a slower rate.
Interestingly, after acute injury of the cortical grey matter, NG2-glia show a fast and very heterogeneous reaction. In vivo 2-photon imaging of NG2-glia reacting to stab wound injury provided new insights into the role of these cells upon lesion: the fast process orientation towards the injury site and their substantial proliferation imply a contribution to wound closure and scar formation.
Taken together our data indicate that adult NG2-glia comprise a very heterogeneous cell population with probably different functions and roles in the brain. This is of high importance as myelin repair in disease or after injury is not sufficient in the adult brain although NG2-glia exist.

Developmental changes in synaptic communication between axons and oligodendrocyte precursor cells in corpus callosum

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Many functions of the central and peripheral nervous system, including motor, sensory and cognitive functions, depend on the myelination of axons. In the central nervous system myelination is performed by oligodendrocytes. Oligodendrocytes arise from oligodendrocyte precursor cells (OPCs) that are widespread throughout the developing and adult CNS and represent 5-8% of the total cell population. Intriguingly, the majority if not all OPCs in grey and white matter areas of the neonatal and adult brain receive glutamatergic and/or GABAregic synaptic input from neurons. Very little information is currently available concerning functional role of synaptic signaling between neurons and OPCs, as well as concerning possible modifications in the properties of this signaling during animal development. We are using patch-clamp recordings, immunohistochemistry and confocal laser scanning microscopy to investigate functional changes at axon-OPC synapses in corpus callosum during postnatal mouse development and maturation, and to correlate these changes with morphological appearance of OPCs. Our findings indicate that as animals mature, OPCs change their morphology. In particular, OPCs occupy larger domains and show more complicated trees of processes in adult vs. neonatal mice. We are currently exploring whether larger trees of processes allow an OPC in adult mice to establish functional contacts with higher number of callosal axons. Furthermore, we are studying possible changes in quantal amplitude, properties of AMPA receptors and release probability at axon-OPC synapses. As it is known that upon animal maturation a switch from synaptic to extrasynaptic transmission occurs at GABAergic synapses between interneurons and OPCs in the barrel cortex, we are trying to find out whether similar changes take place in communication between axons and OPCs in corpus callosum.

Heterogeneity of astrocyte coverage of hippocampal synapses

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The efficiency of neuron-glia interactions is likely to depend on the distance between astrocyte processes and an individual synapse. Interestingly only ~ 60 % of glutamatergic synapses were reported to have astrocyte processes apposed to them in the hippocampus (Ventura and Harris, 1999). Identification of the factors that control variable coverage of synapses by astrocytes could be critical to fully understand neuron-glia interactions. Using electron microscopy (EM) and two-photon excitation (2PE) fluorescence microscopy we could reveal that coverage of thin spines by astrocyte processes is higher than that of thick spines with mushroom morphology. A powerful stimulus to initiate the transformation of thin into thick spines is induction of long-term potentiation of synaptic transmission (LTP). We therefore investigated if induction of LTP in vitro results in an astrocyte process retraction from synapses leading to reduced coverage of spines by astrocyte processes. A significant restructuring of fine astrocyte processes was found to be associated with LTP using diffraction-limited time lapse 2PE fluorescence microscopy of individual astrocytes in parallel to LTP induction at CA3-CA1 synapses. At the ultrastructural level (EM) this astrocyte restructuring was characterized by a reduced abundance of astrocyte processes at synapses after LTP. Because astrocytes take up most of the synaptically released glutamate astrocyte process retraction after LTP may increase glutamate escape from active synapses. Indeed, dual pathway stimulation experiments demonstrated a significant increase of glutamate spill-over between pathways after LTP induction. Together these observations suggest that variable coverage of synapses by astrocyte processes shapes glutamatergic synaptic transmission and can result from synaptic plasticity.

(collaboration of Henneberger lab Bonn, Rusakov lab London, Stewart lab Milton Keynes, Nägerl lab Bordeaux, Oliet lab Bordeaux)

INEX – A computational model to simulate spatial neuronal-astrocytic activity

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Current state of the art *in vitro* neuronal systems apply cultured neuronal networks on multielectrode arrays (MEAs). With MEAs, neuronal cell networks also derived from human pluripotent stem cells (hPSC-NN) can be recorded (Ylä-Outinen et al., Front Neuroengin. 2010). *In vitro* human neuronal cultures provide an excellent tool for basic research, e.g. on development of human neuronal systems, but as well warranted methods for toxicology and drug studies. *In silico* modeling of such neuronal networks will enhance our understanding of the neuronal systems and their development.
In the past, we built a phenomenological model called INEX (INhibitory-EXcitatory) that was used to simulate neuronal activity recorded from frontal cortex tissue of embryonic mice (Lenk, Springer LNCS 2011) and maturating hPSC-NNs (unpublished data). In this paper, we provide a brief overview on *in silico* modeling of mature hPSC-NNs, network topology and the interplay between neurons and astrocytes.

The INEX model is based on inhomogeneous Poisson processes used to simulate neurons which are active without external input or stimulus resembling our in vitro MEA experiments. Based on the INEX framework we present three levels of elaboration: simulation of hPSC-NNs, simulation of network topology and INEX with astrocytes.
Firstly, we describe the simulation results of a network with 1,000 neurons and 10% connectivity but without any spatial topology. For validation, we calculated four statistical features using the burst analysis tool by Kapucu et al. (Front Comput Neurosci. 2012) for both the simulated and the experimental data.
Secondly, we examine the spatial structure of neuronal networks (example in Fig.). Therefore, we built computational model networks with different connection lengths and analyzed, how longer range connections affect the neuronal network behavior.
Thirdly, we look at the interplay between neurons and astrocytes (Fig.). We show how astrocytic effect to single synapses affects the whole network behavior. A version of Tsodyks-Markram presynaptic model and astrocytic effects as described by De Pittá et al. (PLoS Comput. Biol. 2011) was applied to the INEX model with spatial topology.

The simulation results showed that we can simulate typical spike and burst patterns as known from MEA experiments with hPSC-NNs and in particular synchronous bursts. The validation showed that all calculated features of the INEX data are within the lower and upper quartile of the MEA data.
The effect of spatial topology on INEX was similar in 2D and 3D. The burst duration and number of spikes in burst increased remarkably and almost linearly as longer range connections were included in the network. However, longer range connections do not greatly affect the number of spikes or bursts in the network.
Our results on networks with astrocytes show that networks with high activity show reduced activity according to astrocytic glutamate releases to single synapses. As astrocytic glutamate is taken up, the activity increases. As a result additional releases by astrocytes reduce the activity again. This leads to periodic bursting of the network.

To conclude, our results show that even though INEX is a simple framework it offers us means to simulate the neuronal activity of real human neuronal network on MEA. It is an effective computational

model to simulate neuronal activity that can also include realistic concepts of neuronal network topology and astrocytic activity.
(This research has been supported by the 3DNeuroN project in the EU's 7th FP, FET, grant agreement n °296590.)

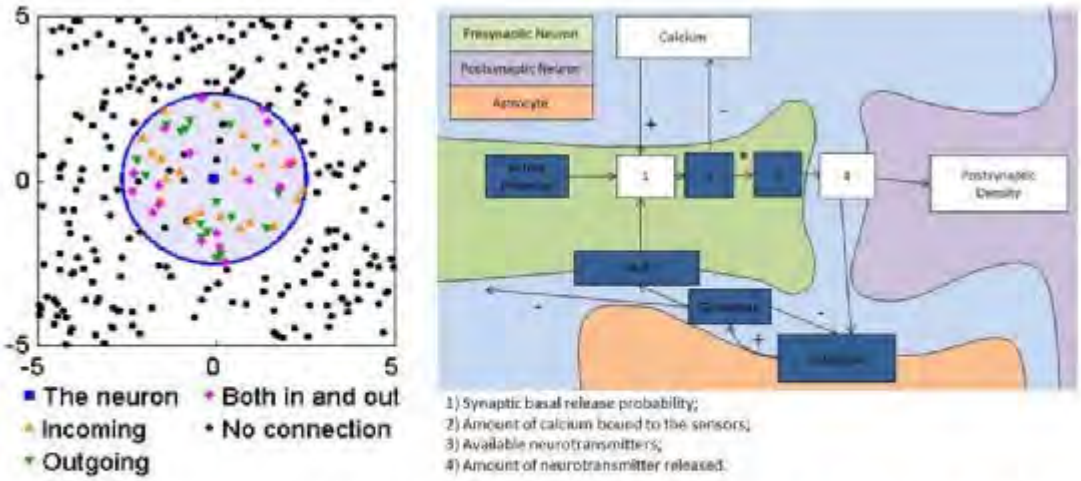

Figure. Left: Demonstration of the spatial topology added to the INEX model. **Right:** Tsodyks-Markram presynaptic model and De Pittà astrocyte effect together with INEX.

Uptake and toxicity of metal oxide nanoparticles in glial cells

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Metal oxide nanoparticles (NPs) have gained considerable interest during the last decade due to their exciting properties as antibacterial agents, for consumer products as well as for technical applications and diagnostics. Due to their frequent use, the likelyhood that brain cells will encounter such NPs has strongly increased. To investigate potential adverse effects of NPs on brain cells, we have studied the biocompatibility, the uptake and the metabolism of iron oxide NPs (IONPs) and copper oxide NPs (CuONPs) on cultured primary brain cells. Cultured astrocytes and microglial cells accumulated IONPs efficiently in a time -, concentration - and temperature -dependent manner. However, despite of an accumulation of large amounts of iron in IONP-treated astrocytes, the viability of these cells was not compromised. In contrast, IONPs in higher concentrations were toxic for microglial cells, most likely due to the rapid uptake and transfer of the IONPs into the lysosomal compartment which facilitates the liberation of iron ions from the accumulated particles. The presence of fetal calf serum in the incubation medium strongly decreased the rate of IONP uptake into brain cells. While some inhibitors of endocytotic pathways lowered IONP accumulation in serum-containing medium, these inhibitors did not affect the rapid IONP accumulation in serum-free conditions. Astrocytes that had been exposed to IONPs for only 4 h remained viable for up to 7 days, hardly released any iron during this incubation period and showed a transient appearance of reactive oxygen species and a strong upregulation of the iron storage protein ferritin. These data demonstrate that cultured brain astrocytes deal well even with large amounts of iron that are accumulated as IONPs. In contrast, a treatment of cultured astrocytes with CuONPs caused severe oxidative stress-mediated toxicity that was prevented by copper chelators, suggesting that rapid liberation of copper from accumulated CuONPs is involved in the observed toxicity of these NPs to astrocytes. These data demonstrate that astrocytes and microglial cells differ in their vulnerability to IONPs and that NP toxicity on a given type of brain cells strongly depends on the type of NPs applied.

Heterogeneity in the response of astrocytes following CNS injury

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Reactive gliosis is a widespread reaction of glial cells to pathological processes in brain parenchyma. Despite the fact that the activation of microglial cells and NG2-glia accompanies a variety of the CNS pathologies, astrogliosis is the most common part of reactive gliosis resulting in phenotypic and functional changes in astrocytes (Götz and Sirko, 2013; Dimou and Götz, 2014). The nature of astrocyte reactions to brain damage is however not stereotypic and exhibits a finely gradated continuum of heterogeneity at multiple levels, including cell morphology and function (Dimou and Götz, 2014; Burda and Sofroniew, 2014; Anderson et al., 2014). Although it is currently unclear how individual astrocytes shape their distinct properties during reaction to the pathological stimuli, observations of astroglial response to the brain injury in real time and within their in vivo environment revealed a heterogeneous behavior of reactive astrocytes and provided first evidence that subset of astrocytes retaining their initial morphology, others directing their processes toward the lesion, and a distinct subset located at juxtavascular sites proliferating (Bardehle et al., 2013). Since parenchymal astrocytes in healthy adult mammalian brain are rather postmitotic (Buffo et al., 2008), the occurrence of some proliferating reactive astrocytes suggests that reactive astrocytes have the ability to de-differentiate and re-enter the cell cycle upon injury, and hence,acquire restrictive stem cell potential. Most important, however, the extent of the proliferative response in reactive astrocytes correlates with the emergence of stem cell capacity therein (Sirko et al., 2013). In order to understand the molecular basis governing astrocyte proliferation at the injury site, we isolate reactive astrocytes from the stab wound injured cerebral cortex gray matter at the peak of their proliferative and stem cell response, and compared their genome-wide expression with the expression profile of adult neural stem cells (NSCs) from the subependymal zone or astrocytes from the healthy brain (Beckervordersandforth et al., 2010). Our comparative analysis revealed a novel regulator of reactive astrocyte proliferation in vivo as well as their stem cell potential measured as neurosphere formation in vitro, and therefore provided new insights into the mechanisms leading to induction of plasticity in astrocytes during their transition from a quiescent to reactive state after CNS injury.

Role of protein translation and protein turnover for astrocyte heterogeneity in the hippocampus, striatum and prefrontal cortex

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Recent work from several groups suggests that astrocytes directly influence the formation, function, and stability of synapses, thereby sculpting axonal and dendritic morphology and balancing the activity levels of neurons. The processes, however, by which this intricate neuron-astroglial network assembles, and the mechanisms underlying the dynamic and refined properties of the mature system, e.g. maintenance of individual specialized circuits or the integration of excitatory and inhibitory neurotransmission, are largely unclear. Astroglial heterogeneity and its morphological, physiological and molecular consequences within and across different brain regions such as the hippocampus or somatosensory cortex might be one possibility of how these processes might be implemented. From our previous studies we know that astrocytes possess the capacity to dynamically react to neurotrophin-induced changes with a global increase in protein synthesis. These newly synthesized proteins can be found in the soma but also in the distal parts of the elaborate astroglial processes close to synaptic contacts. We therefore aim to investigate astroglial heterogeneity on the molecular level by performing cell-selective proteomic profiling of astrocytes in organotypic cultures and in living (transgenic) mice using the recently developed techniques BONCAT, FUNCAT and GINCAT in combination with protein biochemistry, molecular biology and fluorescent in situ hybridization. Especially with GINCAT and the use of the mutant LtoG-MetRS for the cell-selective incorporation of non-canonical amino acids it is possible to distinguish for the first time between the protein population of two different, but functionally interdependent cell-types, and ultimately to obtain a comprehensive analysis of the individual neuronal and astroglial proteomes during development and synaptic plasticity within and across different brain regions.

Symposium

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Keeping it together before and after nerve cord injury: how single neurons help to coordinate locomotor oscillators

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The beauty of an animal in motion reflects the neural choreography needed to coordinate its multiple moving parts. The rhythmic movements of individual body segments or appendages is typically controlled by central pattern generators (CPGs) (i.e., neural oscillators), which require a relatively high degree of coordination. To understand the neural underpinnings of how multiple oscillators are coordinated and how different forms of locomotion are selected, we focused on the brain of the leech, *Hirudo verbana*. We discovered that a single long-distance projecting neuron, called R3b-1, was sufficient to activate either overt crawling or swimming in nearly-intact leeches if their bodies were placed in either shallow or high fluid environments respectively. When R3b-1 was silenced with negative current or the brain was removed by transecting the nerve cord, crawl-specific activity (but not swimming) was lost. We next aimed to determine the contribution of this cell for intersegmental crawl coordination, which involves maintaining a caudally-directed movement of body segments during both the elongation and contraction phases comprising crawling.

Previously, we showed that each and every ganglion of the leech (21 ganglia total) has its own CPG for crawling. We also discovered that DA was a powerful activator of crawling, even in an isolated single ganglion. By activating only a single crawl CPG, with DA, we were able to determine the influence of one crawl oscillator over its neighboring ones, and establish how they might be coupled. We found that although one crawl oscillator could 'drive' its neighbor(s) to crawl, the essential crawl-specific phase relationships were never achieved. These phase relationships could only be achieved when R3b-1 was active. Our data support the idea that R3b-1 is a DA-sensitive interneuron vital for both the activation and coordination of the iterated crawl oscillators, providing the first example of a locomotor command neuron that also controls intersegmental coordination.

As predicted, when the cephalic ganglion and R3b-1 were removed, crawling behavior was lost. Curiously, coordinated crawling returned after 1-2 weeks; a gain of function independent of nerve cord regeneration. Mechanisms underlying the reestablishment of normal phase relationships appear to involve a homeostatic process of reorganization within the ganglion (and its crawl CPG) located directly below the site of nerve cord injury. Our working hypothesis is that this remodeled ganglion becomes the 'lead' crawl CPG and is able to set up the intersegmental phase relationships across the 21 crawl oscillators. Our experiments to date indicate that dopaminergic-related changes occur maximally in the new lead ganglion. When this ganglion is removed, newly acquired coordination is abolished but regained over a similar time course to the first injury. Furthermore, crawl recovery is hypothesized to involve a novel integration of proprioceptive information, as physical therapy involving activation of proprioceptive inputs shortened the time of recovery. Our results may help to shed light on the multiple elements contributing to oscillator coupling during locomotion in healthy animals, and showcase the impressive biological strategies animals can use to 'keep it all together' after injury.

Linking respiration to locomotion

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The basic muscle synergies responsible for propelling the body forward during locomotion are generated by Central Pattern Generators (CPGs) in the spinal cord. Respiration, on the other hand, is generated by CPGs in the brainstem. It is well documented that respiration increases during movement such as locomotion and exercise to compensate for an increased energy demand. A number of feedback mechanisms have been proposed to underlie these respiratory frequency increases during movement. Feed-forward mechanisms have also been proposed. Stimulation of the brain areas involved in the initiation of locomotion was shown to increase respiration. The increases were maintained in paralyzed animals. Moreover, in humans, respiratory increases were shown to occur before movement or when subjects simulated exercise mentally. This suggests that feedback from movement is not necessary and that connections within the central nervous system (CNS) are likely to be responsible for the respiratory increases. We used the lamprey model to identify the central neural structures underlying the respiratory increases in relation to movement. We confirmed that central connections in the brainstem play a crucial role in the respiratory adjustments during movement. We found a population of brainstem neurons that link a locomotor area and the respiratory rhythm-generating area. We first showed that, as with other animal species, intact lampreys displayed respiratory increases in association with locomotion. Changes occured even before swimming began indicating that feed-forward mechanisms were at play. We then isolated the brainstem and upper spinal cord in vitro, thus removing feedback from the muscles, but keeping the neural networks controlling locomotion and respiration intact. Stimulation of the mesencephalic locomotor region (MLR), a brainstem region known to control locomotion, elicited marked increases in respiration. Furthermore, we found that removing the spinal cord and the lower brainstem (corresponding to the medulla oblongata of higher vertebrates) did not abolish the respiratory increases, suggesting that the connections responsible for increasing respiration are located more rostrally in the brainstem, in a region corresponding to the pons and lower midbrain of higher vertebrates. We recorded from a specific group of neurons within the MLR and found that these neurons were active in parallel to the brainstem networks that control locomotion and they connected directly to the brainstem areas that generate breathing movements. Stimulation of the MLR produced a large glutamatergic excitation in individual neurons located in the respiratory rhythm-generating area these cells. These results show, at the single-cell level, a connection between a brain center controlling locomotion and another generating the respiratory rhythm. Therefore, the neural commands that control movements are accompanied by a parallel command sent to the respiratory rhythm-generating centers via a specific neural substrate in the brainstem. Such a direct connection between locomotor and respiratory control centers could provide an advantage in terms of the speed and precision of the respiratory changes related to movement.

Mechanisms of coordination in distributed neural circuits: From encoding through decoding to integration of coordinating information

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Two fundamental goals of neuroscience are to explain, in terms of the organization of their cellular components, how nervous systems work and how they generate overt behavior. Our progress toward these goals has often come from thorough study of specific nervous systems selected because their orderly cellular organization and favorable anatomy allow us to do critical and repeatable experiments or because under experimental conditions they continue to express behaviorally-related activity. Cellular explanations of properties of particular systems have led to insights and the discovery of general principles that apply widely across phyla, even if the links between these systems and overt behaviors sometimes remain unclear. One class of behaviors for which we have outlines of cellular explanations is locomotion.

To understand the coordination of neuronal microcircuits, we study a locomotor system of crayfish: the swimmeret system. The central nervous system of crustaceans consists of a chain of distributed ganglia, each innervating separately one body. The swimmerets of the crayfish occur in pairs from the second to the fifth abdominal segment, with one limb on each side of the abdomen. The central nervous system produces the rhythmic motor pattern on its own, which drives the swimmeret movement in the intact animal as well as in the isolated nerve cord preparation.

Despite the fact that each swimmeret is activated by its own central pattern generator (CPG) in every segment, the combined activity of the four ipsilateral neuronal microcircuits is tightly coordinated in a metachronal wave. The microcircuit in the most posterior segment starts each cycle and the anterior ones follow with a phase lag of a quarter of a cycle. The nervous system is able to keep a stable phase difference between neighboring circuits despite ten -fold changes in the frequency of the rhythm. Coordination is regulated by a simple coordinating network, which connects all microcircuits to each other. In each segment, this coordinating network is composed of three neurons: one Ascending Coordinating Neuron (ASC_E), one Descending Coordinating Neuron (DSC) and one Commissural Interneuron (ComInt1).

ASC_E and DSC are spiking coordinating neurons in each hemiganglion; they carry information about timing, duration, and strength of the motor output of their own segment encoded by burst of spikes to all neighboring segments. They receive the same information from the CPG as the motor neurons which allows them to encode precisely the status of the motor output (Smarandache-Wellmann and Grätsch, 2014).

The coordinating information from ASC_E and DSC is decoded by one nonspiking ComInt1 in each hemisegment. There is a specific gradient of synaptic strength in this system of matching encoders (ASC_E and DSC) and decoders (ComInt1), so that the information from the neighboring posterior segment elicits larger excitatory postsynaptic potentials in ComInt1 than from anterior or more distant segments. This coordinating information is decoded and integrated in ComInt1 and transferred via an electrical synapse to only one of the nonspiking interneurons in the microcircuit's CPG kernel (Smarandache et al., 2009; Smaranrandache-Wellmann et al., 2014). To our knowledge, the swimmeret system is the only circuit of neuronal oscillators where we understand coordination on the cellular level.

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Coordinating central pattern generators: Two neurons, two strategies

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Neural oscillators coordinated to produce meaningful behavior exist in a large variety of animals, ranging from swimming and flying to walking and even brain oscillations. We use the crayfish swimmeret system as a model to study coordination of neural oscillators because the necessary neurons for coordinated motor output are identified on the cellular level.
Movement of the four pairs of swimmerets progresses in a metachronal wave from posterior to anterior with a stable phase lag of 25% between segments, independent of movement frequency. Each swimmeret is controlled by one central pattern generator (CPG) located in each hemiganglion. Exactly three neurons in each hemiganglion are necessary and sufficient to coordinate the CPGs across segments: One ascending (ASC_E) and one descending (DSC) coordinating neuron, as well as one non-spiking Commissural Interneuron 1 (ComInt 1). ASC_E and DSC encode information about the activity state of their home ganglion as bursts of action potentials and send it to the ComInt 1s of the neighboring ganglia, where the information, arriving with a gradient of synaptic strength, is decoded and integrated into the CPG.

Now that the connections in the coordinating microcircuit are identified we wanted to answer further questions concerning the coordinating neurons: (1) So far only inhibitory connections from the CPG to the coordinating neurons have been identified. But are there additional connections and how do those participate in shaping the bursts of action potentials ? (2) *In vivo*, swimmeret movement can change between different speeds. In experimental conditions we can set different frequencies of fictive locomotion by altering the excitation level. We hypothesize that burst properties are changed at different excitation levels in order to maintain the phase lag between segments. (3) What are the transmitters used by the spiking coordinating neurons? To answer the first two questions we recorded ASC_E and DSC intracellularly in an isolated nervous system preparation that was perfused with different concentrations of cholinergic agonists to set the excitation level. For the third task we used MALDI-TOF mass spectrometry after labeling the neurons intracellularly.

(1) Rhythm onset and termination revealed that the bursts of action potentials were shaped by different mechanisms in the coordinating neurons. DSC bursts were driven by phasic inhibition only; ASC_E seemed to receive additional excitatory input.
(2) We could prove that cholinergic agonists acted both directly and indirectly on the number of open ion channels. ASC_E's input resistance was decreasing with increasing excitation level when all synapses were functioning. If ASC_E was isolated from the system by blocking chemical synapses its input resistance was increasing. DSC seemed to have a sweet spot of excitation with lowest input resistances at a medium excitation level.
(3) The fast postsynaptic potentials elicited by the coordinating neurons in ComInt 1 suggest a transmitter with low molecular weight. We could identify acetylcholine as putative transmitter for the coordinating neurons in both the soma and area of dendritic arborization.

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Attention dependent routing by synchrony and dynamic coordination of neuronal processing in monkey's visual cortex

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The strong convergence of afferent synaptic inputs to cortical neurons results in an increase of receptive field (RF) size along visual cortical pathways. For natural scenes there is typically more than one stimulus within larger RFs and neuronal signals representing different stimuli arrive at the same neuron. This raises the question how neurons handle such arbitrary mixtures of signals related to different stimuli, each of which implying different processing and a different response. Selective attention is known to resolve this ambiguity: neurons typically respond as if only the attended stimulus is present within their RF. However, the neuronal mechanism underlying this remarkable capability indicating that processing can be selectively switched between different stimuli that are present simultaneously is strongly debated and not yet understood. Current models of selective attention attribute the observed selective response either to a selective modulation of the neurons input gain for dynamically selected subsets of the afferent inputs or to a modulation of the neurons output gain depending on the neurons feature selectivity and features of the attended stimulus.

We tested the hypothesis that attention dependent selective processing relies on dynamic interaction between oscillating neurons which selectively enhance the input gain of those subset of afferents that convey signals representing the attended stimulus. For this purpose we trained monkeys to a highly demanding shape tracking paradigm which requires covert attention to one of two objects which change their shape continuously and to identify the reoccurrence of the cued objects initial shape. Recordings were taken from areas V1 and V4.

We found that local γ-band activity in area V4 is strongly enhanced if attention is directed to the stimulus inside the RF of a local population of V4 neurons as compared to attention for a stimulus outside this RF. The effect is highly correlated to psychophysical properties of attention and actual behavior. The attention dependent increase in synchrony strengthens the common impact of a local population's output signals at down-stream neurons and corresponds to a selective increase of the input gain for a specific part of the afferents to these down-stream neurons. Further on we reduced the stimulus size to place both stimuli within a single V4 RF and recorded simultaneously from V1 and V4. The results show that four to eight times more synchronization occurred between V4 neurons and the subset of their V1 inputs representing the attended stimulus as compared to those representing the non-attended stimulus. This strong and highly selective attention dependent modulation of inter-areal synchronization is well in line with a selective modulation of the input gain and models of routing by synchrony.

In contrast to models of selective attention based on output gain modulation, selective modulation of input gain allows for selective processing even if both stimuli do not differ systematically in their features. Recordings in V4 show that independent flicker signals imposed on both stimuli are selectively processed for the attended stimulus.

In summary these results suggest that routing by synchrony can explain attention dependent processing and neuronal circuit configuration due to input gain modulations for dynamically selected subsets of neurons afferent synaptic input.

The role of neural synchrony and oscillations in feature-based attention in the primary visual cortex of the macaque monkey

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Understanding the neural mechanism for coordination of brain networks is an important unmet purpose in neuroscience. How do distributed populations of neurons coordinate their activity across a diversity of spatial and temporal dimensions? Unfortunately a complete understanding of dynamic coordination mechanism remains elusive but evidence suggests that oscillations may have a critical role in this process. Several groups have suggested that brain rhythms have a key role in the dynamic coordination of functional brain networks. Attention, which is a mechanism that deals with information overloaded by selecting a stimulus over other existing stimuli in the visual scene, is one of those functions. Columnar organization of neurons in Primary Visual Cortex (V1) has an important role in this process. Generally, each column of V1 has a preferred orientation and direction. There is a hypothesis that columns with the same preferred direction are connected and they enhance each other. Our main purpose of this study is to investigate the connectivity of columns with preferred direction by neural synchrony and oscillations across feature based attention.

Data were recorded from an awake behaving macaque monkey. The monkey was trained to do a feature based attention task. After the monkey fixated at a central point on the screen, a cue which was a static stimulus appeared. Then, after a short delay (270 ms), the stimuli were presented on the screen for 1.33-3 s. The stimuli were two moving random dot patterns with 180 degree direction difference; one of them was inside of the receptive field of the recorded neurons and the other outside of the receptive fields. In each session the direction of movement was varied and there were totally 12 different directions. The monkey should respond to a direction change of the cued stimulus while ignore the direction changes in the un-cued stimulus. A 96-electrode Utah array was implanted in the primary visual cortex and it was used for recoding. The stimulus was selected large enough to include receptive fields of all the electrodes.

For investigating our purpose, both local field potential (LFP) signals and spiking activities were analyzed. First, we selected the channels which were well tuned to the stimulus motion direction. Second, we considered the conditions in which anti-preferred stimulus was presented in the receptive field while either preferred or anti-preferred stimulus was presented outside of the receptive field and the monkey should attend to the stimulus which was outside of RF. If the stimulus outside of RF has preferred direction, we call it attend to preferred otherwise we refer to it as attend to anti-preferred. Finally, we analyzed LFP and spiking activity in these two conditions. There was a significant difference in gamma band activity of LFP between attend to preferred and attend to anti-preferred conditions. Importantly, spiking activity of the simultaneously recorded channels with the same preferred direction were analyzed. The population results show that feature attention causes significant difference in spike-coherency between channels with the same preferred direction around 60 Hz. Besides, coherence index which is the difference of spike-coherency in the mentioned conditions normalized by their sum significantly differs from zero as shown in fig1. This result shows that synchrony has a role in brain activity

coordination and functions such as feature based attention.

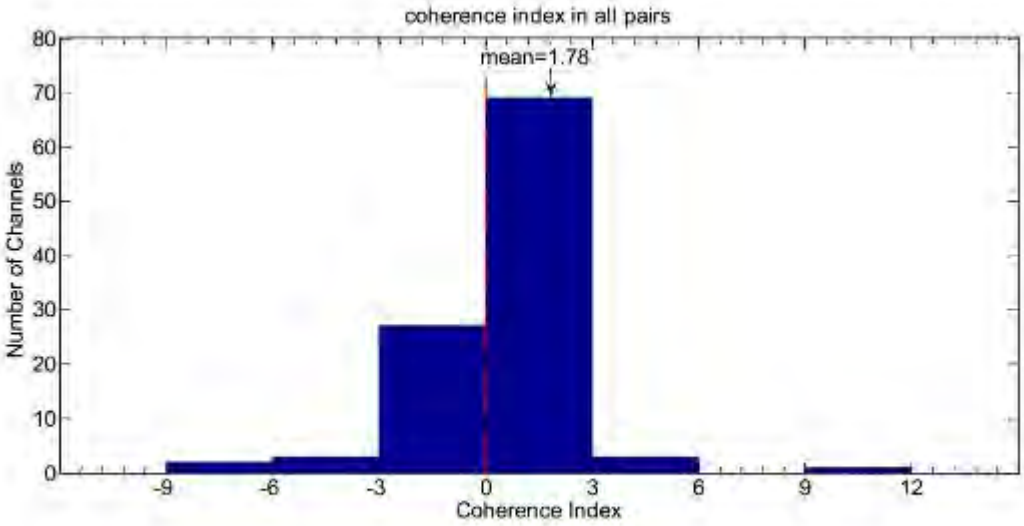

Figure 1

Symposium

S30: Adaptation and plasticity in a distorted sense of hearing during tinnitus and hyperacusis

S30-1 Noise-induced hearing loss and the development of tinnitus in Mongolian gerbils and rats
Manuela Nowotny

S30-2 Interactions of arousal and traumatic stress with tinnitus related hearing disorders in animal models
Lukas Rüttiger, Ksenia Varakina, Dorit Möhrle, Dan Bing, Marlies Knipper

S30-3 Tinnitus and Hidden Hearing Loss
Roland Schaette, Lara Li Hesse, Warren Bakay, Jennifer Linden, David McAlpine

S30-4 Abnormal sound processing in tinnitus patients suggests thalamic dysfunction: result from fMRI
Pim Van Dijk

Noise-induced hearing loss and the development of tinnitus in Mongolian gerbils and rats

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One central theme of our work is the development of a reliable tinnitus model to identify key factors for tinnitus induction. Tinnitus in humans is often related to acoustic overstimulation, and co-occurs with (temporary) hearing loss. Characteristics of the over-stimulating noise critical for the induction of tinnitus include frequency content and sound pressure level (SPL) in addition to duration of application and frequency bandwidth. We present here results from our study of the relationship between hearing loss and the duration of acoustic overstimulation for up to 2 h. Specifically, we explored the correlation between the bandwidth of the noise and the risk of tinnitus development using Mongolian gerbils and rats as model organisms.

To verify physiological conditions in the hearing organs, we tested the hearing ability of Mongolian gerbils (Meriones unguiculatus) and laboratory rats (Rattus norvegicus) by means of auditory brainstem response (ABRs) measurements from 2 to 20 kHz before, during and after noise overstimulation. The acoustic stimulus was composed of bandwidths of 0.5 and 1 oct around a center frequency of 8 kHz (105 and 115 dB SPL). To document the development of tinnitus in our animals we used behavioral tests in the same frequency range.

Acoustic overstimulation led in all our animals to hearing impairment, as indicated by a significant threshold shift at frequencies above 6 kHz. Exposing animals longer than 30 min (rats) or 60 min (gerbils) shows no further effect on the threshold values. Additional deterioration of the hearing was achieved only through increasing the sound pressure level by 10 dB (115 dB SPL). Regarding the bandwidth of the applied noise band, we could show in another experimental trail that noise with smaller bandwidths lead more often to permanent threshold shifts several weeks after the overstimulation than broad band signals. However, the risk to develop tinnitus was lower in the narrowband group. In contrast, repeated acoustic overstimulation increases the risk for tinnitus development only in the narrowband noise group. Although the center frequency of the applied noise was at 8 kHz in both groups, the tinnitus frequency developed differently. While the noise-induced threshold shift after the acoustic overstimulation was not significantly different, the narrow-band noise signal leads to a tinnitus perception at 10 to 12 kHz, the broad-band noise results in a tinnitus perception at 8-10 and 16-18 kHz.

Conclusion

The frequency of the tinnitus perception is related to the bandwidth of the stimulating noise. An overstimulation with a narrow-bandwidth noise leads to a tinnitus perception of frequencies above the center frequency of the stimulus. In contrast, broadband noise stimuli lead to a twofold tinnitus perception, one at the center frequency of the noise stimulus and another one octave above.

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Interactions of arousal and traumatic stress with tinnitus related hearing disorders in animal models

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The loss of auditory sensation is one of the substantial challenges in aging populations since the loss of perception may socially isolate affected people. Beside the reduced hearing sensitivity and the altered sound processing of temporally and spatially modulated auditory stimuli leading to limited speech understanding, also abnormal perception of sound or phantom perceptions, like hyperacusis and tinnitus may arise [1,2].

Inner ear physiology and hearing sensation can be lost as a consequence of even moderate auditory overexposure. Recent studies demonstrated a close relation between aging, loss of inner hair cell synaptic contacts, and auditory brainstem response (ABR) wave amplitudes in the mouse [3] and in the rat (own studies) even after full recovery of normal audiometric threshold sensitivity following a transient hearing loss after mild noise exposure. Even a mild noise exposure can lead to a loss of cochlear function at supra -threshold sensation levels and a loss of IHC synaptic contacts in an age related manner. By this, auditory thresholds are increased and the auditory response range is reduced.

We studied the age and trauma related loss of synaptic structures, afferent synaptic contacts, and auditory fibers by the auditory evoked brainstem response (ABR) and the otoacoustic emissions (DPOAE) over a longer life span in the aging rat and the Mongolian gerbil. Furthermore, the hearing sensation in rats was studied by a behavioral approach [4] that tested for hyperacusis and tinnitus sensation.
A differential vulnerability of a particular subgroup of auditory fibers for age and sensitivity for acoustically induced insult became evident that was correlated with differences in the brain's capacity to compensate sensory deprivation, observations that are discussed in the context of changing risk for hyperacusis and tinnitus.

In the rat, results from behavior studies on young and aged rats showed that the brain may compensate for the peripheral loss [5], preventing tinnitus or hyperacusis. In the gerbil, age related hearing loss may be influenced by cognitively demanding housing conditions (enriched environment).
In summary, the age related decline of hearing function observed in the animal model was correlated with morphological specifications of hair cell molecular phenotype. Behavior studies can test for a correlation of the observed functional and structural changes with tinnitus and hyperacusis.

After noise exposure, the activation of a protective cyclic guanosine monophosphate (cGMP) dependent molecular cascade has been described in the rat and mouse [6]. We therefore tested the protective potential of cGMP increase by pharmacological stimulation of soluble guanylyl cyclase (sGC) activation for age and noise induced progression of hearing deficits. As an alternative approach, we studied the hearing of animals held in tightened environmental conditions (enriched environment), promoting the activation of stress related molecular cascades.

The data show that the otoprotective effect of the cGMP signalling cascade after noise induced damage of the ear needs to be carefully evaluated in the context of aging and environmental demands.

This work was supported by Deutsche Forschungsgemeinschaft (Forschergruppe FOR-2060) and Action on Hearing Loss (RNID G54-Ru)

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Tinnitus and Hidden Hearing Loss

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Several lines of evidence suggest that the occurrence of subjective tinnitus is linked to cochlear damage, as up to 90% of tinnitus patients have impaired hearing, and animal studies have shown that the induction of hearing loss can lead to behavioural signs of tinnitus. On the other hand, about 10% of the tinnitus patients present with clinically normal hearing thresholds, i.e. with audiometric thresholds less than 20 dB HL for frequencies up to 8 kHz (Barnea et al., 1990; Sanchez et al., 2005), which has been interpreted as evidence that hearing loss might not be required for tinnitus to occur. However, a normal audiogram does not necessarily indicate absence of cochlear damage. It has recently been shown in mice that a mild acoustic trauma, which only caused a temporary shift in hearing thresholds, could lead to permanent deafferentation of some 50-60% of the auditory nerve (AN) fibers in the high-frequency region of the cochlea (Kujawa and Liberman, 2009). AN fibres with high response thresholds have been shown to be particularly vulnerable to this kind of damage (Furman et al., 2013).

Based on the results from animal studies, we have investigated whether such “hidden hearing loss” might also be found in tinnitus subjects with normal audiograms. When compared to a control group matched for age, sex, and hearing thresholds, the auditory brainstem responses (ABRs) showed a significant reduction of the wave I potential (generated by the AN fibres) in the tinnitus group, indicating deafferentation. On the other hand, the amplitudes of wave V, which is generated in the central auditory system, were not significantly different between tinnitus and control group. This investigation has provided physiological evidence for hidden hearing loss in tinnitus subjects with normal audiograms, and for a compensatory increase in gain in the central auditory system that might underlie the generation of tinnitus (Schaette and McAlpine, 2011).

To study putative mechanisms for the development of tinnitus after hidden hearing loss, we have used a computational model. The model demonstrates that activity stabilization through homeostatic plasticity can account for the development of increased spontaneous firing rates, a putative neural correlate of tinnitus, in the central auditory system after AN fibre deafferentation. The same mechanism can also explain the development of tinnitus after hearing threshold increase.

We have then tested the model prediction that hidden hearing loss leads to the development of increased spontaneous firing rates in a mouse model. Mice were exposed to octave-band noise (8-16 kHz) at 100 dB SPL for 2 hours under anaesthesia. Hidden hearing loss was confirmed through ABR recordings and cochlear histology. Recordings from the inferior colliculus 4 weeks after noise exposure showed significantly increased spontaneous firing rates, demonstrating that hidden hearing loss can indeed trigger the development of a putative neuronal correlate of tinnitus.

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Abnormal sound processing in tinnitus patients suggests thalamic dysfunction: result from fMRI

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Tinnitus is typically related to peripheral hearing loss. Abnormal adaptive mechanisms in the brain presumably lead to abnormal patterns of neural activity, which may result in tinnitus. It is unclear, why some people with hearing loss develop tinnitus, while others with similar hearing loss do not develop tinnitus.

We explored differences in brain activity between subjects with and without tinnitus in two studies with a similar design. In the first study, the subjects had near normal hearing thresholds. This study included 16 subject with and 16 subjects without tinnitus. In the second study, the subjects had a moderate sensorineural hearing impairment, where 34 subjects experienced tinnitus, and 19 did not report tinnitus. In both studies, functional MRI scans were obtained while the subjects listened to broad-band sound stimuli of varying intensity and lateralization (left/right ear). This was expected to produce responses of varying amplitude in auditory brain areas, which allows for an analysis of functional connectivity. A sparse imaging paradigm was used to reduce the effect of scanner noise. In both studies, the principle finding is that in subjects with tinnitus, the correlation between the activity in the auditory cortex and the brainstem is reduced in comparison to subjects without tinnitus.

These connectivity results illustrate the abnormal sound processing in tinnitus patients. The reduced connectivity may be related to reduced activity in the thalamus in tinnitus patients with gaze-evoked tinnitus (Van Gendt et al., 2012). Abnormal function of the thalamus would presumably also result in abnormal connectivity across the brainstem -thalamus-cortex pathway, as reported here. Abnormal thalamic function is consistent with tinnitus models, based on thalamo-cortical dysrythmia (Llinas et al., 1999) or abnormal thalamic gating (Leaver et al., 2011). Thalamic dysfunction has also been found in patients with neuropathic pain (reviewed in Garcia -Larrea et al., 2013), which suggests common mechanisms across pain and tinnitus.

Symposium

S31: Integrative study of the social insect brain - combining neuroethological and computational approaches

S31-1 Exploratory learning in bees, and the search for neural correlates.
Randolf Menzel, Aron Dür, Benjamin Paffhausen, Jacqueline Degen, Uwe Greggers, Nanxiang Jin

S31-2 From Insect Neuroethology to Neurotechnology: Computations in Small Brains.
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Exploratory learning in bees, and the search for neural correlates.

Randolf Menzel[1], Aron Dür[1], Benjamin Paffhausen[1], Jacqueline Degen[1], Uwe Greggers[1], Nanxiang Jin[1]

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Social bees (honeybees, bumble bees) navigate between the hive and foraging places after exploring the environment. We examined under natural conditions and in the laboratory how bees acquire the necessary information, and searched for potential neural correlates by recording from multiple mushroom body extrinsic neurons. The exploratory orientation flights of honeybees in a natural environment were tracked with harmonic radar. In the lab, walking bees (bumble bees or honeybees) were video tracked in an arena setting or on a wax comb within a functional mini-colony. Natural exploration of honeybees consists of short- and long-range orientation flights. Short-range flights are likely to be related to learning of the specific features of the immediate surroundings of the hive, long-range flights to learning of the ground structure as indicated by their guidance by parallel landscape structures. During sequential long-range orientation flights honeybees explore novel sectors of the terrain. Learning is documented by the finding that the time bees need to return back to the hive after a displacement is positively correlated with the angular deviation to the orientation flight. Navigation in a laboratory setting has been studied in an arena consisting of a local cue and extra maze landmarks. Bees use both cues for navigation. Mushroom body extrinsic neurons indicate exploration dependent activity depending on the location and the walking behaviour of the recorded animal. Neural correlates of exploration and social interactions were studied in mushroom body neurons (A1/A2) of animals in a mini-colony. We found that spontaneous neural activity is lower in these animals as compared to similar neurons recorded in harnessed bees. None of the units was selectively active for any of the social interactions, the location on the comb or the body direction. We conclude from these recordings that mushroom body extrinsic neurons code physical parameters (location, body directions) or social interactions in a combinatorial way.

From Insect Neuroethology to Neurotechnology: Computations in Small Brains.

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One primary goal of computational neuroscience is to uncover fundamental principles of computations that are performed by the brain. Social Insects show a rich repertoire of goal-directed and adaptive behaviors in a social context. They exhibit a number of cognitive abilities (Menzel 2012) such as spatial cognition and complex forms of learning. The nervous systems of insects feature a moderate complexity. At the same time invertebrates and vertebrates share many basic computational mechanisms at the single neuron and circuit level such as e.g. cellular adaptation, short term plasticity, or spike timing dependent plasticity. I thus argue that insect models are particularly well suited for studying principles of neural information processing and for translating those principles into functional models that can perform brain-like computations. I will present some of our ongoing work in this direction.

In a first approach we follow the idea of insect-inspired 'minibrains' for the control of autonomous robots (Webb, 2000; Floreano, Ijspeert & Schaal, 2014). In a spiking neural network simulation we combined sparse coding at the level of Kenyon cells and reward-based plasticity in the mushroom body. A robot equipped with this network can rapidly establish reliable associations of sensory objects with reward (Helgadottir et al., 2013) typically within a single learning trial, replicating behavioral results in honeybees (Pamir et al., 2014).

In a related project we made use of neuromorphic hardware – electronic versions of spiking neurons and synapses on a microchip—to implement a neural network inspired by the sensory processing architecture of the insect nervous system. We demonstrate that this network achieves classification of real-world data —a widespread problem with many technical applications (Schmuker, Pfeil & Nawrot, 2014). An analysis of the network dynamics showed that stable decisions in output neuron populations are reached within less than 100 ms of biological time, matching the time-to-decision reported for the insect nervous system (Strube-Bloss, Nawrot & Menzel, 2011; Nawrot et al., this volume).

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The parallel systems in the primary auditory center of the honeybee

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Honeybees learn the place of nectar or pollen-bearing flowers from their hivemates. The foragers returning from foraging to the hive display waggle dances in order to inform the dance-attendees (followers) of the floral odor, the direction and distance from their hive to the site of the flowers, and the followers are recruited to a remote food source according to these informations (Frisch 1967; Riley, Greggers et al. 2005; Menzel, De Marco et al. 2006). Karl von-Frisch (Frisch 1967) also analyzed which elements of the waggle dance were correlated with the distance from the hive to the flower, and suggested the duration of waggling is one indicator of distance. Since then, ethological studies on waggle-dance communication have suggested that “airborne vibration” generated by the wing vibration during waggling are important cues in dance communication (Dreller and Kirchner 1993; Michelsen 2003). These results suggest that the distance is encoded in the duration of airborne vibration pulses generated during the waggle dance. The auditory organ of the honeybee is Johnston’s organ (JO) on the antennae, which enables detecting airborne vibration during waggle dance communication and also detecting air current during flight. The sensory afferents of the JO send their axons to three distinct areas of the bee brain, the dorsal lobe (DL), the dorsal subesophageal ganglion (dSEG), and the posterior protocerebral lobe (PPL) (Ai et al., 2007). Within these termination fields, sensory axons in the medial PPL are characterized by both thick processes with large varicosities and somatotopy, while those in the DL and dSEG (DL-dSEG) show thin processes with small varicosities and no somatotopy, suggesting that vibratory signals detected by the JO are processed in dual parallel pathways in these primary sensory centers. In order to clarify the characteristics of the auditory processing, the response properties of the interneurons arborizing in these primary sensory centers have been investigated (Ai et al., 2009). DL-Int-1 and DL-Int-2 densely arborize in DL-dSEG and can monitor the duration of the vibratory stimulation by these response patterns. On the waggle dance communication, the duration of airborne vibration caused by the dance is thought to be one of the index of distance. These DL interneurons might be related with the neural circuits for decoding the “distance” from the vibratory signals. The afferents of neck hair plate, the gravity sensing organ, also terminate in DL-dSEG, suggesting DL-dSEG might be not only primary center of vibration but also the integration center of vector information (Ai and Hagio, 2013). In contrast, PPL-D-1 has dense arborizations in the PPL and sends axons into the ventral nerve cord, with blebby terminals in the contralateral dSEG and PPL, and responds to vibratory stimulation on the ipsilateral antenna with long-lasting excitation (Ai and Itoh, 2012). Thus the auditory pathway through DL-dSEG might be related with the neural circuits for decoding the “distance” from the vibratory signals and also the integration center of vector information. The PPL is the opto-motor reflex center because the visual interneurons and motion-sensitive interneurons arborize in the PPL. The auditory pathway through PPL is thought to be a region for integrating the visual signals and mechano-sensory signals detected by JO for controlling the stable flight. This work is supported by the Ministry of Education, Science, Technology, Sports and Culture of Japan (Grant Number 22570079) and Strategic International Cooperative Program, Japan Science and Technology Agency (JST).

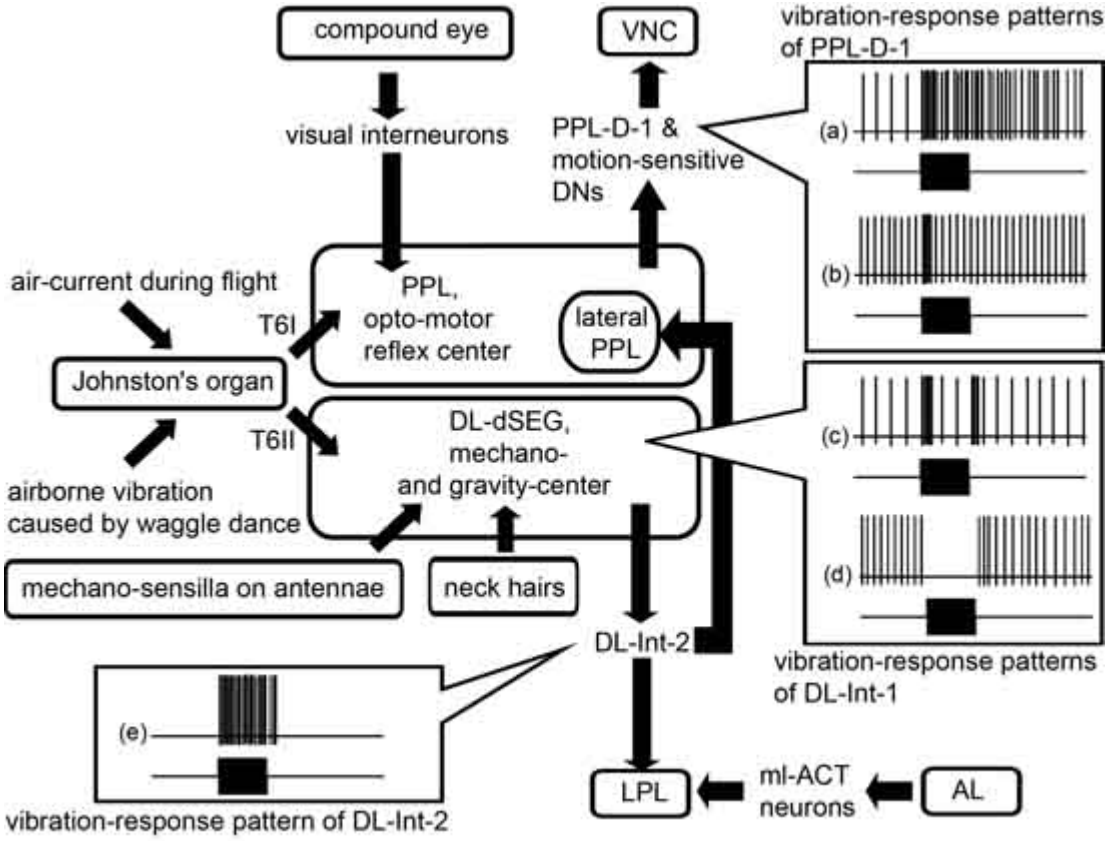

Fig. 1. Parallel pathways in the honeybee auditory system

Evidence for morphological refinement of neurons encoding waggle dance communication signals in the honeybee

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Honeybees exhibit a unique communication behavior, the waggle dance [1], to convey information about food sources to their hive mates. Airborne vibration signals, generated by fast wing beats, play an important role in this dance communication [2]. Those vibration signals are detected by Johnston's organ and processed by neurons in the dorsal lobe (DL) and other areas of the honeybee brain [3]. Honeybees participate in the dance behavior only after reaching the age where they start to forage. This gives rise to the hypothesis that these vibration sensitive neurons acquire their processing capabilities with age and undergo developmental changes to facilitate this. We investigated age-related morphological changes of an identified interneuron in the dorsal lobe, DL-Int1, comparing morphologies obtained from young newly emerged honeybees and older forager honeybees. We first compared these two sets of neurons using common morphological measures such as neuron size, total surface area, number of bifurcations, etc. These measures did not show any systematic differences between the age groups. We then compared the neurons based on the spatial distribution of their neurites. We aligned neurons pairwise[4] and defined similarity measures on the aligned neurons based on mean euclidean distance and other measures. We then applied a spectral clustering method based on these similarity measures. Neurons of forager bees formed a clear cluster, while neurons from newly emerged bees neither clustered with those of foragers nor formed a cluster of their own. This suggests that the morphologies of DL-Int1 neurons vary strongly in young honeybees but refine to a common structure with experience or age.

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Dopamine signalling and the survival of honey bee queens

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Exposing young worker honey bees to queen mandibular pheromone (QMP) lowers brain dopamine levels and reduces expression of the D1 -like dopamine receptor gene, *Amdop1*. We have found in addition that one of the key components of QMP (homovanillyl alcohol, HVA) activates the D2-like dopamine receptor, *Am*DOP3. These results are consistent with evidence showing that dopamine plays a critical role in associative learning in insects, but why does QMP reduce aversive learning while leaving appetitive learning intact? We have used *in vivo* voltammetry to confirm that dopamine is released in the mushroom bodies of the bee in response to aversive stimuli (electric shock) and to determine whether dopamine release or clearance rate are affected by HVA. We have been examining also the effects of dopamine on mushroom body neurons, and whether responses to dopamine are influenced by hormone levels in the bee. This talk will provide a synthesis of our recent findings, and explore what QMP's actions can tell us about dopamine signalling in the insect brain.

How the clock develops: The PDF-network in honeybee brains of different developmental stages

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The seasonal and daily changes in floral resources are a great challenge for honeybees. To adapt to these changes bees have evolved a circadian clock. This clock is involved in different processes like the sun-compass orientation or the age related polyethism in honeybees.

The molecular basis for the honeybee's circadian rhythm is a transcriptional/translational negative feedback loop in which four putative honeybee clock genes *period*, *cryptochrome-m*, *cycle* and *clock* are involved. But other factors like the neuropeptide PDF (Pigment Dispersing Factor), which has been shown to be part of the circadian clock in different insects, seem to be important for the normal circadian timekeeping, too.

Here we characterize the development of the PDF-network of *Apis mellifera* on an anatomical level via whole mount immunoassays. PDF- positive neurons of the honeybee master clock were identified in brains at different time points of the honeybee development: Larval, pupal and adult stages. The larval and pupal stages were reared *in vitro* to achieve a higher standardization. We could observe PDF-positive stainings from larval stage 3 on. The number of PDF-positive cellbodies, which are positioned in the lateral brain between optical lobe and protocerebrum, increased throughout development and the arborization-network gained complexity. In the adult bee fibers run through the protocerebrum, the optic lobes, to the antennal lobes and the ocelli and connect the two brain hemispheres. To get a more precise idea how the PDF-network and therefore the honeybee circadian clock develops the arborization pattern was also reconstructed and a 3D Image was created.

Symposium

S32: Microglia and brain tumors: friends or foes?

S32-1 Targeting the immunosuppressive glioma microenvironment
Michael Platten

S32-2 Microglia and macrophages as modulators of glioma vascularization and progression
Peter Vajkoczy

S32-3 Buzzing the buddy: chemokines in the interplay of TAMs and glioma cells
Janka Held-Feindt, Kirsten Hattermann, Maximilian Mehdorn, Rolf Mentlein

S32-4 MIF signaling and the brain tumor microenvironment
Nicolai E. Savaskan

S32-5 Microglia-glioma cells interactions in a 3D co-culture model
Anne Régnier-Vigouroux, Liliana Cisneros

S32-6 MIF signaling and the brain tumor microenvironment
Ali Ghoochani, Nicolai E Savaskan, Michael Buchfelder, Ilker Y Eyüpoglu

Targeting the immunosuppressive glioma microenvironment

Michael Platten[1]

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Gliomas are typically viewed as inaccessible for an effective antitumor immune response as they grow in a site protected from infiltrating immune cells. Moreover, the glioma microenvironment constitutes a hostile environment for immune response. Glioma-derived factors such as transforming growth factor-beta and catabolites of the essential amino acid tryptophan paralyze T cell function. However, there is increasing evidence that a meaningful antitumor immunity exists in glioma patients and that it can be activated by immunotherapy. Based on encouraging results from other tumor entities there is now a realistic and promising option to combine active immunotherapy, such as targeting IDH1 or EGFRvIII with agents blocking the immunosuppressive microenvironment in patients with gliomas to allow a peripheral antitumor immune response induced by vaccination to become effective. These data and concepts from preclinical models are presented.

Microglia and macrophages as modulators of glioma vascularization and progression

Peter Vajkoczy[1]

Glioblastoma multiforme is one of the most malignant brain tumors. This cancer is characterised by high vascularisation and strong invasiveness. In addition, the tumor mass consists of about one third of microglia. Until now, the importance of these cells on tumor growth and angiogenesis is not completely understood.

We used the intracranial implantation of syngenic tumor cells investigating the putative role of microglia on tumor vascularisation. The microglia cells of naïve and tumor bearing mice were purified, RNA was isolated and Real-time PCR was performed. Regulated pro-angiogenic factors were analyzed by FACS and immunofluorescence stainings, subsequently. Furthermore, we studied the migratory behavior of microglia cells in vitro.

Microglia cells accumulated in and around the brain tumor tissue and displayed a high affinity to the perivascular niche. Approximately 20% of the tumor blood vessels were associated with two or more Iba-1 positive cells. Using a CD11b-HSVTK transgenic mouse model, depletion of these cells led to a reduced vessel count further indicating a central role on brain tumor vascularisation. Moreover, we found different surface molecules as well as soluble factors like chemokines involved in angiogenesis that are up-regulated on the mRNA and protein level in microglia of tumor bearing mice. These identified molecules showed a high expression particularly in the brain tumor area. Additionally, we established an in vitro culture of microglia cells and defined CCL2 as a positive migration signal. Especially tumor microglia responded to this chemokine.

Our results demonstrate that microglia cells accumulate in the perivascular niche, up-regulate pro-angiogenic factors and are able to modulate the brain tumor vascularisation. A better understand of the interaction between microglia, tumor cells and endothelial cells might lead to novel strategies for the future treatment of glioblastoma multiforme.

Buzzing the buddy: chemokines in the interplay of TAMs and glioma cells

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In the recent past several studies have sustained the hypothesis that glioma progression is not only driven by tumor cells themselves but also by their interaction with tumor-associated macrophages and microglia cells (TAMs). Moreover, it seems that small chemotactic cytokines, known as chemokines, are important key players in glioma progression. To deepen this issue in more detail we focused on the point how chemokines – exemplarily described here for the two chemokines CX3CL1/fractalkine and CXCL12/stromal cell-derived factor-1 (SDF-1) - impair to glioma pathology.

Initially, we found that CX3CL1 is produced in GFAP-expressing glioma regions whereas its receptor CX3CR1 is exclusively expressed on TAMs in situ and in vitro. Additionally, freshly isolated human TAM-enriched fractions separated by CD11b MACS technology displayed high CX3CR1 mRNA expression levels in vitro. Functionally, cultured human TAMs responded in vitro to CX3CL1-triggered activation of CX3CR1 with adhesion, migration and enhanced expression of matrix metalloproteinases (MMP) 2, 9, and 14 contributing indirectly to glioma progression.

Next, we analysed if CX3CL1/CX3CR1 could promote tumor progression by directly influencing the glioma cell themselves – maybe by a “reverse signaling” mechanism. This reverse signal transduction which is known from other transmembrane ligands like TNFalpha, ephrins and semaphorins occurs when transmembrane ligands bind to their classical receptors and responses are elicited also in the ligand-carrying cells. Thus, we exposed adherent glioma cells expressing CX3CL1 to non-adherent monocytic cells expressing CX3CR1, and monitored signal transduction and proliferation of glioma cells. In these experiments both classical and reverse signaling were clearly observed and could be omitted by siCX3CL1 knock down strategies. Moreover, proliferation of glioma cells was considerably promoted.

Summarized, these results support the hypothesis that the CX3CL1/CX3CR1 axis is an important factor in glioma progression – firstly indirectly by attracting CX3CR1-positive TAMs to the tumor facilitating glioma cell invading though activation of TAM-related MMPs and – secondly by directly promoting glioma cell proliferation though reverse signaling mechanisms.

In a second topic we focused on the significance of CXCL12 and its both receptors CXCR4 and CXCR7 in the interplay between TAMs and glioma cells. We showed that CXCR7 - which was discovered as a novel, alternative receptor for CXCL12 - is highly expressed on tumor cells and increased with malignancy. In contrast, CXCR4 expression is much more restricted and found to be predominately expressed in glioma stem-like cells from which it diminished upon differentiation whereas CXCR7 rose drastically. Since CXCL12 is abundantly expressed and produced in TAMs we considered CXCL12 function on glioma cells especially focussing on CXCR7. We revealed that CXCR7-positive glioma cells were activated by CXCL12 indicating that the receptor is functionally active. Additionally, CXCL12 prevented Camptothecin- and Temozolomide-induced apoptosis, and the selective CXCR7-antagonist CCX733 reduced this anti-apoptotic effects. Thus, CXCR7 is a functional receptor for TAM-secreted CXCL12 in gliomas and mediates resistance to apoptosis.

Taken together, all these results indicate that chemokines play a pivotal role in the interaction of TAMs and glioma cells resulting in progression of these fatal tumors via several mechanisms.

MIF signaling and the brain tumor microenvironment

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Malignant glial brain tumors proliferate and infiltrate brain parenchyma by evading the inert immune system. Here we searched for paracrine mediators contributing to this immune escape phenomenon and identified macrophage migration inhibitory factor (MIF) as an essential player in glioma-induced microglia paralysis. Analysis of human samples reveals that MIF is expressed at high levels in malignant gliomas and is present in cerebrospinal fluids of glioma patients. Gene silencing of MIF does not disrupt glioma cell proliferation or survival, however knock down of MIF in vivo leads to alleviated tumor growth and prolonged survival compared to wild-type gliomas. Microglia, the resident inert immune cells of the brain, show increased infiltration into tumor bulk in MIF deficient gliomas. We investigated MIF signaling in microglia and identified CD74 as the mediator for MIF-induced microglial paralysis. RNA interference with CD74 in microglia leads to allayed downstream ERK1/2 activation and enhanced microglial migration. Furthermore, CD74 comprised microglia attacks glioma cells in vitro and reduces glioma proliferation in a contact-independent manner. Microglial cells with inhibited CD74 infiltrate brain tumors and reduce glioma proliferation, leading to prolonged survival in vivo. Further analysis revealed that inhibition of CD74 receptor enhances IFN-γ expression in microglia. Moreover, IFN-γ treatment shows tumor growth inhibition activity and alleviates tumor infiltration into brain parenchyma. Thus, we identified glioma derived MIF secretion which is essential in immune escape of brain tumors and tumor microenvironment shaping. Interference with glioma-induced MIF or microglial CD74 expression in either way provides the potential for disrupting microglial paralysis in malignant brain tumors.

Microglia-glioma cells interactions in a 3D co-culture model

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Glioblastoma and its microenvironment form a complex, dynamic system, largely but not only controlled by immune molecular and cellular players, such as glioma-associated microglia/macrophages. The establishment of tight links between cancer cells and its environment emphasizes the need for appropriate models for studying glioblastoma biology and for predicting its response to multiple therapeutic treatments. Contrary to cell monolayers, spheroids of cells provide a three-dimensional environment that more closely resembles that of a tumour mass. We have developed a culture model composed of mono- and multicellular spheroids embedded in the presence or absence of microglia in a collagen matrix. Spheroids consist either of tumor cells only or of a mixture of tumor and microglial cells. Our aim is to investigate the potential of such spheroid models as a platform to assess the functional relevance of microglia-glioma cell interactions in glioma development and therapy. To illustrate our approach, I will report on experiments performed to analyse the effects of temozolomide combined with an inhibitor of the sphingosine kinase in the presence or absence of photon irradiation. These effects were analysed on human glioma cells spheroids and mixed human microglia-tumor cells spheroids. Invasion profile, levels of cell proliferation and cell death as well as signalling pathways were monitored by imaging, flow cytometry, qPCR and ELISA analyses. Irradiation enhanced the decrease in invasion and proliferation and the increase of cell death induced by the combination of temozolomide and sphingosine kinase inhibitor; these effects were further potentiated by the presence of microglia in the spheroids. In absence of any treatment, the presence of microglia in the spheroids did not impair but rather supported its development (growth and invasion). This observation suggests that, in the current experimental conditions, microglia/macrophages may acquire anti-tumor activities after chemo- and radiation treatment. Preliminary data suggest the involvement of a feed-back loop between the sphingosine 1 phosphate receptor and STAT-3. These and other data indicate that the spheroid model we sat up is a robust system, which can be used to investigate molecular mechanisms in an in vitro multicellular environment. As such, it represents an attractive model for the assessment of various treatments and possibly for the prediction of their impact.

MIF signaling and the brain tumor microenvironment

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Malignant glial brain tumors proliferate and infiltrate brain parenchyma by evading the inert immune system. Here we searched for paracrine mediators contributing to this immune escape phenomenon and identified macrophage migration inhibitory factor (MIF) as an essential player in glioma-induced microglia paralysis. Analysis of human samples reveals that MIF is expressed at high levels in malignant gliomas and is present in cerebrospinal fluids of glioma patients. Gene silencing of MIF does not disrupt glioma cell proliferation or survival, however knock down of MIF *in vivo* leads to alleviated tumor growth and prolonged survival compared to wild-type gliomas. Microglia, the resident inert immune cells of the brain, show increased infiltration into tumor bulk in MIF deficient gliomas. We investigated MIF signaling in microglia and identified CD74 as the mediator for MIF-induced microglial paralysis. RNA interference with CD74 in microglia leads to allayed downstream ERK1/2 activation and enhanced microglial migration. Furthermore, CD74 comprised microglia attacks glioma cells *in vitro* and reduces glioma proliferation in a contact-independent manner. Microglial cells with inhibited CD74 infiltrate brain tumors and reduce glioma proliferation, leading to prolonged survival in vivo. Further analysis revealed that inhibition of CD74 receptor enhances IFN-γ expression in microglia. Moreover, IFN-γ treatment shows tumor growth inhibition activity and alleviates tumor infiltration into brain parenchyma. Thus, we identified glioma derived MIF secretion which is essential in immune escape of brain tumors and tumor microenvironment shaping. Interference with glioma-induced MIF or microglial CD74 expression in either way provides the potential for disrupting microglial paralysis in malignant brain tumors.

Symposium

S33: Balancing change and stability: homeostatic plasticity in the central nervous system

S33-1 Activity-dependent plasticity of the axon initial segment and its synapses
Juan Burrone, Winnie Wefelmeyer

S33-2 Inhibitory axons as dynamic structures adapting to activity
Corette J Wierenga

S33-3 Homeostatic plasticity of subnetworks of excitatory and inhibitory neurons in mouse visual cortex in vivo
Tara Keck

S33-4 Stability matters - homeostatic plasticity in denervated neuronal networks
Andreas Vlachos

S33-5 Homeostatic regulation of synaptic function and reconfiguration of gene expression upon ketamine treatment: Relevance to antidepressant effects.
Santosh Pothula, Denny Schanze, Anna Fejtova

S33-6 Staggered development of SPON neurons in mice lacking L-type Ca2+-channels
Sara Leijon, Neil Portwood, Anna K Magnusson

Activity-dependent plasticity of the axon initial segment and its synapses

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The axon initial segment (AIS) is a structure at the proximal end of the axon with a high density of sodium and potassium channels that defines the site of action potential generation. It has recently been shown that this structure is plastic and can change its position along the axon as well as its length in a homeostatic manner (Grubb & Burrone 2010; Kuba et al. 2010). Here, we investigated AIS plasticity in CA1 neurons of rat hippocampal organotypic cultures, using optogenetics to excite individual pyramidal neurons expressing channelrhodopsin -2 (ChR2). 48 hour photostimulation (20 ms blue light flashes delivered in bursts of 5 at 20 Hz, mean of 1 Hz) led to an outward shift of the AIS by around 12 µm. This effect was cell-autonomous and independent of network activity. The structural plasticity of the AIS was accompanied by a decrease in input resistance, resulting in an increased current threshold for eliciting an action potential and a shift in the input/output curve towards higher input currents.

In parallel to this, we also mapped and characterised the axo -axonic synapses that Chandelier interneurons form exclusively onto the AIS of pyramidal cells. We find that these synapses, labelled with either presynaptic (vGAT) or postsynaptic (gephyrin, $GABA_AR$ a2) markers, did not translocate with the AIS after chronic stimulation. As a result, a subset of synapses formed onto the proximal end of the AIS were left behind, creating a partial mismatch between axo-axonic synapses and the AIS. We are currently exploring the functional consequences of this intriguing form of plasticity.

Inhibitory axons as dynamic structures adapting to activity

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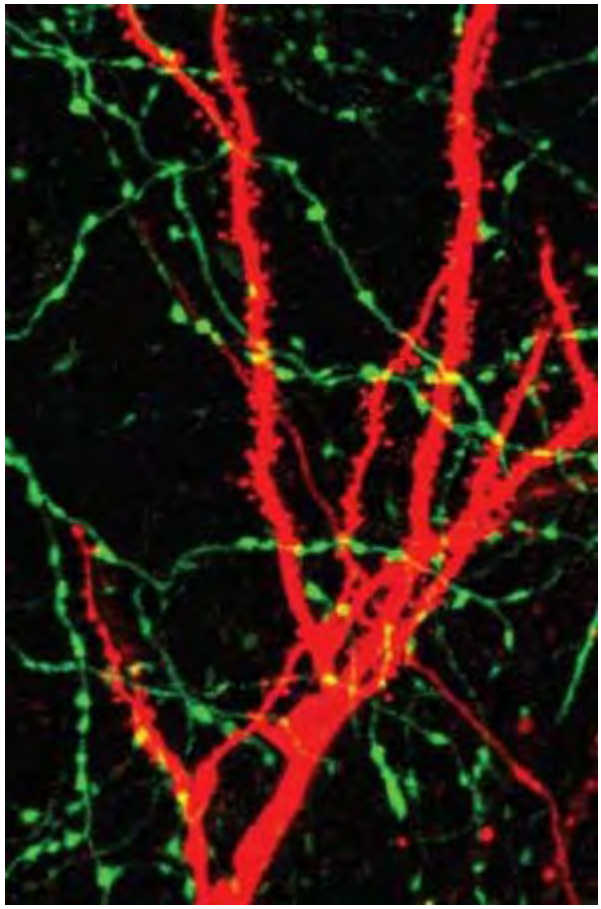
For a healthy brain changes at inhibitory and excitatory synapses need to be well-coordinated at all times to ensure the stability of neuronal circuits. Subtle defects in the coordination of excitation and inhibition can cause aberrant neuronal activity or circuitry and may underlie psychiatric disorders. Inhibitory axons may act as a dynamic structure that can quickly adjust to a changing environment, by responding to local signals from postsynaptic cells via adhesion molecules and to global signals from the local neuronal network. A highly dynamic inhibitory system may serve to quickly respond to changes to allow circuit rearrangements by excitatory connections.



Homeostatic plasticity of subnetworks of excitatory and inhibitory neurons in mouse visual cortex in vivo

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Homeostatic plasticity is believed to be essential to prevent extreme activity levels as activity levels in the brain adapt in response to changes in the surrounding environment, learning, or following alternations to sensory input. While homeostatic plasticity has been demonstrated to occur in vivo following sensory deprivation, whether all, or only a subset of cells undergo homeostatic recovery associated with homeostatic mechanisms is unclear. Here, we examine individual excitatory and inhibitory neurons' activity levels over time, using chronic imaging of genetically encoded calcium indicators expressed in the visual cortex of behaving mice. We find that not all excitatory cells recover their activity following sensory deprivation, and those that do, are strongly correlated with one another prior to deprivation. These recovering excitatory cells are also strongly correlated with highly active inhibitory cells prior to deprivation, suggesting that there is a subnetwork of excitatory and inhibitory cells that homeostatically recover their activity after sensory deprivation. Recovery of activity is also associated with a shift in the balance between synaptic excitation and inhibition, towards a reduction in inhibition, suggesting that classic homeostatic mechanisms, as well as network properties, play a role in the homeostatic recovery of activity following sensory deprivation.

Stability matters - homeostatic plasticity in denervated neuronal networks

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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 neuronal networks is considered to play an important role for the postlesional recovery. Although lesion-induced synaptic reorganization has long been described, its dynamics and the underlying molecular mechanisms are still insufficiently understood. Using in vitro entorhinal cortex lesion we were recently able to demonstrate that neurons respond to the loss of afferent input with a compensatory, i.e., homeostatic increase in excitatory synaptic strength, which accompanies the structural reorganization of denervated neurons. These results indicate that homeostatic synaptic plasticity could be of relevance for many neurological diseases. My presentation will summarize our current knowledge on the role of inflammation and coagulation in denervation-induced homeostatic synaptic plasticity. I will discuss the possibility that factors known to play an important role in the vascular and immune system may act by modulating neuronal intracellular calcium stores to affect the postlesional reorganization of neuronal networks (supported by DFG, CRC1080).

Homeostatic regulation of synaptic function and reconfiguration of gene expression upon ketamine treatment: Relevance to antidepressant effects.

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Major depressive disorder (MDD) is the most common psychiatric disorder with lifetime prevalence of 16%. Conventional antidepressants require prolonged treatment and have only limited efficacy. Thus development of broadly potent rapidly acting antidepressants is needed. Recent evidences revealed that the rapid, but transiently persistent antidepressant effects of a single low dose of ketamine might be associated with synaptogenesis, changes in synaptic transmission and neuronal plasticity but the underlying molecular and cellular mechanisms are not completely understood [1,2]. Here, we investigated the effect of ketamine on synaptic function, molecular composition, intracellular signaling and gene expression in dissociated rat cortical cultures. Synaptotagmin1 Ab uptake assay, electrophysiological recordings and quantitative immunostainings were used to determine the changes in pre and postsynaptic function and molecular composition. Genome-wide microarray-based expression analysis was performed to screen for changes in gene expression at several time points after ketamine treatment. Results of the study revealed rapid changes in synaptic function and concomitant molecular remodeling and reconfiguration of cellular gene expression. Interestingly, our analyses revealed biphasic changes and opposite regulation at early (30 min after treatment) and at delayed (24 hrs after treatment) time points. While acute effects were common to both excitatory and inhibitory synapses, delayed effect was specific to excitatory synapses, suggesting that acute interference with NMDA-mediated transmission by ketamine induces adaptive functional and structural changes that seem to rely on homeostatic neuronal plasticity mechanisms and lead to a shift in the excitation-inhibition balance. In line with the involvement of homeostatic mechanisms the regulation of key genes relevant to synaptic transmission, neuronal plasticity, calcium and MAPK signaling pathways, and antidepressant mechanisms was observed. Our results also suggest that changes in the expression of activity -dependent genes upon ketamine treatment might be partially dependent on transcriptional corepressor C -terminal binding protein 1 (CtBP1). The results of this study provide better understanding of cellular and molecular mechanisms involved in rapid antidepressant actions of ketamine and may identify potential targets for exploring the novel therapeutic approaches for MDD.

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Staggered development of SPON neurons in mice lacking L-type Ca2+-channels

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Background
Congenital hearing loss is a common hereditary disease. Detailed knowledge of how sensorineural hearing loss specifically affect the central auditory pathways involved in the processing of vocal communication is scarce. This study investigates how auditory deprivation affects SPON neurons in the brainstem that extract and preserve the temporal patterns in sounds (Felix et al. (2011) J Neurosci: 35:12566-78), important cues for meaningful sounds such as speech or animal vocalizations. To better understand how the biophysical properties of SPON neurons are shaped during postnatal development we studied SPON in congenitally deaf mice lacking cochlear-driven activity in the auditory nerve (Platzer et al. (2000) Cell:102:89-97).

Methods
Whole-cell patch-clamp recordings were performed on postnatal (P) mice lacking the alpha1D subunit of the Cav1.3 ion channel. This calcium channel is essential for inner hair cell release of neurotransmitter onto auditory afferents, and the mice are consequently born deaf (Platzer et al. (2000) Cell: 102:89-97). For comparison, age-matched (P5-P15) wild type mice were used as controls in the electrophysiological characterization of the respective SPON neurons. To investigate the expression of membrane proteins we used immunohistochemistry.

Results
There are significant differences in intrinsic membrane properties related to rebound spiking in alpha1D-KO (KO) SPON neurons compared to wildtype (WT) controls. The voltage sag induced upon hyperpolarization, indicative of the h-current and important for SPON rebound spiking (Felix et al. (2011) J Neurosci: 35:12566-78), was greatly reduced or sometimes absent in SPON neurons of the KO compared to in pre-hearing WT animals. By isolating inwardly rectifying currents, a developmental up-regulation of the h-current was measured in the KO SPON neurons compared to in post-hearing WT animals. Although, the kinetics of the h-current normalized, its voltage-dependence was reduced in KO SPON neurons. Immunolabeling against HCN-subunits demonstrated normal levels of the HCN2 subunit whereas the reactivity to HCN1 subunit was greatly reduced in KO compared to age-matched controls. indicating a compensatory regulation of HCN2 in the KO animals. SPON neurons furthermore have a longer rebound duration, indicative of larger Ca2+-currents, in KO animals than WT SPON neurons. Analyzed over age, the rebound duration is significantly reduced at pre-hearing onset ages (

Conclusions
The alpha1D-KO mice are born congenitally deaf and thus lack activity in the auditory nerve. The results of this study demonstrate that, throughout development, two different mechanisms play a role in compensating for the loss of auditory input in the SPON. At earlier ages, when h-currents are still small in the KO neurons, Ca2+-currents contribute more than in control animals to rebound spiking in SPON neurons. As the SPON neurons in the KO develop, the h-current is up-regulated via HCN2-subunits, which contribute to depolarize the resting membrane potential of the neurons. By bringing the membrane potential closer to spike threshold voltage the neurons rescue their ability to trigger rebound spiking,

which is the functional output of SPON neurons. Whether these homeostatic compensatory effects are driven by input-specific activity or if they are instructed by an intrinsic molecular-genetic machinery remains to be investigated.

Symposium

S34: Modeling evolution, neuronal development and neurodegenerative disorders using mammalian induced pluripotent stem cells

S34-1 Using human neural cells to model for autism spectrum disorders with accelerated brain growth
Maria Carolina Marchetto, Haim Belinson, Yuan Tian, Karen Pierce, Eric Courchesne, Daniel Geschwind, Anthony Wynshaw-Boris, Alysson Muotri, Fred Gage

S34-2 Understanding cortical malformations: Combining Human iPSCs Technology and Mouse Models
Silvia Cappello

S34-3 Mitochondrial Function In iPSC From PD Patients
Constantin Alexander Stautner, Martin Jastroch, Beate Winner, Jürgen Winkler, Daniela Vogt Weisenhorn, Wolfgang Wurst

S34-4 Pericytes as an alternative cell source for direct neural reprogramming
Marisa Karow

S34-5 Gene dosage-dependent rescue of HSP neurite defects in SPG4 patients' neurons
Beate Winner

S34-6 Derivation of early neuroepithelial precursors from fetal tissue to assess novel neural reprogramming pathways
Katharina Günther, Philipp Wörsdörfer, Sandra Meyer, Antje Appelt-Menzel, Heike Walles, Frank Edenhofer

Using human neural cells to model for autism spectrum disorders with accelerated brain growth

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Autism spectrum disorders (ASD) are complex neurodevelopmental diseases that affect about 1% of children in the United States. The precise mechanisms that cause autism, however, remain unknown. Neuropathological imaging and genetic studies have led to two major hypotheses for autism pathogenesis: altered brain growth and dysfunctional neuronal networks. The major impediment to testing these hypotheses is the lack of relevant animal and cell models. Recently, reprogramming of human somatic cells to a pluripotent state (induced pluripotent stem cells or iPSC) has provided an exciting opportunity to produce relevant cellular models of human complex neurogenetic diseases. We have generated iPSCs lines for ASD (Rett syndrome and idiopathic autistic patients) and detected cellular phenotypes that can directly test the current models for autism pathogenesis abovementioned. Knowledge of the biological causes of neural maldevelopment in ASD would likely lead to the development of clinically useful biomarkers of risk for this disorder in pre-symptomatic infants, which may lead to the development of novel therapies.

Understanding cortical malformations: Combining Human iPSCs Technology and Mouse Models

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The regulation of proliferation, differentiation and neuronal migration is of crucial importance for the formation of a functional cerebral cortex. Periventricular neuronal heterotopia and subcortical band heterotopia can be indicative of a failure of correct neurogenesis and neuronal migration. Although in the last decades many genes responsible for cortical malformation in human and mouse have been identified, the mouse model often failed to closely mimic the human phenotype, making impossible to extrapolate the molecular and cellular mechanisms underlying these developmental disorders.
We recently found that mutations in genes encoding the receptor-ligand cadherin pair DCHS1 and FAT4 lead to Van Maldergem syndrome, a recessive multiple malformation syndrome in humans that includes periventricular heterotopia. Down-regulation of DCHS1 and FAT4 in the embryonic mouse developing cortex and in human cerebral organoids increased neural stem cell proliferation and reduced differentiation and migration of neurons, resulting in the heterotopic accumulation of cells below the neuronal layers in the neocortex.

Mitochondrial Function In iPSC From PD Patients

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In recent years Parkinson ’s disease (PD) has become the second most common neurodegenerative disorder, with a prevalence of up to 5% at the age of 85. Its characteristic clinical symptoms - resting tremor, rigidity, postural instability and bradykinesia - occur once about 60% to 80% of dopaminergic neurons in the Substantia Nigra (SN) are lost. So far, a big variety of factors have been identified to take part in neurodegenerative processes, among which age, lifestyle, various other environmental factors and genetic predisposition take the most important places. The interplay of several of these factors is thought to fuel a variety of pathological molecular processes like neuroinflammation, dysfunction of protein degradation, increased oxidative stress and mitochondrial dysfunction.

Bioenergetics and, in particular, mitochondrial function have been consequently linked to PD since the discovery that mice, exposed to 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP), which causes selective inhibition of mitochondrial respiration in dopaminergic (DA) neurons, develop motor symptoms resembling, to an extent, symptoms of PD patients. Over time, both sporadic and familial PD have been linked to mitochondrial dysfunction. Sporadic PD patients have been discovered to i.e. show age-dependent increased rates of mutation and deletions in the mitochondrial genome, reduced mitochondrial complex 1 activity in the SN of autopsied patients or development of PD-like symptoms in patients who were exposed to a selection of (agricultural) toxins like rotenone or paraquat. Similar, a variety of pathogenic genetic mutations associated with PD could be linked to mitochondrial dysfunction. For example *Pink1* and *Parkin* are involved in mitochondrial fission and fusion events, *LRRK2* mutations lead to increased mitochondrial genomic mutation rates and loss of *DJ-1* results in destabilization of the cellular redox balance. However, it is not clear whether these mitochondrial dysfunctions are cause or consequence of possible bioenergetic deficits.

Thus, in order to elucidate these causal links we will first bioenergetically profile idiopathic PD patient’s as well as familial PD patient’s cells. To this purpose, a cluster of idiopathic PD patients and corresponding controls was assembled, skin biopsied and screened for PD-associated risk factors or mutations. Patient’s cells passing this screen were reprogrammed into human induced pluripotent stem cells (iPSCs) *(Kohl, Winkler, Winner; Erlangen)*. Preliminary results in our lab indicate bioenergetic deficits in patient’s iPSCs over corresponding controls, yet not in primary patient material, indicating that the choice of the proper in vitro model is crucial. Therefore, our lab addresses the analysis of various differentiation products from corresponding iPSCs, thereby gaining a broad overview over several PD associated cell types and their bioenergetic profile and possible contribution to the pathogenesis of Parkinson’s disease.

Pericytes as an alternative cell source for direct neural reprogramming

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Pericytes play an important role in the response to central nervous system (CNS) injury. For instance, they have been shown to contribute to glial scar formation upon spinal cord lesion (Göritz et al., Science, 2011) as well as ischemic stroke (Fernandez-Klett et al., J Cereb Blood Flow Metab, 2013). We have previously shown that cells expressing pericyte markers can be isolated from the adult human brain and reprogrammed into induced neurons by forced expression of Sox2 and Ascl1 (Karow et al., Cell Stem Cell, 2012). Our recent work focuses on further using these somatic cells as alternative cellular target to generate oligodendroglial cells via direct reprogramming strategies. As opposed to skin fibroblasts, pericytes represent a cell population that has the advantage of being resident within the tissue undergoing neurodegeneration, e.g. the diseased brain and the spinal cord. We will show how the choice of transcription factors employed, allows instructing the genesis of cells of the neuronal or oligodendroglial lineage. This cellular plasticity combined with their ubiquitous distribution endows pericytes with essential prerequisites for being considered a promising target cell population for the development of cell-based therapies for neurodegenerative diseases. A special focus will be laid on the generation of human striatal interneurons obtained via transcription factor-driven direct reprogramming of pericytes as an approach to restore the basal ganglia activity that is detrimentally affected in patients with Parkinson´s disease.

Gene dosage-dependent rescue of HSP neurite defects in SPG4 patients' neurons

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We model a motor neuron disease called hereditary spastic paraplegia (HSP) utilizing iPSC-derived cells with a mutation in the SPG4 gene, encoding Spastin, the most frequent autosomal dominant cause of HSP. We show that the complexity of SPG4 neurites is decreased, which is accompanied by an imbalance of axonal transport, with less retrograde movements and prominent neurite swellings with disrupted microtubules. Of note, upregulation of another microtubule severing protein, p60 katanin, partially compensates microtubuli dynamics in SPG4 neurons. Neurite complexity and maintenance in HSP patient-derived neurons are critically sensitive to Spastin gene dosage. Furthermore, our human model offers an ideal platform for pharmacological screenings with the goal to restore physiological Spastin levels in SPG4 patients.

Derivation of early neuroepithelial precursors from fetal tissue to assess novel neural reprogramming pathways

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Cellular reprogramming offers novel promising strategies for modeling complex neurodegenerative and neuropsychiatric diseases as well as cell replacement therapies. The Yamanaka approach allows the generation of induced pluripotent stem cells (iPSCs) and their differentiation into neural progenitor cells (NPCs). More recently, direct conversion of somatic cells into NPCs emerged into a new promising, more straightforward strategy to derive patient-specific cells for biomedical applications. Those directly converted NPCs exhibit advantages over iPSCs as they can be generated faster and additionally carry a strongly reduced tumorigenic potential. Though established in mouse cells, directly converted human cells are not fully characterized and seem to be limited in their differentiation and proliferation potential. Therefore there is a need for a primary derived population, which might represent a bona fide model to provide a standardized basis for comparative validation studies of progenies arising from novel direct conversion protocols. Moreover, such cells could help to elucidate mechanisms of early neural development. In this study we hypothesized that the modulation of the BMP, TGFß, WNT and SHH signaling is instrumental in stabilization of early progenitors. Thus, we assessed the potential of small molecules to enable the generation of early NPCs from isolated human fetal brain cells in culture. Indeed, we identified conditions that allow robust formation of colonies, which could be isolated and monoclonally expanded for more than 25 passages displaying a homogeneous morphology and a high proliferation rate. Further characterization by immunofluorescent stainings and quantitative PCR show a characteristic neural stem cell profile expressing SOX1, PAX6, Nestin and SOX2. We show a high neurogenic potential by differentiating into a high percentage of TUJ1-positive neurons among which rare Peripherin-positive cells were found. By application of targeted differentiation protocols we could expand the spectrum of neuronal subtypes also to Peripherin- and TH-positive neurons as well as GFAP-positive cells representing the glial lineage.
In conclusion, we here present a protocol for stabilization of a novel fetal-derived early NPC population. These cells do not only serve as a new comparative cell population for direct conversion experiments, but might also provide a source of cells for medical application, and tissue engineering.

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

T1-1A Are olfactory bulb and brain volume growing proportionally?
Elke Weiler, Willi Bennegger

T1-2A Axonal pathology in patient-derived neurons harboring SPG11 mutations: An iPSC model for spatacsin-linked Hereditary Spastic Paraplegia
Himanshu Kumar Mishra, Francesc Pérez-Brangulí, Iryna Prots, Steven Havlicek, Zacharias Kohl, Jonatan Dorca-Arevalo, Martin Regensburger, Elisabeth Sock, Juan Blasi, Teja W Groemer, Ursula Schlötzer-Schrehardt, Jürgen Winkler, Beate Winner

T1-3A Calcium response profile of different cell types
in the mouse subventricular zone
Nanette Messemer, Laura Fritz, Joachim W. Deitmer

T1-4A Chemically defined differentiation of human astrocytes from iPSC derived neural stem cells for disease modeling
Pretty Garg, Kurt Gottmann, Katja Nieweg

T1-5A Direct Conversion of Adult Cortical Oligodendrocyte Progenitor Cells to Functional Neurons
Daniel Peterson, S Bazarek, A Mehta, RA Marr, GE Stutzmann, RM Howard, SR Whittemore

T1-6A Efficient generation of human iPSC derived Parvalbumin interneurons for disease modelling
Debia Rajnath Wakhloo, Katja Nieweg

T1-1B Expression and Function of Lin28B in the Autonomic
Nervous System
Melanie Hennchen, Ikram Abarchan- El Makhfi, Hermann Rohrer

T1-2B Filopodia-based Wnt transport during patterning of the neural plate in vertebrates
Steffen Scholpp, Eliana Stanganello, Alexander Schug

T1-3B Inducible formation of hippocampal oligodendrocytes from pre-existing local precursors
Christoph Ott, Imam Hassouna, Liane Dahm, Meike Hütte, Miso Mitkovski, Sandra Göbbels, Klaus-Armin Nave, Hannelore Ehrenreich

T1-4B Retracted

T1-5B Interactions between the meninges and the cortical neuroepithelium time gliogenesis during cortical development
Alexander von Holst, Richard Sturm

T1-1C Mutations in mouse Cdk5rap2; more than just microcephaly
Sami Zaqout, Nadine Krämer, Gisela Stoltenburg, Jessica Fassbender, Gregor Willerding, Angela Kaindl

T1-2C MYCN in Sympathetic Neurogenesis and Neuroblastoma Development
Marco Kramer, Marie Arsenian-Henriksson, Hemann Rohrer

T1-3C Neuronal organization of the I layer of human neocortex during prenatal development
Alena A. Kozlova, Nadezhda A. Sidorova, Lyubov A. Tkachenko

T1-4C Protocadherin18a influences neurogenesis in zebrafish thalamus
Bernadett Bösze, Steffen Scholpp

T1-5C Role of TGF-ß on the Development and Survival of Mouse Hindbrain Serotonergic Neurons
Enaam Chleilat, Eleni Roussa

T1-1D Role of TGF-ß on the Development of Midbrain Dopaminergic Neurons
Fabian Josue Cardenas Lara, Eleni Roussa, Kerstin Krieglstein

T1-2D Targeted disruption of the serine/threonine kinase ULK4 gene leads to abnormal neurogenesis and congenital hydrocephalus-like phenotype
Min Liu, zhenlong Guan, Timothy O'Brien, Sanbing Shen

T1-3D Tcf7l2 steers neuronal diversity required for asymmetric brain formation and function
Matthias Carl, Ulrike Hüsken, Stephen Wilson

T1-4D Tgfbr2 conditional knock-out in the developing telencephalon results in neurovascular defects
Tanja Vogel, Nicole Hellbach, Stefan Weise

T1-5D The ectonucleotidase NTPDase2 controls progenitor cell proliferation in neurogenic niches of the adult mouse brain
Jennifer Stefani, Kristine Gampe, Klaus Hammer, Peter Brendel, Alexandra Pötzsch, Grigori Enikolopov, Keiichi Enjyoji, Amparo Acker-Palmer, Simon C. Robson, Herbert Zimmermann

Are olfactory bulb and brain volume growing proportionally?

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Brain growth continues in mammals far beyond birth, especially in altricial species. The American mink (*Neovision vison var. atratus*) is born with eyes and ears closed and depends primarily on its sense of smell for nutrition and neonatal survival. Thus we were interested whether the olfactory bulb, the main station for olfactory information processing, is already more developed than the rest of the brain at an early postnatal stage and/or if the increase of the olfactory bulb size compared to the total brain is proportional during postnatal development.

Therefore, we investigated the total brain volume and the portion of the olfactory bulbs in 63 male minks ranging from newborns (postnatal day 1, P1) up to seven months (P210). Animals were sacrificed according to the German Animal Welfare Act, brains removed from the skull, and brain volumes determined. Tissues were fixed and cryosections performed. We analyzed the volume of the right bulbs by means of a morphometric system and appropriate correction factors. Because there is no lateralization of the olfactory bulbs, the volume was doubled and the proportion of both olfactory bulbs on the whole brain determined.

The volume of the olfactory bulbs in newborn minks is 3.69±0.08 mm^3, increasing continuously during postnatal life: A 23.0 fold increase from P1 to P15-30 (84.82±5.91 mm^3) is followed by a 2.2 fold increase up to P60-90 (189.27±7.69 mm^3). Afterwards an increase of 1.2 fold (that means 25% more volume) is observed for every two months: P120-150 (236.43±8.43 mm^3), P180-210 (293.02±19.20 mm^3), so that the total bulb volume increases 82.3 fold from P1 to P210.

The brain, in contrast, shows a different development. The brain volume in newborns is 331±24 mm^3, with an increase of only 16.2 fold up to P15-30 (5371±243 mm^3), followed by an increase of 2.1 fold to P60-90 (11026±476 mm^3), when adult levels are reached and no further increase is observed (P120-150: 11109±358 mm^3; P180-210: 10997±484 mm^3). Thus the total brain volume increases only 33.6 fold from P1 to P210.

As a consequence, the proportion of the olfactory bulbs on the whole brain changes dramatically postnatally. From an initial value of 1.12±0.10% in newborns, the proportion continuously increases up to P15-30: 1.58±0.07%, P60-90: 1.72±0.08%, P120-150: 2.11±0.11%, P180-210: 2.66±0.13%. The differences among the age groups are statistically highly significant ($p<0.001$).

Thus the olfactory bulb shows a different developmental pattern compared to the rest of the brain. While the brain exhibits the S-shaped growth reaching adult size, which is typical for mammals, the olfactory bulb continues to increase in both, the absolute size and the portion on the total brain.

This indicates that the rostral migratory stream does not only replace dying neurons within the olfactory bulb, but is continuously adding new neurons, which results in a continuous growth of the olfactory bulb. This suggests an increasing importance of the olfactory sense in postnatal life. While in newborns

olfaction is needed just for nutrition and social odors, juveniles need to detect and identify prey and predators. Adults olfaction is further needed in marking of territory, identify rivals and sexual cues. The gain of olfactory function requires increasing information processing and thus more structure, explaining the un-proportional and continuing growth of the olfactory bulb compared to the rest of the brain.

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Axonal pathology in patient-derived neurons harboring SPG11 mutations: An iPSC model for spatacsin-linked Hereditary Spastic Paraplegia

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Hereditary spastic paraplegias (HSPs) are a heterogeneous group of inherited motor neuron diseases characterized by progressive spasticity and weakness of the lower limbs. They are classified genetically as autosomal dominant, autosomal recessive and X-linked HSP. Mutations in the Spastic Paraplegia Gene11 (SPG11), encoding spatacsin, cause the most frequent form of autosomal recessive HSP. Partly due to lack of a relevant disease model, the underlying molecular mechanisms have not been studied in detail. To overcome this limitation we, for the first time, generated induced pluripotent stem cells (iPSCs) from two SPG11 patients, having heterozygous nonsense and/or splice site mutations, and two age matched controls. We differentiated these iPSCs into forebrain neurons and investigated the neuronal pathology associated with the disease. SPG11 patients' derived neurons exhibited severely impaired outgrowth and branching of axonal processes, implicating a compromised neuritic complexity compared to controls. Gene expression analysis further revealed down regulation of specific motor, synaptic and microtubule associated genes. A reduced expression of acetylated tubulin in the neuronal cells was indicative of the axonal instability, which was further corroborated by ultra structural analysis of these cells showing pathological accumulation of membranous bodies within axonal processes. Finally, time lapse assays performed in SPG11 patients' derived neurons highlighted a reduction in the anterograde vesicle trafficking indicative of impaired axonal transport. Altogether, our SPG11-iPSC model provides the first evidence that mutations in SPG11 have a detrimental effect on the homeostasis of neuronal cells and more importantly disturb the critical balance of transport activity in SPG11 patients' neurons. Furthermore, our human model offers an ideal platform to define new targets to intervene the course of this progressing motor neuron disease.

Calcium response profile of different cell types in the mouse subventricular zone

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Adult neurogenesis takes place in two major regions of the mammalian brain, in the dentate gyrus of the hippocampus and in the subventricular zone (SVZ) of the lateral ventricles. Mouse SVZ consists mainly of 4 different cell types: astrocyte-like stem cells (type-B), transit-amplifying progenitor cells (type-C), neuroblasts (type-A) and ependymal cells.
The aim of this project was to distinguish the different neural stem cell types and ependymal cells by their Ca^{2+}-response profile.
Therefore acute brain slices from mice of different ages (~P15, 2 month and 10 months) were prepared and loaded with the calcium-sensitive dye Fluo-4. For identifying GFAP-positive cells (astrocytes and astrocyte-like stem cells) a transgenic mouse line (hGFAP-mRFP1; Hirrlinger *et al.* Mol. Cell. Neurosci. 2005) was used.
Based on morphological, RFP-staining (RFP: red fluorescent protein) and agonist activity, cells could be classified into three different groups: ependymal cells (located on the border of the ventricle, ciliated), RFP-positive cells and yet unidentified but responding cells. Calcium responses were elicited by different agonists, i.e. phenylephrine, glutamate and ADP. Most cell types responded to ADP and/or ATP, suggesting the presence of metabotropic P2Y receptors in all SVZ cell types. The responses to other agonists were diverse and changed with the developmental stages. Thus, calcium responses may serve as an additional criterion to identify certain cell types in the SVZ.

Chemically defined differentiation of human astrocytes from iPSC derived neural stem cells for disease modeling

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Human astrocytes have a 25 times increased cell volume, develop 10 times more processes, support 20 times higher number of synapses and come in a broader variety of morphologically distinct subtypes than their rodent counterparts (Oberheim et al, 2006, Bushong et al, 2002, Oberheim et al, 2009). These differences indicate a very distinct role of human astrocytes which is still not revealed completely. However, in vivo studies from human samples are very restricted due to ethical reasons and limited availability. Thus, in vitro approaches become more important.
Several researchers have explored the prospective of human induced pluripotent stem cells (iPSCs) to differentiate into neurons and astrocytes, with neurons being the center of focus among brain cells. Only a few groups have successfully differentiated mature astrocytes from iPSC derived neural stem cells. These reports show that it takes a long period of about eight months to obtain functionally active, mature astrocytes from the progenitor cells (Krencik et al, 2011). This brings in a big limitation to the use of these long-term cultured cells in disease modeling.
The reason for the necessity of the extended culturing period can be found, when looking at the time neural stem cells need to undergo the gliogenic switch in vivo. Only then, astrocytic differentiation factors like CNTF will be effective in switching on the expression of genes like GFAP. Several in vitro studies have shown that epigenetic modifications are responsible for triggering the gliogenic switch.
In this study, we have developed a protocol to induce this gliogenic switch at an early stage in human iPSC derived neural stem cells. For final astrocytic differentiation, we further invented a cost-effective small molecule based approach and compared the differentiated cells with astrocytes generated by the conventional CNTF protocol. Astrocyte differentiation was evaluated by expression of key markers like GFAP. Maturation of these cells was further confirmed by a significant abrogation of the proliferation marker, Ki67. Functionality of the in vitro generated astrocytes was investigated by measuring spontaneous as well as ATP induced changes of intracellular calcium concentrations. In addition, we studied their capability of adopting a reactive stage after exposure to inflammatory molecules like TNF-a and found a significant upregulation of Lcn2 and Serpina3a. With these functional characteristics and short differentiation time, these small molecule differentiated glial cells illustrate a promising application in understanding the role of astrocytes and neuron-glia interaction in fundamental biology as well as pathophysiology.

Direct Conversion of Adult Cortical Oligodendrocyte Progenitor Cells to Functional Neurons

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Direct conversion of resident glia to neurons has recently emerged as a potential strategy for repair in the adult CNS. Oligodendrocyte Progenitor Cells (OPCs) are the most abundant proliferating resident neural cell population in the adult CNS. Their proliferative status and minimal functional contribution to the mature CNS (as currently known) suggest OPCs as an ideal target population for conversion. Adult rat cortical OPCs were isolated using Magnetic Activated Cell Sorting for O4 antigen selection and maintained as a primary culture for screening of putative neurogenic transcription factors. Retroviral delivery of the single factor neurogenin2 resulted in BIII-tubulin expression in OPCs 3 days post transduction. Mature neuronal markers NeuN and MAP2 were expressed by 7 days post transduction. Electrophysiological recordings of patch clamped transduced cells revealed evoked repetitive action potentials at 14 days post transduction. Spontaneous activity was recorded from transduced OPCs co-cultured with P1 rat cortical neurons and absent upon blockade of glutamatergic input with CNQX. Direct in vivo delivery of retroviral neurogenin2 to the adult rat cortex demonstrated co-expression of immature neuronal marker, doublecortin, in transduced cells one week following gene delivery. Transduced cells expressed NeuN at three weeks and exhibited mature neuronal morphology with extensive processes and dendritic spines. Direct in vivo conversion of resident OPCs may provide an alternative to cell transplantation for neuronal replacement or modulation of dysfunctional circuitry.

Efficient generation of human iPSC derived Parvalbumin interneurons for disease modelling

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Inhibitory GABAergic interneurons are capable of regulating the neuronal firing activity within the cerebral cortex. Parvalbumin (PV) expressing interneurons show fast-spiking properties that control the flow of excitation within the cortex and are implicated in the generation of gamma frequency oscillations which might further organize neural ensembles. The role of PV^+ interneurons has been implicated in various neurodegenerative and psychiatric disorders like Alzheimer's disease, Bipolar disorder and Epilepsy, which makes them an important target when trying to model these diseases, using stem cell based technologies. However, efficient generation of PV^+ interneurons in vitro using human induced pluripotent cells (iPSCs) has been a challenge thus far. The protocols already published *(Maroof et al, 2013; Nicholas et al, 2013 and Liu et al, 2013)* generate moderate amounts of PV^+ interneurons, only when co-cultured with mouse glutamatergic neurons or glial cells after an extensive culture time. We aim at developing an efficient, time optimized protocol for the generation of induced pluripotent derived PV^+ interneurons in an entirely human system. Our strategy involves two steps: I) providing early patterning cues to determine the fate of medial ganglionic eminence (MGE) neuronal progenitors and II) final maturation of PV^+ interneurons within synaptically active neuronal networks. We optimized the timing and the concentration of Purmorphamine treatment, which has been shown to effectively mimic sonic hedgehog in inducing ventral forebrain fate. In this way, we were able to generate cultures, comprised of 100% Nkx2.1 (MGE marker) and Foxg1 (forebrain marker) positive cells, which further differentiated into higher percentage of GABAergic interneurons. As soon as these cells adopt a post-mitotic neuronal stage, we purified them by immunopannning and co -culturing them with human iPSC derived glutamatergic neurons and astrocytes. These cultures are obtained separately by standard small molecule based protocols, using SB431542 and Dorsomorphin, but omitting ventralizing factors. Importantly, by multi-electrode recordings we could show, that mature glutamatergic cultures develop synaptically active networks, which provide the necessary excitatory input for the final maturation of PV^+ interneurons. Since direct functional analysis of these interneurons in humans is not possible, being able to efficiently generate and study the role of PV^+ interneurons in synaptically active human networks appears an exciting prospect when trying to model neurodegenerative or psychiatric diseases.

Expression and Function of Lin28B in the Autonomic Nervous System

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Lin28 is an evolutionary conserved RNA binding protein expressed in many developing tissues controlling the timing of differentiation. It is a major negative regulator of the let-7 family of microRNAs, which is involved in the control of differentiation. The mammalian homologues Lin28A and Lin28B are expressed in embryonic stem cells and have essential functions in the maintenance of stem cell pluripotency. Lin28A/B are not expressed in adult tissues but are activated in diverse tumors, including neuroblastoma. Neuroblastoma is a childhood tumor of the developing autonomic nervous system that arises from sympathoadrenal neuroblasts in sympathetic ganglia and adrenal medulla. Forced expression of Lin28B in mouse sympathetic neuroblasts elicits neuroblastoma formation during postnatal development. Lin28B-induced tumors are characterized by reduced let-7 expression and stabilization of the MYCN oncogene (Molenaar et al., (2012). However, the cellular and molecular mechanisms involved in tumor initiation by Lin28B are unclear. Lin28B is generally expressed in undifferentiated progenitor cells, maintaining progenitor identity by the inhibition of differentiation.
As proliferating sympathetic neuroblasts, the candidate tumor founder cells, display many characteristics of differentiated neurons (Scg10, Th, Dbh, Islet1) it was unclear how forced Lin28B expression in these cells can elicit tumor development. Here, we demonstrate that Lin28B is expressed throughout the development of sympathetic ganglia. Overexpression of Lin28B in sympathetic neuroblasts from the chick embryo leads to increased proliferation. Interestingly, this effect is restricted to a small time window in sympathetic ganglion development. The proliferation effect is not associated with increased expression of early progenitor markers, which argues against a role of Lin28B in lineage progression. We also exclude an action of Lin28B on the expression of IGFII, which is known to control neuroblast proliferation.

To address the action of Lin28B in the control of sympathetic neuroblast proliferation in more detail we study the role of let-7, the major Lin28B antagonist. Let-7 expression is analyzed in vivo during neurogenesis and the effects of let -7 knockdown and overexpression on sympathetic neuroblasts proliferation are investigated in vitro, as well as let-7 effects on Lin28B expression.

Filopodia-based Wnt transport during patterning of the neural plate in vertebrates

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Tissue development is a key process in living organisms. An essential component for these developmental processes - but also for tissue regeneration and stem cell regulation - is the communication of cells by paracrine signaling. Following the Wolpertian French flag model, these signaling processes are responsive to concentration gradients of signal carrying molecules, so-called morphogens. The highly conserved family of Wnt proteins can act as morphogens and represents an important regulator of anterioposterior patterning in the central nervous system. After secretion, specific transport mechanism must ensure proper distribution of the morphogen. Experimental studies in zebrafish embryos and human kidney cells have given first evidence for a novel short-range transport of Wnt morphogens from the Wnt active tissue towards receiving cells using filopodia, as signal transmitting structure. These specialized filopodia transport signaling proteins between communicating cells and allow a high degree of control of propagation speed, direction and concentration of the transmitted ligand. The crucial question is how this novel short-range mechanism can result in a long-range gradient of morphogen molecules covering the entire neural plate.

In order to give an answer to this question and address the theoretical feasibility of the hypothesis we have set up complementary Monte Carlo simulations. The simulation iteratively reproduces ligand production, cell migration, and a slight ligand decay in concordance with experimentally measured boundary conditions. In a filopodia mediated transport system the major parameters are not anymore diffusion rate, cell adhesion, and concentration of the ligand but length, angle distribution, and growth frequency of filopodia. During the simulation we were able to identify key parameters of the underlying mechanism and we were able to quantitatively reproduce previous experimental data. These results provide evidence that a filopodia based short-range transport system for Wnt has long-range signaling function and is therefore able to pattern the vertebrate neural plate during development.

Inducible formation of hippocampal oligodendrocytes from pre-existing local precursors

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In a process called myelination, oligodendrocytes wrap myelin sheaths around axons in the central nervous system (CNS). This axonal insulation increases the velocity of neuronal signal transmission by action potentials. Demyelination can occur as a result of disease, intoxication or injury. Subsequently, neuronal networks cannot communicate properly anymore and affected subjects experience persistent cognitive and motor impairments. Postnatally, oligodendrocyte precursor cells (OPCs) represent the largest endogenous source of progenitor cells in the CNS. Therefore, research in demyelinating diseases like multiple sclerosis (MS) has focused on the therapeutic potential of these adult OPCs. In chronic progressive MS patients, we could show that treatment with the hematopoietic growth factor erythropoietin (EPO) improved motor performance and memory functions. Translating these findings to experimental animals, we found that intraperitoneal application of EPO enhanced cognition and hippocampal LTP in juvenile mice. At the cellular level, we made the unexpected observation that a 3-week EPO treatment leads to an increase in the absolute number oligodendrocytes in the hippocampal CA1/CA3 subfields. This EPO -induced increase in oligodendrocyte number was based on direct differentiation of OPCs without prior cell proliferation. Indeed, labelling resident oligodendrocyte precursor cells with a tamoxifen-inducible NG2-CreERT2 knock-in approach confirmed that EPO caused pre-existing precursors to differentiate into mature oligodendrocytes. Additionally, this increase in hippocampal oligodendrocytes was accompanied by a significant increase in myelin proteins. These results suggest that the differentiation of OPCs can be directly driven by EPO treatment and lays the ground for future therapies of demyelinating diseases.

Interactions between the meninges and the cortical neuroepithelium time gliogenesis during cortical development

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Many organs develop by defined signaling events that depend on the timed interaction between epithial and mesenchymal tissues (kidney or tooth development for example). The development of the nervous system and that of the cortical neuroepithelium in particular, however, have rather been viewed as intrinsic and self regulatory. This view has been challenged by the analysis of Foxc1 mutant lines, which show a lateral expansion of the cortical neuroepithelium and consequently a delayed onset of neurogenesis. The important point is that Foxc1 is selectively expressed in the developing meninges that cover the cortical neuroepithelium. We have employed a co-culture system that allowed us to assess the cell biological consequences of the interaction between meningeal cells and cortical neural stem and progentor cells (NSCs). Surprisingly, we recorded an increase in gliogenesis rather than in neurogenesis as we had expected. We could biochemically identify three novel factors that appear to drive cortical NSCs out of the cell cycle and to promote their astroglial fate. Therefore, we think that the meninges represent a source for extracellular signaling cues that are important for cortical development.

Mutations in mouse Cdk5rap2; more than just microcephaly

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Patients with homozygous mutations in the Cyclin -dependent kinase 5 regulatory subunit-associated protein 2 (CDK5RAP2) gene suffer from autosomal recessive primary microcephaly type 3 (MCPH3) and intellectual disability at birth. Microcephaly is due to a reduction of brain volume which affects disproportionately the grey matter. This phenotype is most likely caused by a stem cell defect with a premature shift from symmetric to asymmetric progenitor cell divisions leading to premature neurogenesis, a depletion of the progenitor pool, and a reduction of the final number of neurons. In addition, we reported recently that a reduced propagation and survival of neural progenitors also contribute to the disease. *Cdk5rap2* mutant or *Hertwig's* anemia mice (*an/an*) have small brains and thin cortices already at early stages of neurogenesis during embryonic development. These mice were originally known for their hematopoietic phenotype and the recent identification of an exon 4 inversion in the *Cdk5rap2* gene led to their neurological assessment and their identification as a MCPH3 model. However, as Cdk5rap2 is a centrosomal protein and expressed in various tissues, other organs may be affected as well. Indeed mutant mice (*an/an*) show peripheral blood cytopenias, spontaneous aneuploidy, and a predisposition to hematopoietic tumors. The (*an/an*) males are infertile secondary to a severe germ cell deficiency, and we found a significant reduction in the testicular size compared to the control. The (*an/an*) females cannot deliver pups and, in line with this, we detected neither the uterus nor ovaries in these females. Additionally, we found that (*an/an*) mice exhibit eye abnormalities ranging from reduced size of one or both eyes (microphthalmia) to total absence of both eyes (anophthalmia). Our findings indicate that Cdk5rap2 functions not only as a regulator of neural progenitor proliferation but also affects the development of other organs in mice. Further studies in humans are warranted to analyze the significance of these findings for individuals with biallelic CDK5RAP2 gene mutations.

MYCN in Sympathetic Neurogenesis and Neuroblastoma Development

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Neuroblastoma (NB) is a childhood tumor that arises from developing sympathetic ganglia and adrenal medulla. Amplification of MYCN correlates with poor prognosis and MYCN overexpression in embryonic mouse sympathetic ganglia results in the postnatal generation of tumors that closely resemble human NB. MYCN cooperates with mutational activation of anaplastic lymphoma kinase (ALK) in animal models, promoting progression to NB. Our previous work demonstrated an essential role for Alk signaling in the proliferation of neuroblasts during neurogenesis in chick sympathetic ganglia, but the function of Mycn and its interaction with Alk signaling was unclear. Here, we analyze the expression of c-myc and Mycn and demonstrate an essential role for Myc proteins in embryonic sympathetic neuroblast proliferation using the BET bromodomain protein inhibitor JQ1 and selective siRNAs to interfere with c-myc and Mycn expression. The effects of MYCN overexpression is investigated both in vivo and in vitro using the PiggyBac transposon system. MYCN overexpression leads to significantly increased proliferation of sympathetic neuroblasts in vivo and supports long-term neuroblast proliferation in vitro. Interestingly, MYCN-induced proliferation is completely blocked by the addition of the Alk inhibitor TAE-684. These results reveal a cooperative interaction between Alk signaling and Myc function in sympathetic neuroblasts during development, resulting in the dependence of neuroblast proliferation on both Myc and Alk signaling. Thus, aberrant activation of proliferation regulators that are active during normal development of sympathetic ganglia and adrenal medulla may be sufficient to induce NB.

Neuronal organization of the I layer of human neocortex during prenatal development

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The first layer of the neocortex is a complex structure. There is growing evidence showing its importance in the development of the cerebral cortex. Studies of early prenatal ontogenesis (up to GW 20) in humans indicate a more complex structure of the layer I of the neocortex in humans than in animal models, even in comparison with primates.
The present researchs aims to identify the patterns in the development of the first layer of the cortex in the fetal brain during the second half of gestation.
The research was conducted on embryonic autopsy material 24-34GW. For identify neuronal organization was used immunohistochemical methods of analysis based on the neuronal markers: neurofilament heavy chain protein N200, microtubule-associated protein MAP2 and calcium-binding proteins calbindin and parvalbumin, and reelin.
The laminar structure of the cortex was revealed by DAPI nuclear staining and a location of immunoreactive cells within the cortex layer was identified. Cytoarchitecture was visually revealed by Nissl staining and a cortex stratification was assessed using optic density measuring.
Morphologically, three types of Cajal -Retzius neurons were identified, classified by Marin -Padilla as triangular cells, bitufted horizontal and irregular cells. There are two types of processes in the lower and the upper part of the plexus by cells of layer I: thick long straight processes which are always located within in the most lower part of the plexus, and the thin long straight processes which are located in both parts.
Our results suggest a more complicated structure of layer I than it was previously thought, and it suggests that these are area specific and age specific features of layer I.

Protocadherin18a influences neurogenesis in zebrafish thalamus

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In the past years, significant progress has been made in understanding molecular aspects of neurogenesis and brain development of several vertebrate models; however, there are many basic questions that remain to be answered. How is neurogenesis regulated in a developing brain part? Does the rate of proliferation versus differentiation alter the size of a brain area? Does cell adhesion influence neuronal differentiation? We address the question how neuronal cell adhesion is linked to neurogenesis.
It is known that cell adhesion molecules have an essential role in establishing and maintaining brain complexity. Protocadherins are Ca^{2+}-dependent transmembrane proteins, playing a role in cell-cell interactions. Focusing on the developing thalamus in zebrafish, we found a novel member of non-clustered protocadherins, *protocadherin18a* (*pcdh18a*) that is strongly enriched in the ventricular zone of the diencephalon. Our groundwork suggests a role for Pcdh18a in the establishment of thalamic complexity via Delta/Notch mediated neurogenesis.

Role of TGF-ß on the Development and Survival of Mouse Hindbrain Serotonergic Neurons

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Hindbrain 5-HT neurons produce serotonin and release of this neurotransmitter in the brain is thought to contribute to happiness and well-being. When 5-HT neurons fail to function properly, neurological disorders like depression and anxiety may arise. The role of specific 5-HT neurons sub-populations in these disorders is poorly understood and the drugs designed to act on the 5-HT system target globally rather than modulating neurotransmission at specific sub-populations. A better understanding of the development of serotonergic neurons might contribute to a better understanding of mood disorders and the development of more selective therapies. The development of serotonergic (5-HT) neurons however, is not fully understood. Transforming growth factor beta (TGF-ß) may influence this process as double knockout mice for TGF-ß 2/3 display a phenotype in dopaminergic neurons, a neuronal subpopulation that shares common developmental signals with hindbrain 5-HT neurons.

The aim of this study is to investigate the role of TGF-ßs in the development and survival of hindbrain 5-HT neurons and elucidate the underlying molecular mechanisms. Several conditional knockout mouse lines have been generated to characterize the phenotypic impact of TGF-ß family members on 5-HT neurons in the rostral and caudal hindbrain. The mouse model utilized Cre-Lox technology to specifically delete TGF-ß receptor 2 (TGFBR2) and TGF-ß ligand 2 (TGF-ß2) in specific brain regions. In the TGF-ß2 flox/flox: Krox20/Cre model and TGFBR2 flox/flox: Krox20/Cre, TGF-ß2 and TGFBR2 was knocked out in the caudal group of the hindbrain (rhombomeres 3 and 5), respectively. In the TGFBR2 flox/flox: En1/Cre model, TGFBR2 was knocked out in the rostral group (rhombomere 1). By deleting TGFBR2 or TGF-ß2 from various parts of the hindbrain, it was thought that 5-HT neuronal developmental would be disrupted.

The results show that in the TGFBR2 flox/flox: Krox20/Cre mice, absence of TGFBR2 causes no differences in the number of 5-HT neurons at embryonic day (E) 13.5 compared to control. Also, no difference in the number of 5-HT neurons was observed in the TGFBR2 flox/flox: En1/Cre model at E13.5 compared to control. Our data also demonstrate that the TGF-ß2 flox/flox: Krox20/Cre mice reveal significantly decreased numbers of 5-HT neurons in the caudal group in comparison to control. Moreover, the number of 5-HT neurons counted using immunohistochemistry was consistent with what was qualitatively seen by *in situ* hybridization of the serotonergic marker gene Pet1.

The results propose a TGF-ß2 dependency for proper 5-HT neuron development.

Role of TGF-ß on the Development of Midbrain Dopaminergic Neurons

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The dopaminergic (DA) neurons of the midbrain are involved in voluntary movement, as well as in regulation of behaviour. Their dysregulation has been linked to the development of depression and drug addiction, while their degeneration leads to the characteristic symptoms of Parkinson's disease. A better understanding of the molecular mechanisms that lead to the development of the DA neurons might provide valuable information for the generation of new and better therapeutic alternatives for these ailments, as well as give further insights for the regenerative medicine field. It is known that the formation of the midbrain DA neurons is regulated by diffusible factors like Sonic Hedgehog and Fibroblast Growth Factor 8. Increasing evidence supports that Transforming Growth Factor ßs (TGF-ßs) also participate in the formation of these neurons. However, a molecular mechanism by which TGF-ß acts on these neurons is yet to be elucidated.

In this work we aim to understand the role that TGF-ß signalling plays on the development of midbrain DA neurons. For this purpose, we have generated a conditional knockout mouse model in which a region of the midbrain, including the DA neurons, lacks the TGF-ß Receptor II (TGFBR2). This model was generated using the Cre-lox system by crossing the En1-Cre line, in which the expression of the recombinase Cre is controlled by the Engrailed 1 promoter, and the TGFBR2 -flox line, in which the TGFBR2 gene is floxed.

Through the use of immunohistochemistry on brain cryosections, we found that these conditional knockout mice have a decreased number of DA neurons in the ventral midbrain at embryonic stages. This provides further evidence that TGF-ß signalling is necessary for a proper development of these neurons. In order to understand the molecular mechanism by which TGF-ß acts in this process, we performed a cDNA microarray to analyse the transcriptome of the ventral midbrain of conditional knockout mice and compared it with that of wild type mice. Among the genes differentially regulated by the loss of the TGFBR2, we found genes that fall into the category of receptors, ion transporters, phosphatases and molecular chaperones, among others. Some of the differentially regulated genes have been shown to be implicated in processes like inflammation and endosomal sorting. Future analysis will be focused on understanding the function these genes have during the generation of the midbrain DA neurons, in order to shed some light onto the mechanism by which TGF- ß acts on these process. Another goal of this project is to fully characterize the phenotype of the mouse line.

Targeted disruption of the serine/threonine kinase ULK4 gene leads to abnormal neurogenesis and congenital hydrocephalus-like phenotype

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Previous genetic studies demonstrate that *ULK4* gene in humans is associated with a range of neuropsychiatric conditions including schizophrenia, autism, bipolar and depression. Unc-51-like kinase 4 (*ULK4*) gene encodes a novel serine/threonine kinase. Functional analyses of the *ULK4* gene by knockdown approach in neuroblastoma cells show that depleted ULK4 expression disrupts the composition of microtubules, compromises neuritogenesis and cell motility, and modulates multiple signalling pathways. Additional evidence of gene expression suggests that Ulk4 is regulated by developmental cues, and there is a developmental switch for Ulk4 isoform expression during mouse brain development and neuronal maturation, highlighting potential roles of *Ulk4* in brain formation and function. In this report, we have carried out neuroanatomical and pathophysiological studies in *Ulk4* targetedly disrupted mice, by comparison with wildtype littermates, which uncovered a range of abnormalities in the *Ulk4*$^{-/-}$ mice. Here we showed that *Ulk4* deficiency led to significant pre-weaning loss, and postnatal *Ulk4* null mice displayed reduced body size. The majority of *Ulk4*$^{-/-}$ mice exhibited typically hydrocephalus -like phenotype with domed heads. Neuroanatomical analysis revealed a congenital hydrocephalus phenotype, which become severer during early postnatal period. We have detected abundant *Ulk4* expression in the ependymal cells lining the ventricular systems including lateral ventricle, third ventricle, aqueduct and subcommissural organ (SCO), suggesting that *Ulk4* may play a vital role in the production and circulation of the cerebrospinal fluid. *Ulk4* was also widely expressed in the cortex and hippocampus, and thinner cortex was observed in *Ulk4-/-* mice. This was correlated with a decreased pool of proliferating progenitors at E15.5, which was demonstrated by reduced BrdU (S-phase marker) and PH3 (M-phase marker) positive cells in the ventricle zone (VZ). BrdU birthdating analysis of P0 *Ulk4*$^{-/-}$ mice showed a mild but significant modulation of neuronal migration during cortical genesis, however, the cortical lamination architecture was largely preserved in P12 *Ulk4*$^{-/-}$ mice. These data demonstrate that *Ulk4* plays critical roles in neural proliferation, neuronal migration and CSF circulation.

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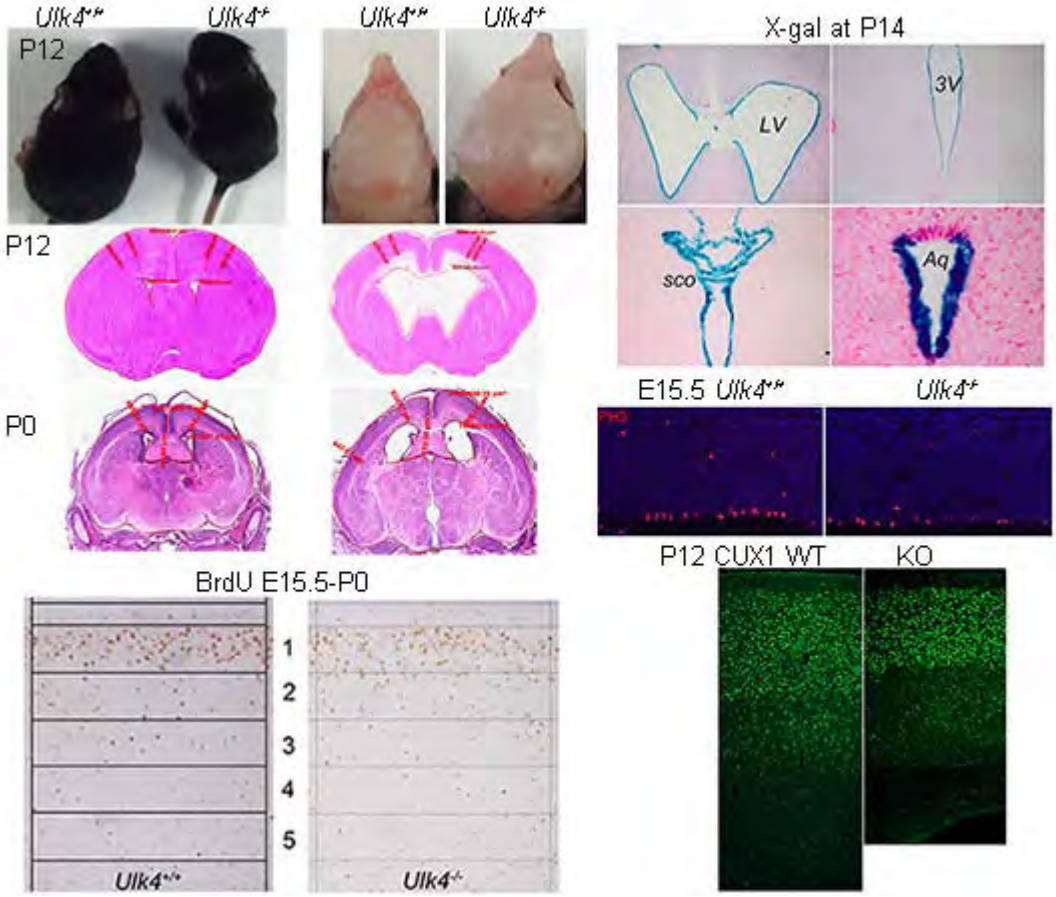
Ulk4+/+
Ulk4-/-
P12
Ulk4+/+
Ulk4-/-
X-gal at P14
LV
3V
sco
Aq
P12
P0
E15.5 Ulk4+/+
Ulk4-/-
BrdU E15.5-P0
1
2
3
4
5
Ulk4+/+
Ulk4-/-
P12 CUX1 WT
KO

Tcf7l2 steers neuronal diversity required for asymmetric brain formation and function

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Anatomical differences between left and right hemispheres and functional lateralization are conserved features of the vertebrate brain. Yet, the molecular mechanisms underlying brain asymmetries and their functional connection are poorly understood.

Like in many animals, the bilateral habenular nuclei in the zebrafish forebrain are asymmetric such that distinct neuronal populations of the dorsal habenula differ in size across the left-right axis. We find that this difference is abrogated in the absence of Tcf7l2 function. Tcf7l2 is a transcriptional regulator of the Wnt/beta-catenin signaling pathway and functions to activate the pathway during the establishment of habenular asymmetry. We show that it acts cell-autonomously in presumably all post-mitotic habenular precursor cells and influences neuronal cell-type outcome. The differences in this outcome between left and right is due to varying Wnt signaling activity in the environment the precursors are born into. As a result, visual information is not relayed by the left habenula only like in normal embryos but bilaterally in the symmetric *tcf7l2* mutant brain. These observations are a step towards understanding the molecular mechanisms underlying brain asymmetry and the generation of neuronal diversity. Moreover, it is one of the first links between anatomical and functional asymmetries of the brain.

The habenular neural circuit is a highly conserved neurotransmitter system, which relays cognitive information into mid- and hindbrain structures. In humans, mutations in *tcf7l2* and habenular dysfunction have been independently linked to pathophysiological syndromes such as depression and schizophrenia. Thus, it is tempting to speculate on an evolutionarily conserved link. We are now imaging habenular network development over four days in the living zebrafish embryo. The understanding of the circuit's temporal-spatial formation together with the underlying genetic cascades required for this process is the prerequisite to investigate the information flow in the healthy and the manipulated brain.

Tgfbr2 conditional knock-out in the developing telencephalon results in neurovascular defects

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To understand the role of transforming growth factor beta (TGFß) in forebrain development, we studied TGFß-signalling in vivo via a Foxg1-cre knock-in mouse to conditionally knock-out the TGFß receptor 2 (Tgfbr2). Surprisingly, the ventral telencephalon of Foxg1cre/+;Tgfbr2flox/flox (Tgfbr2-cKO) mutants displayed intracerebral hemorrhages, although FOXG1 is mainly expressed in progenitors and neurons of the telencephalon. Clustering of endothelial cells and reduced vessel branching were hallmarks of the impaired vasculature of mutant embryos. In contrast, pericytes covered endothelial tubes as in control animals and cell junctions connected endothelial cells. However, the integrity of the extracellular matrix (ECM) was disturbed in Tgfbr2-cKO. Endothelial cells and pericytes did not express Foxg1 in significant amounts. Thus, the vascular defects arose from disturbed signals between neurons and vessels, involving an altered secretome. Accordingly, we observed a variety of expression changes in mutant brains, which affected growth factors such as IGFs and IGF -binding proteins, TGFß, and VEGF-A. Furthermore, ECM-associated molecules were altered such as THBS-2, ADAMTS-1, integrins as well as integrin-associated proteins. We determined the altered secretome as well as the neural proteome to reveal molecular factors involved in this complex phenotype. We studied the effects of the altered secretome using conditioned medium (CM) from primary neuronal cultures of different regions derived from Tgfbr2 -cKO and control forebrains. HUVECs that were treated with this CM displayed less branching points and showed less migration towards it. Among the candidates that we explored in greater detail were VEGF-A, IGFs and TGFß. VEGF-A supplementation reverted the observed alteration of HUVEC branching and migration, whereas application of TGFß severed these conditions. In conclusion, Tgfbr2 -cKO mice give the opportunity for analysing neural alongside endothelial development and factors that are mediating synchronized development.

The ectonucleotidase NTPDase2 controls progenitor cell proliferation in neurogenic niches of the adult mouse brain

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In the adult rodent brain nerve cells are continuously generated from stem cells in the subventricular zone (SVZ) of the lateral ventricles and the hippocampal dentate gyrus. Increasing evidence suggests that extracellular purine and pyrimidine nucleotides are involved in the control of both embryonic and adult neurogenesis[1]. These nucleotides act via ionotropic P2X or metabotropic P2Y receptors. We have previously noted that stem/progenitor cells in both the SVZ and the subgranular layer (SGL) of the dentate gyrus express high levels of plasma membrane-bound nucleoside triphosphate diphosphohydrolase 2 (NTPDase2)[2]. NTPDase2 hydrolyzes extracellular nucleoside di - and triphosphates, thus modulating their effect on nearby nucleotide receptors. Deletion of this ectoenzyme would increase local extracellular nucleoside triphosphate concentrations, perturbing purinergic signaling and boosting progenitor cell proliferation and neurogenesis. Using newly generated mice globally null for *Entpd2*, we demonstrate that NTPDase2 is the major ectonucleotidase in these progenitor cell rich regions[3]. Loss of NTPDase2 abrogates ectonucleotidase activity in the neurogenic niches but does not affect levels of activity and protein of other ectonucleotidases. It causes increased progenitor cell proliferation in both the SVZ and the SGL without affecting long-term progeny survival and new neuron formation. Lack of NTPDase2 leads to expansion of the hippocampal stem cell pool as well as of the intermediate progenitor type -2 cells. Cell expansion is subsequently lost at around type -3 stage, paralleled by decreases in CREB phosphorylation in the doublecortin-expressing progenitor cell population and by increased apoptosis as demonstrated by increased labeling for activated caspase-3.
We propose that NTPDase2 has functionality in scavenging mitogenic extracellular nucleoside triphosphates in neurogenic niches of the adult brain. The enzyme thus functions as a homeostatic regulator of nucleotide-mediated neural progenitor cell proliferation and expansion.

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Poster Topic

T2: Axon and Dendrite Development, Synaptogenesis

T2-1A Alpha-synuclein is associated with the synaptic vesicle apparatus in the human and rat enteric nervous system
Martina Böttner, Tobias Fricke, Melanie Müller, Martina Barrenschee, Thilo Wedel

T2-2A Axogenesis in the antennal sensory system of the grasshopper: pioneer neurons
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T2-3A Cdh13 in the developing mouse nervous system
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T2-4A Cortical progenitors release the chemokine CXCL12 (SDF-1) to promote ingrowth of thalamocortical afferents
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T2-1B Fascicle switching: an ancient pattern of axogenesis in a modern brain
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T2-2B Generation and Analysis of a Novel Transgenic Mouse Line with Defective Fiber Tracts
Maria Eleni Kastriti, Marina Theodosiou, Marina Vidaki, Kostas Theodorakis, Domna Karagogeos

T2-3B LIM-domain-binding proteins: Interaction in neuronal development and epileptogenesis
Barbara Karoline Iwaniuk, Robert Maresch, Alexander Grote, Rebecca Kulbida, Karen MJ van Loo, Heinz Beck, Susanne Schoch, Albert J. Becker

T2-1C Local stimulation of mouse hippocampal neurons by recombinant prion protein induces rapid neurite outgrowth
Ladan Amin, Xuan Thi Anh Nguyen, Gabriele Giachin, Giuseppe Legname, Dan Cojoc

T2-2C Molecular mechanisms of exocytosis of large dense core vesicles in dorsal root ganglion neurons
Ali Hussein Shaib, Margarete Klose, Jens Rettig, Barbara Niemeyer, Ute Becherer

T2-3C Neuronal functions of RIM3γ and RIM4γ
Katrin Michel, Sara Ferando-Colomer, Johannes A. Müller, Ana-Maria Oprisoreanu, Albert Becker, Dirk Dietrich, Susanne Schoch

T2-1D Neuroplastins interact with TRAF6 to initiate cell signaling
Sampath Kumar Vemula

T2-2D Postnatal development and plasticity of anatomical pathways suitable for multisensory integration processes in rodent primary sensory cortices A1, S1, and V1
Julia U. Henschke, Patrick Kanold, Henning Scheich, Eike Budinger

T2-3D Quantification of hippocampal and cortical network activity in awake neonatal mice.
Robin Till Hinsch, Stephan Marguet, Walid Fazeli, Dirk Isbrandt

T2-4D Reelin induces branching of neurons and radial glial cells during corticogenesis
Xuejun Chai, Li Fan, Hong Shao, Xi Lu, Wei Zhang, Jiawei li, Jianlin Wang, Shulin Chen, Michael Frotscher, Shanting Zhao

Alpha-synuclein is associated with the synaptic vesicle apparatus in the human and rat enteric nervous system

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Background & aims:
Aggregation of alpha-synuclein (a-syn) has been implicated in the development of neurodegenerative diseases including its spread from the enteric nervous system (ENS) to the brain. Physiologically, a-syn is located at the presynapse and might be involved in regulating neurotransmission. Therefore, the aim of the study was to characterize the physiological ontogenetic and regional expression pattern of a-syn in the ENS and its association with the synaptic vesicle apparatus.
Material & methods:
Ontogenetic mRNA expression of a-syn and synaptophysin was determined in the rat intestine. Myenteric plexus cultures treated with glial cell line-derived neurotrophic factor (GDNF) were assessed for mRNA expression of a-syn, co-localization of a-syn with the pan-neuronal marker PGP 9.5 and the synaptic vesicle marker synaptophysin and studied by scanning electron microscopy (SEM). Human colonic specimens were subjected to co-localization studies of a-syn with synaptophysin.
Results:
a-syn and synaptophysin intestinal gene expression levels were highest during early postnatal life and also detectable at adult age. a-syn was co-localized with PGP 9.5 and synaptophysin in myenteric plexus cultures and up-regulated after GDNF treatment. SEM confirmed the presence of neuronal varicosities to which a-syn was associated. Consistently, a-syn and synaptophysin showed partial co-localization in the human ENS.
Conclusions:
The ontogenetic and locoregional expression pattern as well as the regulation by GNDF give evidence that a-syn is physiologically associated to the synaptic vesicle apparatus. The data suggest that a-syn is involved in the regulation of synaptic plasticity in the ENS during early postnatal life and adult age.

Axogenesis in the antennal sensory system of the grasshopper: pioneer neurons

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In axogenesis, pioneer cells generate axons which first navigate a pathway for subsequently developing neurons. Pioneers have been identified via neuron-specific labels in various nervous systems[1,2] and found to use similar navigational cues to reach their targets[3,4]. The earliest pioneer neurons in the grasshopper antenna are two pairs of HRP-positive cells which delaminate from the neuroepithelium at the tip at about one third of embryogenesis and extend their axons towards the base of the antenna (Fig.1). The pioneer neurons establish the initial dorsal and ventral nerve tracts of the antenna onto which the axons of sensory neurons later project. Near the base of the antenna, another neuron, the base pioneer, originates from the mesectoderm, as shown by Mes3 immunolabeling. Both sets of pioneers also express the cell surface lipocalin Lazarillo. Immunoblocking of Lazarillo in whole embryo culture disrupts axogenesis and demonstrates that the base pioneer guides the axons of the tip pioneers.

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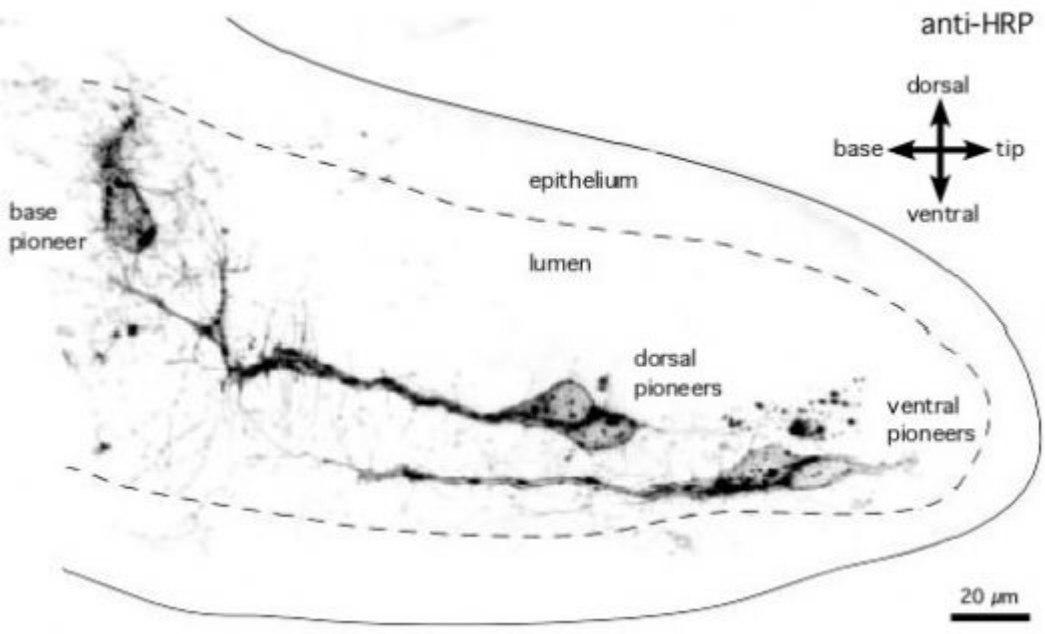

Cdh13 in the developing mouse nervous system

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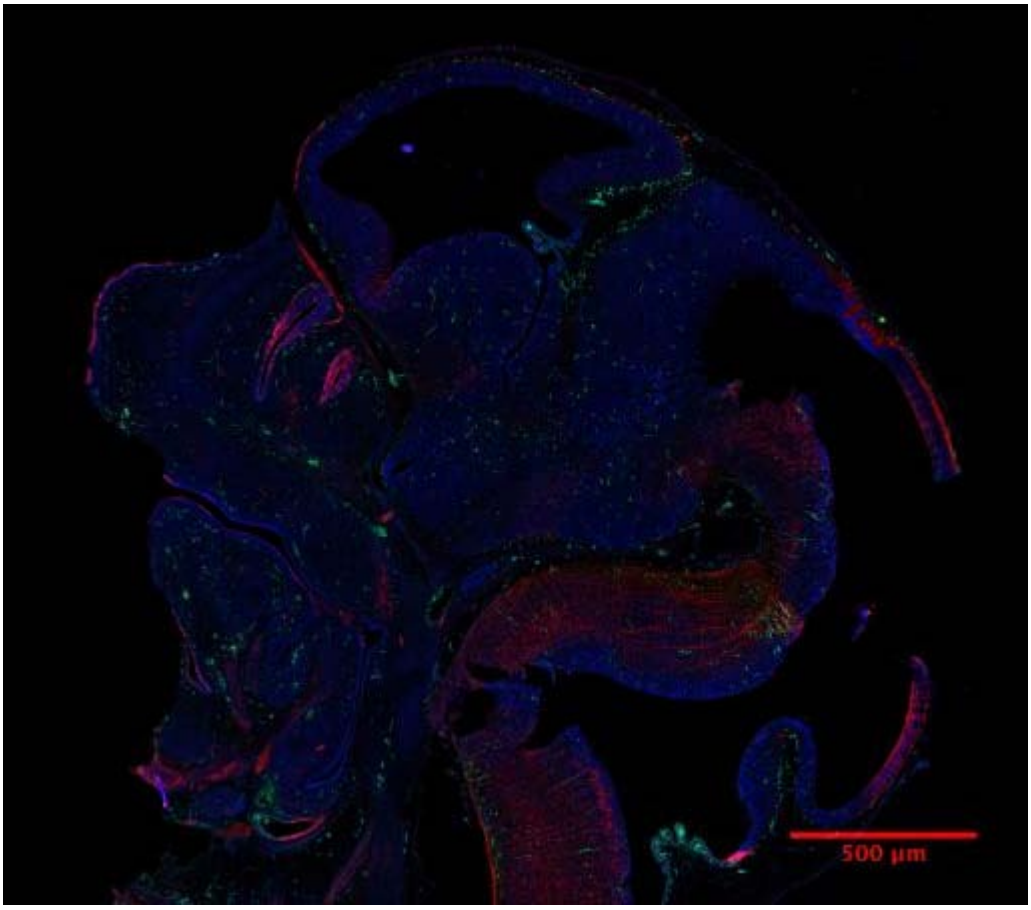
Bachground: During development, the pre and postnatal brain is shaped by many different factors for growth, differentiation, migration and survival. Together, such factors have also been related to the pathogenesis of many (developmental) brain disorders, such as attention-deficit/hyperactivity disorder (ADHD), autism and depression. An interesting new genetic factor we would like to investigate in this context is CDH13, which encodes Cadherin -13, a member of the calcium dependent cell adhesion protein family. Cadherins are important molecules for tissue formation, proper cell adhesion and neuronal growth.

Aim and Method: While previous evidences support the role of Cdh13 in the development and guidance of motor axons, there is no equivalent information regarding the central nervous system. It was our aim to make a observational study to identify locations and the role of CDH13 in the developing mouse brain using immune-histological methods.

Results: Here we show/report the regional and temporal expression pattern of Cdh13 in the mouse developing brain, from embryonic day E13.5 to E17.5. Currently, we are also investigating how Cdh13 deficiency affects the anatomy and neuronal subtype distribution of different developing brain areas. CDH13 expression first appears in regions like the spinal cord, from where the expression expands to the hindbrain and then becomes more abundant in the midbrain and telencephalon. During this time, CDH13 appears not only in fiber tracts, but also at regions like the mid-hindbrain border. Starting E15.5, at E17.5 a distinct pattern in the isocortex of differently layers of CDH13 intensity emerge, while older regions of CDH13 expression intensities start lessening.

Conclusion: The observed anatomical and cellular expression pattern suggests the importance of Cdh13 in the formation and innervation of areas tightly linked to emotional regulation, attention and cognition. Analysis of the corresponding regions might reveal insights into critical time points for development of different brain circuits. Expression in the raphe nuclei for example coincides with the migration of 5HT positive cells in a along a ventro-dorsal axes.


500 µm

Cortical progenitors release the chemokine CXCL12 (SDF-1) to promote ingrowth of thalamocortical afferents

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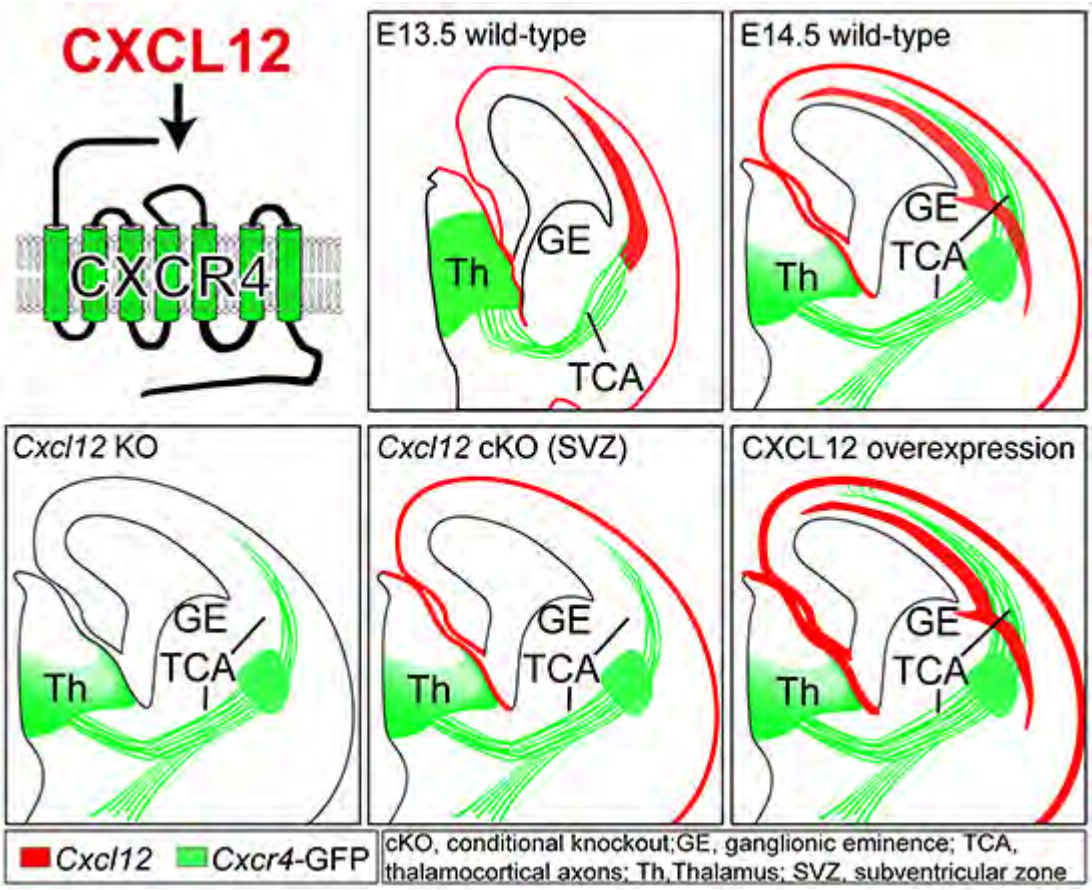
Little is known about factors that coordinate the production of new neurons with the supply of long-range afferents. Here, we demonstrate that chemokine receptor CXCR4 and its only known endogenous ligand CXCL12 (stromal cell-derived factor-1, SDF-1), a system known to regulate neuronal migration, enables cortical progenitors to stimulate ingrowth of thalamocortical axons (TCAs). First, we established *Cxcr4* gene expression patterns in the developing thalamus. From E12 to E14, *Cxcr4* was detected in the ventricular zone and in postmitotic neurons forming thalamic nuclei. After E14, *Cxcr4* decreased strongly in medial nuclei but continued at a high level in lateral nuclei including the lateral geniculate. *Cxcl12* was expressed in the meninges covering thalamus and cortex, and in cortical intermediate progenitor cells (IPCs). Next, we analyzed CXCR4 activation in wild-type- and *Cxcl12*-deficient mice using a phospho-sensitive anti-CXCR4 antibody. This revealed CXCL12-dependent CXCR4 activation in thalamic nuclei that were juxtaposed to the meninges. Examination of the gene expression of neuronal guidance factors in the thalamus of *Cxcl12*$^{-/-}$ embryos showed increased transcript levels of *Robo1*, a receptor of the axonal repellant Slit1. We thus hypothesized that Slit1- and CXCL12-pathways might interact to regulate growth of TCAs and examined their effects in thalamic explants. We found that CXCL12 dose-dependently stimulated axonal outgrowth and that Slit1 reduced this CXCL12 growth promoting effect, suggesting that the Slit1/Robo-pathway inhibits TCA growth downstream of CXCR4. Next, we examined TCAs in vivo using *Cxcr4*-GFP reporter mice. *Cxcr4*-GFP^{+} TCAs crossed the pallium/subpallium boundary and reached the territory of *Cxcl12*-expressing cortical IPCs at E13.5. Tracing technology and examination of *Cxcr4*-GFP^{+} TCAs in Cxcl12$^{-/-}$ mice revealed attenuated intracortical TCA elongation in the absence of CXCL12. Overexpression of CXCL12 in vivo enhanced intracortical TCA elongation and caused premature cortical plate invasion. Strikingly, conditional ablation of Cxcl12 in cortical IPCs decreased intracortical TCA elongation. Taken together, we demonstrate that Slit1 and CXCL12 exert opposing effects on the growth of thalamic axons. CXCL12, emanating from the meninges stimulates CXCR4 receptors in the cell bodies of thalamic neurons to regulate gene expression of guidance cues while cortical progenitors release CXCL12 to promote ingrowth of TCAs. Thus, CXCL12 coordinates production of cortical neurons and intracortical growth of TCAs.


CXCL12
CXCR4
E13.5 wild-type
GE
Th
TCA
E14.5 wild-type
GE
TCA
Th
Cxcl12 KO
GE
TCA
Th
Cxcl12 cKO (SVZ)
GE
TCA
Th
CXCL12 overexpression
GE
TCA
Th
Cxcl12
Cxcr4-GFP
cKO, conditional knockout;GE, ganglionic eminence; TCA, thalamocortical axons; Th,Thalamus; SVZ, subventricular zone

Fascicle switching: an ancient pattern of axogenesis in a modern brain

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The midbrain neuropils of Panarthropoda exhibit a wide spectrum of neuroarchitectures - from rudimentary to highly elaborated - and which at first sight defy a unifying neuroarchitectural principle. Developmental approaches have shown that in model arthropods such as insects[1,2], conserved cellular and molecular mechanisms first establish a simple axon scaffold for the midbrain. However, to be adapted for adult life, this immature groundplan must be restructured into a modular, chiasmal/columnar neuropil known as the central complex. The transformation is executed by a process known as "fascicle switching", in which subsets of neurons systematically redirect their growth cones from anterior to posterior commissural fascicles at stereotypic locations across the midbrain.
Although the mode of generating neurons can differ significantly across the Panarthropoda - individual precursor cells in insects, crustaceans and onychophorans, as opposed to invaginated proliferative clusters in chelicerates and myriapods - the molecular mechanisms regulating precursor selection may be conserved. Equally, fascicle switching also features in the midbrain of all panarthropods studied despite the different modes of neurogenesis and varying degrees of neuropilar elaboration. The differences likely reflect both the extent to which fascicle switching has progressed during development and the lifestyle of the individual organism, but the preserved mode of axogenesis argues for the process being an ancient, conserved, feature of the Panarthropoda.

Support: Deutsche Forschungsgemeinschaft, Graduate School of Systemic Neuroscience

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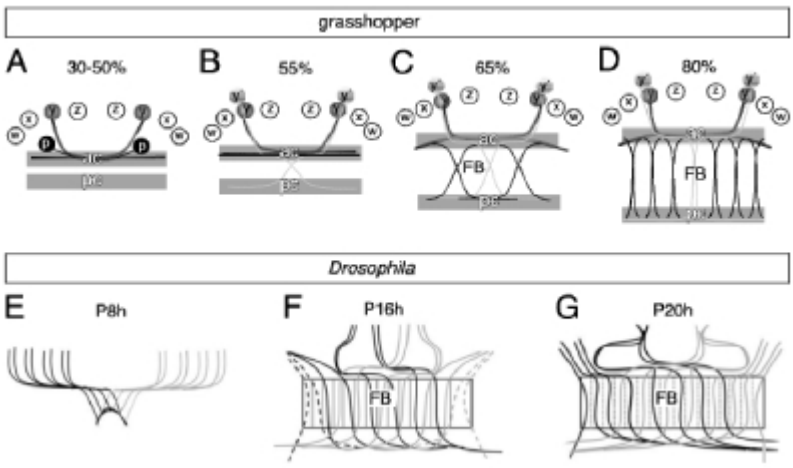

Generation and Analysis of a Novel Transgenic Mouse Line with Defective Fiber Tracts

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The mammalian cortex receives major sensory input from the thalamus through thalamocortical axons (TCAs) and sends information back to the thalamus via cortical efferents (corticothalamic axons or CTAs). Based on the "handshake" hypothesis, these two axonal systems are thought to constitute a case of codependent axonal growth and guidance. Early generated CTAs from the pioneer neurons of the subplate interact with TCAs to navigate correctly.
Cortical efferents are expressing high levels of the protein Transient Axonal Glycoprotein-1 or Contactin-2 (TAG-1/Cntn2), a neuronal recognition molecule of the immunoglobulin superfamily which is involved in neurogenesis, neurite outgrowth and fasciculation. Among other neuronal subpopulations, it is expressed early by pioneer neurons in the preplate and later on in the marginal zone and subplate of the developing cortex.
We took advantage of the unique expression of TAG-1 by CTAs to study the formation and function of these axons in vivo by generating the transgenic mouse line *Tag-1*$^{loxP\text{-}GFP\text{-}loxP\text{-}DTA}$. This line expresses GFP under the *Tag-1* promoter, also encompassing the coding sequence of Diptheria Toxin subunit A (DTA) under quiescence. We crossed these mice to the *Emx1::Cre* line, which expresses the Cre recombinase specifically in the embryonic cortex. Upon crossing, GFP expression is eliminated and the toxin starts being expressed in TAG-1$^+$ neuronal cells of the neocortex resulting in their death.
We summarize the examination of the coincidence of the transgene expression with that of endogenous TAG-1 and the profile of the cells that express the transgenic GFP. Additionally, we performed a preliminary analysis on *Emx1::Cre;Tag-1::DTA* mice. We show that transgenic GFP is expressed according to the endogenous pattern and that upon Cre recombination, extensive cell death takes place in the cortex. As a result, there are a number of defects associated with this formation.
Analysis of the developing cortex reveals extensive cell death resulting in a significantly smaller cortex. Although some CTAs remain, they are significantly reduced as shown both by immunohistochemistry and DiI tracing experiments in the embryonic neocortex. Postnatally, absence of the anterior commissure, abnormal corpus callosum formation and a smaller hippocampal formation are observed. Moreover, cortical layering seems to be preserved in part, since the lamination is present in the normal sequence but the thickness of each layer is reduced. Therefore, the partial elimination of TAG-1$^+$ cortical neurons results in a severe phenotype. Analysis is in progress to reveal the full phenotype and understand the functional consequences of the lack of TAG-1$^+$ neurons in the developing and adult mouse cortex.

LIM-domain-binding proteins: Interaction in neuronal development and epileptogenesis

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LIM-domain-binding (LDB) proteins are multi adapter proteins, critical for brain development, that interact with different transcription factors and kinases like the STE-20 like kinase (SLK). LDBs and SLK are strongly expressed during brain development especially in cortical brain regions, suggesting a critical role during neurogenesis. It is described that LDB1 and LDB2 interact with each other and that they have functional redundancy, however, only little is known about LDBs interaction with SLK and their functional role during mammal brain development. Here, we examined in detail the functional interplay of LDB1 and -2 with SLK in brain development and potentially aberrant patterns in the emergence of cortical malformations. shRNA-mediated silencing of mouse LDB1, LDB2 or SLK in cortical neurons resulted in aberrant morphology affecting axons and dendrites. Whereas single knock down of LDB1 or LDB2 could be rescued by SLK overexpression double knock down could not be completely rescued indicating the necessity of LDB-SLK interaction for regular neurite growth in vitro. Intraventricular in utero electroporation of SLK shRNAs resulted in morphological as well as functional deficits, in affected cortical neurons and cortical architecture and aggravates pentylenetetrazol (PTZ)-induced seizures. Furthermore functional analysis revealed reduced miniature inhibitory postsynaptic currents in SLK knock down pyramidal neurons together with a postnatal loss of gephyrin-positive puncta. These findings indicate fine tuned interactions between LDB and SLK during neuronal development and might therefore contribute to the emergence of a functional neuronal network. Conversely, perturbation of this network leads to dysplastic and hyperexcitable neurons. Our finding of reduced expression of LDB1 and LDB2 in human dysplastic neuronal ganglioglioma components may render this mechanism also to certain extent and functional equivalent in human gangliogliomas. Such new insights in epileptogenicity of focal lesions may provide a basis for improved therapy development in pharmakoresistant epilepsy patients.

Local stimulation of mouse hippocampal neurons by recombinant prion protein induces rapid neurite outgrowth

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The cellular prion protein (PrP^C) is a (GPI)-anchored protein which is highly expressed in the nervous system and plays an important role in the development of the central and peripheral nervous systems. While its abnormal conformer is associated with neurodegeneration and prion diseases, its physiological function still remains elusive. In order to investigate the role of PrP^C in neuronal guidance and growth cone steering we developed an experimental procedure to deliver controlled amount of purified full-length recombinant prion protein (recPrP) to specific targets such as mouse hippocampal growth cones. In this technique, recPrP at low concentration was encapsulated in phospholipid micro-vesicles which were manipulated by a laser tweezer and positioned near an exploring hippocampal growth cone. The molecules were released by vesicle membrane photolysis and reached to the growth cone by free diffusion. The growth cone dynamics and neurite elongations were monitored by time-lapse phase contrast and FRET microscopy during the entire duration of the experiment.

Our results indicated that local delivery of recPrP enhanced neurite outgrowth and growth cones turned towards the source. On the other hand neither the N-terminal nor C-terminal domains of recPrP enhanced the neurite elongation. Inhibitors of Src kinases, including p59Fyn, Rho kinase, ERK, protein kinase A (PKA) and phosphatidylinositol 3-kinase (PI3k) blocked the effect of recPrP on neurite elongation, suggesting that multiple signaling pathways were involved in transduction of recPrP-mediated signals. Neurite outgrowth enhancing was completely abolished when recPrP was delivered to neurons knock-out for the prion protein ($Prnp^{0/0}$) suggesting that recPrP required physiologically active PrP^C on the membrane to exert its functions. All together, our findings predicted that full-length recPrP function as signaling molecules and membrane-anchored PrP^C function as cellular adhesion molecules and could be signaling receptor for recPrP. Therefore their homophilic interactions promoted cell adhesion to extra cellular matrix and facilitated neurite outgrowth and cytoskeleton rearrangement.

Molecular mechanisms of exocytosis of large dense core vesicles in dorsal root ganglion neurons

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Dorsal root ganglion (DRG) neurons transmit sensory information to the central nervous system through glutamatergic synaptic transmission that can be modulated by neuropeptides. They are contained in large dense core vesicles (LDCVs) and can be as diverse as calcitonin gene -related peptide (CGRP), neuropeptide Y (NPY), substance P, etc. LDCVs are exocytosed in response to specific stimulus at the cell somata and in afferent terminals along with synaptic vesicles (SVs). The release machinery of these LDCVs is poorly understood. Thus, we investigate the role of the priming factors CAPS1 and CAPS2. Using PCR, we verified that both CAPS isoforms are expressed. With total internal fluorescence reflection microscopy, we visualize in real time the release of LDCVs that are labeled through Lenti virus driven expression of NPY-Venus. We found that the amount of secreting neurons was raised by 20% upon CAPS 1 or CAPS 2b overexpression and that the number of secreted LDCV per responding neuron was more than twice as high in comparison to WT control. CAPS1 and 2 double KO showed mild or no reduction in the number of secreted vesicles but a significant decrease in the amount of secreting cells was observed. Since the density of LDCVs at the plasma membrane was not affected by CAPS expression level, our result suggest that CAPS2b can be a priming factor for LDCVs in DRG-neurons. We also examine the role of CAPS in SV priming. For this we have established a DRG-dorsal horn neurons co-culture to allow synapse formation. Additionally, we designed a Lenti virus encoding for vGlut tagged to the pH sensitive fluorescent protein mNectarin to specifically label SVs and visualize their exocytosis. With these tools we are investigating whether CAPS plays a differential role in priming of LDCV vs. SV.

Neuronal functions of RIM3γ and RIM4γ

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The morphology of neurons is immensely complex and essential for the proper development and function of neuronal networks in the brain. Neurite outgrowth requires the polarized trafficking of newly synthesized membrane and cargo to the tip of the neurite and of signaling molecules back to the soma. This cellular transport is mediated through several independent intermediate processes, each of which requires its own set of regulatory proteins. We have previously shown that the short variants of the RIM protein family, RIM3γ and RIM4γ, which are composed of only the RIM specific C_2B domain and an isoform specific N-terminus, play an essential role during neuronal arborization and the development of dendritic spines (Alvaréz-Baron et al., 2013). However, to date it is not understood how the two proteins contribute to the regulation of these processes.

For the large RIM family members, RIM1 and RIM2, a role in the regulation of several aspects of presynaptic neurotransmitter release, like in docking and priming of synaptic vesicles as well as in the recruitment of Ca^{2+}-channels to the active zone, is well established. A potential synaptic function of RIM3γ and RIM4γ was suggested by the finding that γ-RIMs modulate Ca^{2+}-channel opening times by binding to the ß subunit of voltage gated Ca^{2+}-channels (Uriu et al., 2010). However, the role of RIM3γ and RIM4γ in synaptic transmission at native central nerve terminals has not yet been determined.

Here, using live cell imaging experiments on RIM3γ and RIM4 γ knockdown neurons we observed changes in vesicular transport routes suggesting, that the correct supply of new membrane and proteins to sites of neuronal outgrowth is disrupted after loss of RIM3γ and RIM4γ. In order to directly evaluate the functional role of RIM3γ and RIM4γ in vivo and during different developmental stages we generated RIM3γ and RIM4γ knockout mice. RIM4γ knockout mice develop a strong phenotype of episodic ataxia and seizure-like episodes at around P21, accompanied by reduction in weight and body size. RIM3γ knockout mice in contrast seem to be healthy and show no obvious phenotype. In order to address synaptic function of γ-RIMs we performed electrophysiological measurements in the hippocampal CA1 region on acute brain slices of knockout mice and wild type littermates. The differential sensitivity of field potentials to ω-conotoxin-GVIA suggests a presynaptic phenotype in RIM4γ but not in RIM3γ knockout mice.

In summary, these results provide new insights into the role of γ-RIMs in neurite outgrowth and network formation and explore the largely unknown synaptic fuction of the small members of the RIM protein family, RIM3γ and RIM4γ.

Neuroplastins interact with TRAF6 to initiate cell signaling

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The cell adhesion molecule neuroplastin (Np) is associated with cortical thickness and intellectual ability in humans. Np has four isoforms generated by alternative splicing. Np55 possesses two extracellular immunoglobulin-like domains. Np65 has three such domains. Np55 and Np65 also may contain a four amino acid insert DDEP in their cytoplasmic domains. Np expression has been shown to promote neurite outgrowth in cultured hippocampal neurons. In particular Np55 is highly expressed when neuronal circuits have been formed after birth. Additionally, Np65 expression starts later and coincides with the major period of synaptogenesis to regulate synapse formation/stabilization, e.g. via trans-synaptic adhesion. We recently showed that Np deficiency leads to decreased synapse numbers and alterations in actin cytoskeleton in hippocampal neurons. Here we report a putative binding motif for the tumor necrosis factor receptor-associated factor 6 (TRAF6) in the Np cytoplasmic domain. TRAF6 is an E3 ligase and adaptor protein expressed in neurons. We show Np binding to TRAF6 and consequences for signaling events involved in synapse formation/stabilization. Over-expression of either Np55- or Np65-GFP increased the dendritic complexity as well as the number and length of dendritic protrusions in neurons and promoted filopodia formation in HEK cells. Np-induced filopodia were reduced by 70% when TRAF6 was knocked down using siRNA. All GFP-tagged Nps recruited cytoplasmic TRAF6 to the cell membrane and filopodia in transfected HEK cells. In co-precipitation assays TRAF6-Flag and Np-GFP were co-immunoprecipitated from extracts of co-transfected cells. Mutations of key amino acids in the putative TRAF6 binding motif of Np65 reduced the co-precipitation of TRAF6-Flag compared to wild-type Np65-GFP. Moreover, either inhibition of ERK1/2 (by PD98059), PI3K (Wortmannin), p38MAPK (SB202190), and NF-KB (SN50) patways reduced the number of filopodia in Np-transfected cells. Currently we are also evaluating the underlying signaling pathways involved in Np-dependent dendrite branching. Therefore, we propose that Nps bind TRAF6 and thus initiate signaling cascades including NF-KB and/or PI3K/Akt/WASP pathways leading to changes in actin cytoskeleton and gene transcription during synapse formation or stabilization and synaptic plasticity.

Postnatal development and plasticity of anatomical pathways suitable for multisensory integration processes in rodent primary sensory cortices A1, S1, and V1

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Multisensory integration does not only recruit higher-level association cortex, but also low-level and even primary sensory cortices. Recently, we showed that the primary auditory (A1), somatosensory (S1), and visual (V1) cortex of adult Mongolian gerbils receive convergent inputs from brain structures of non-matched senses (Henschke et al., Brain Structure and Function, 2014). The underlying anatomical pathways include a thalamocortical (TC) and a corticocortical (CC) system, which might preferentially serve short latency integration processes in the primary sensory areas. Here, we ask how the multisensory TC and CC systems develop and change during the total lifespan of the animals. Knowledge about mechanisms underlying these changes during development will provide insights into plastic processes caused by experience, learning, and sensory deprivation.
We approached the issue by stereotaxic pressure injections of the retrograde tracer Fluorogold into A1, S1, and V1 at postnatal days 1 (somatosensation already active), 9 (before ears and eyes open), 15 (ear canals open), 21 (eyes open), 28 (weaned), 120 (young adult), and 1000 (old age) to identify cells that project to the particular areas. Our cell counts demonstrated especially in the first postnatal weeks large changes of the developing multisensory TC and CC connections; mainly due to an initial competition between the developing senses and a subsequent structural consolidation of the sensory pathways during the critical and sensitive phase. At adult stages, many crossmodal TC and CC connections remain; however, most of them disappear during aging.
To test if the changes in crossmodal connectivity are due to a selective generation of projection neurons, a loss of these neurons, a reorganization of axonal branches, and/or changes in the nature of the transmission systems (driving, modulatory, inhibitory) we counterstained the histological sections with antibodies against markers for neurogenesis (Doublecortin), cell apoptosis (Caspase-3), axonal plasticity (GAP34), and calcium-binding proteins (Calbindin, Parvalbumin). Our results show that during normal development TC and CC projection neurons are not newborn, do not die, and major axonal reorganization processes are mediated by neurons within the non-lemniscal thalamic nuclei and primary sensory cortices itself

Quantification of hippocampal and cortical network activity in awake neonatal mice.

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The mouse is a well-established model organism used in neuroscientific research. The generation and characterization of genetic mouse models have specifically advanced the understanding of the pathophysiology of human neurological diseases. Neonatal mice offer the opportunity to postnatally study developmental disease stages whose corresponding time window would be the prenatal period in humans.

Physiological, and pathological, developmental changes in electrical brain activity of mouse pups have mostly been studied in in-vitro, or anesthetized in-vivo, preparations. To characterize the temporal dynamics of electrical brain activity in healthy neonates, we established head-fixed multi-channel (silicon probe) in-vivo recordings from hippocampus and cortex of locally anesthetized, awake mice between postnatal days P3 and P14.

We recorded local field potential (LFP) dynamics from the primary visual cortex (V1), which, prior to eye opening (at P12-P13), mostly reflect intrinsic retinal, thalamic, and cortical activity. Using a second electrode, we simultaneously recorded the LFP depth profile along the CA1-dentate gyrus/CA3 axis in the hippocampus.

In V1, we found two major types of oscillatory events. The first type comprised 5 to 10-s events with frequencies between 4 to 15-Hz and a spectral peak frequency that increased with age, which were similar to the so-called spindle bursts described in neonatal rats. Oscillations with frequencies in the 15 to 20-Hz beta (ß) range were the second, less frequently occurring type of oscillatory events, whose spectral frequency was consistent across ages. ß-oscillations are pronounced between ages P5 and P7, at which time the events are longer in duration than at other ages. By P12 to P14, the period of eye opening, the incidence of both event types became highly variable, which was concomitant with an increase in baseline activity. The higher variability in activity between P12 and P14 may represent transitions occurring with eye opening.

In the hippocampus, we observed oscillations in stratum radiatum of CA1 with 10 to 30-Hz power that lasted 2-30 s and had their longest durations between P9 and P14. Sharp wave-ripple oscillations, signature events in the CA1 pyramidal layer of adult rodents, were first detected at P9, peaking between ~100 to 150-Hz. By P12 sharp waves displayed two depth profiles with distinct amplitude maximums. The first had a maximum in stratum radiatum, which is likely to reflect the population activity in CA3; whereas the second group had an amplitude maximum in str. lacunosum moleculare, indicating the maturation of the distal dendrites of CA1 pyramidal cells and perforant path input from entorhinal cortex.

Overall, the pronounced dynamic changes in cortical and hippocampal in-vivo network patterns (increased variability and event length) from P9 to P12 parallel the previously described developmental switch from “discontinuous” to “continuous” activity, in which solitary, stereotyped events give way to persistent and diverse activity.

In summary, our data provide a basis for the qualitative and quantitative characterization of neonatal network activity in genetic mouse models.

Reelin induces branching of neurons and radial glial cells during corticogenesis

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Neurons born in germinal zones migrate for long distances along the processes of radial glial cells (RGCs) to reach their final positions in the cortex. Here, we visualized individual migrating neurons and RGCs using in utero electroporation. Combined with immunocytochemistry against Reelin, we demonstrate that intensive branching of migrating neurons and RGCs is closely correlated spatiotemporally with the distribution of endogenous Reelin. Time-lapse imaging shows that the leading processes of migrating neurons give rise to more and more branches in the Reelin-containing zone accompanied by gradual shortening of the leading processes after the growth cone has contacted the Reelin-containing zone. Absence of Reelin in reeler mice results in severe migration defects and morphological abnormalities of neurons in the reeler cortex. The neurons have lost their bipolar orientation and were disoriented. Moreover, in reeler mice the branching of the basal processes of RGCs is severely disrupted in the marginal zone that in wild-type animals is strongly immunoreactive for Reelin. Consistent with previous reports, we show that in dissociated reeler cortical cultures exposure to recombinant Reelin enhanced dendritic complexity and glial branching. Our results suggest that Reelin induces branching of the leading processes of migrating neurons and of basal processes of RGCs when they arrive at the Reelin-containing marginal zone. Branching of these processes may be crucial for the termination of nuclear translocation during the migratory process and for correct neuronal positioning.

Poster Topic

T3: Developmental Cell Death, Regeneration and Transplantation

T3-1A Cell type-specific differences in activity-dependent postnatal apoptosis in neocortical cultures
Oriane Blanquie, Anne Sinning, Heiko Luhmann

T3-1B Influence of the nicotinamid-nucleotid-transhydrogenase on the manifestation of perinatal hypoxic-ischemic brain lesions in the mouse
Sandra Semar, Thomas Tschernig, Carola Meier

T3-1C Neuroprotective effect of grafted murine induced pluripotent stem cells on injured spinal motoneurons following ventral root avulsion in rats
Antal Nógrádi, Krisztián Pajer, Csilla Nemes, Sára Berzsenyi, András Dinnyés

T3-1D Regeneration of the subgenual complex in the stick insect *Sipyloidea sipylus*
Reinhard Lakes-Harlan, Stefanie Weis, Johannes Strauß

Cell type-specific differences in activity-dependent postnatal apoptosis in neocortical cultures

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A wave of neuronal apoptosis occurs in all mammals, ensuring the proper development of the nervous system. This physiological process, which triggers the loss of up to 50% of the neurons in certain brain areas, happens mostly during the last trimester of gestation in humans and the first two postnatal weeks in rodents.

Here, we studied the developmental profile of apoptosis in different neuronal subpopulations using immunochemistry in dissociated cortical cultures from neonatal mice. We were particularly interested in a transient neuronal subpopulation, the Cajal-Retzius neurons (CRNs), which disappear by the end of the second postnatal week *in vivo*. Our current data suggest that the time course and the rate of postnatal apoptosis also vary between different neuronal subpopulations *in vitro*. Whereas about 60% of GABAergic neurons undergo apoptosis between DIV 6 and 12, the majority of CRNs (80 %) has already undergone apoptosis by DIV 9. Moreover, about 30% of the overall population of neurons is lost from DIV 3 to DIV 14.

While the regulation of early apoptosis is well understood in the peripheral nervous system, the mechanisms underlying apoptosis in central neurons remain rather elusive. Previous work from our group and others suggest that electrical activity is a major regulator of neuronal cell death in the brain. Based on this, we first studied how chronic blockade of electrical network by application of TTX affects the rate of cell death in two different neuronal populations. We also studied the effect of chronic application of gabazine on the electrical activity pattern using MEA recordings, and then investigated the effect of these activity modulations on the rate of apoptosis.

In summary, our data demonstrate that neuronal populations *in vitro* are dissimilarly affected by modulations of the electrical activity patterns. Thus, distinct mechanisms seem to control cell survival and cell death in the different populations of neurons. To conclude, this in vitro study can provide further insights into the mechanisms underlying the neurodevelopmental activity-dependent apoptosis occurring in rodents as well as in humans.

Influence of the nicotinamid-nucleotid-transhydrogenase on the manifestation of perinatal hypoxic-ischemic brain lesions in the mouse

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In the brain, ischemia and oxygen deficiency lead to neuronal cell death within minutes. This process is irreversible and causes crucial deficits for the affected patient. The impact of a hypoxic-ischemic insult is especially fatal if it occurs during the early postnatal phase of life. Hypoxic-ischemic brain damage results in cognitive and sensorimotor impairment of the surviving child, including spasticity, malfunction in coordination and movement, and cognitive deficits. As a result of the oxygen deficiency, homeostasis of glutamate and calcium is disturbed and formation of reactive oxygen species (ROS) is increased, leading to necrotic and apoptotic cell death.
Nicotinamid-nucleotid-transhydrogenase (Nnt) is a mitochondrial protein that promotes the reduction of ROS by catalyzing the regeneration of NADPH. The lack of Nnt, in turn, is known to result in increased ROS levels (Nickel et al., 2013). We therefore hypothesize that a hypoxic-ischemic insult will have a larger manifestation in mice lacking Nnt as compared to Nnt-positive mice.
To investigate this hypothesis, a hypoxic-ischemic insult was induced in Nnt-positive mice (Nnt+) and insult manifestation was compared to that of mice, which have no functional Nnt (Nnt-). In addition to lesion size, expression of cleaved-caspase-3, a marker for apoptosis, and CD68, a marker of activated microglia and macrophages, were compared.
There were significant differences in all three parameters investigated between the Nnt+ and Nnt- mice. However, results revealed that – in contrast to the original hypothesis – lesion size, apoptosis and microglia activation were more pronounced in Nnt+ mice than in Nnt- mice.
In summary, Nnt clearly has an effect on the outcome of hypoxic-ischemic lesions in the early postnatal mouse brain. As the insult is associated with excitotoxicity and calcium increase, one possible explanation for our finding is that for instance changes in ATP requirement and calcium release modulate processes at the inner mitochondrial membrane. Further studies will focus on ROS-pathway associated proteins and investigate their action under hypoxic-ischemic conditions.

Reference:

Nickel A, Kohlhaas M, Maack C (2013) Mitochondrial reactive oxygen species production and elimination. J Mol Cell Cardiol 73: 26-33.

Neuroprotective effect of grafted murine induced pluripotent stem cells on injured spinal motoneurons following ventral root avulsion in rats

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Avulsion of one or more ventral roots induces dramatic motoneuron loss. Novel therapeutic approaches aiming at the alleviation of motoneuron death involve transplantation of stem cells, including induced pluripotent stem cells (iPSCs). Here we investigated the neuroprotective potential of transplanted undifferentiated mouse iPSCs following avulsion of the lumbar 4 ventral root, an injury known to induce the death of the majority of affected motoneurons. Transplantation of iPSCs induced an improved reinnervation of reimplanted ventral root by host motoneurons as compared with controls (number of retrogradely labelled motoneurons: 503 ± 45 [grafted] vs 48 ± 5 [control]). Functional reinnervation was confirmed by muscle force measurement of the reinnervated muscles. The extensor digitorum longus (EDL) and the tibialis anterior (TA) muscles of grafted rats were innervated by significantly greater numbers of motor axons than the muscles of control animals (15±0,8 [grafted] vs 5±0,6 [control] motor units for EDL, and 17±0,7 [grafted] vs 6±1 [control] for TA). The grafted cells differentiated into neurons and astrocytes in the injured cord. These findings suggest that iPSCs are able to rescue damaged motoneurons and promote the regeneration of their axons into the reimplanted ventral root.
TÁMOP-4.2.4.A/2-11/1-2012-0001 'National Excellence Program' and EU FP7 projects (STEMMAD, PIAPP-GA-2012-324451; STEMCAM, PIAP-GA-2009-251186, Anistem, PIAPP-GA-2011-286264; EpiHealth, HEALTH-2012-F2-278418; EpiHealthNet, PITN-GA-2012-317146).

Regeneration of the subgenual complex in the stick insect *Sipyloidea sipylus*

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Insects can regenerate neuronal elements after lesion to different extend. Stick insects are able to regenerate lost body appendages to a seemingly normal appearance. Here we investigate in detail, whether this peripheral regeneration also comprises the regeneration of neuronal architecture and that of complex sensory organs.

Nymphs of different stages had one midleg ablated. The anatomy of the nervous system in the proximal tibia of the regenerated leg was evaluated in adult animals by tracing the nerves with cobalt chloride. The neuronal architecture in regenerated legs was surprisingly similar to that of the control legs. In the regenerates (except after amputation in stage 6) a main leg nerve can be found, which may be named nervus cruris, like in normal legs. A nerve branch from the nervus cruris innervates a complex sensory organ, the subgenual organ complex, again in both, the normal and regenerated leg. In controls, this sensory complex is a very sensitive vibration receiver and consists out of different subparts (Strauss & Lakes-Harlan, 2013, J Comp Neurol 521, 3791). The complex organisation with subgenual organ proper, anterio-ventral subgenual organ and Distalorgan is completely regenerated. However, the size of the organs is reduced compared to controls. Independent of the stage of amputation, the number of sensory units of the subgenual organ is about 50% of the controls. Similarly, the length of the Distalorgan is less than 50% of the control length.

In summary, the general anatomy of the nerves and sensory organs is regenerated, however, the number of cells is reduced. Whether regeneration occurs depends on the stage and the architecture seems to be outlined in the beginning of regeneration, with no improvement or continuous growth during further postembryonic development.

Poster Topic

T4: Neurotransmitters, Retrograde messengers and Cytokines

T4-1A CAPS1 critically regulates BDNF release and intragranular pH of secretory granules
Robert Eckenstaler, Volkmar Leßmann, Tanja Brigadski

T4-1B Muscarinic receptor control of pyramidal neuron membrane potential in medial prefrontal cortex (mPFC) in young rats.
Przemyslaw Norbert Kurowski, Maciej Gawlak, Pawel Szulczyk

T4-1C No evidence for role of extracellular choline -acetyltransferase in generation of gamma oscillations in rat hippocampal slices *in vitro*
Jan-Oliver Hollnagel, Rizwan ul Haq, Christoph J Behrens, Anna Maslarova, Istvan Mody, Uwe Heinemann

T4-1D Pre- and postsynaptic effects of norepinephrine on neurotransmission between Layer 4 excitatory neurons in rat barrel cortex
Jiali Tang, Gabriele Radnikow, Dirk Feldmeyer

CAPS1 critically regulates BDNF release and intragranular pH of secretory granules

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The mammalian neurotrophin brain-derived neurotrophic factor (BDNF) is an important regulator of a variety of brain functions including neuronal development and synaptic plasticity. The protein is stored in secretory granules and released in an activity-dependent manner. Although the activity-dependent release of BDNF is assumed to be a key element for the induction of synaptic plasticity processes in neurons, the molecular mechanisms of secretory granule exocytosis in neurons largely remained elusive.

We now investigated the relevance of the priming factor CAPS1 for the exocytosis of BDNF-containing secretory granules in dissociated hippocampal neurons from mice. The cells were transfected with plasmids coding for BDNF-GFP and shRNA directed against CAPS1. Depolarization-induced secretory granule exocytosis was analyzed by monitoring the intragranular GFP-fluorescence intensity using time-lapse video microscopy. Fusion pore opening of BDNF-containing secretory granules and BDNF-GFP content release were strongly affected following CAPS1 knockdown. Furthermore, although the average size and protein content of BDNF-GFP secretory granules remained unchanged after CAPS1 knockdown, the intragranular pH value was significantly higher in hippocampal neurons transfected with CAPS1-shRNA. We also determined the role of CAPS1 in transmitter release by destaining of synaptic boutons loaded with the styryl dye FM®1-43. While density and size of synaptic boutons was unaffected after CAPS1 knockdown, the efficiency of synaptic vesicle release was significantly reduced.
Our results demonstrate that endogenous CAPS1 plays an important role in regulating both, neurotransmitter release from synaptic vesicles as well as protein release from single secretory granules and that CAPS1 has a previously unrecognized additional function in regulating intragranular pH.

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Muscarinic receptor control of pyramidal neuron membrane potential in medial prefrontal cortex (mPFC) in young rats.

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Aim: Cholinergic muscarinic input to the medial prefrontal cortex (mPFC) neurons controls learning, working memory and attentional processes. Damage of the cholinergic system has been implicated in several neuropsychiatric disorders such as schizophrenia, Alzheimer's disease and other forms of senile dementia. Attenuation of this system also occurs during physiological aging. The aim of this study was to clarify the mechanism responsible for control of the medial prefrontal cortex (mPFC) pyramidal neurons by muscarinic receptors.

Material and methods: Experiments were performed on mPFC pyramidal neurons in slices isolated from young (18-22-day-old) male rats. Recordings of membrane potential were performed with the gramicidin perforated-patch method in the absence of Ca++ ions and in the presence of tetrodotoxin (TTX, 1 μM) in extracellular solution. Glutaminergic and GABAergic transmission were blocked.

Results: The application of cholinergic receptor agonist (carbamoylcholine chloride, CCh, 100 μM, n=33) evoked membrane potential depolarization (10.0±1.3 mV), which was completely eliminated by the M1/M4 (pirenzepine dihydrochloride, 2 μM, n=6) and M1 (VU 0255035, 5μM, n=6) muscarinic receptors antagonists, and by removing Na+ ions from the extracellular solution. In the presence of Nav1.9 antibodies (4 μg/ml, n=7) in the intracellular solution, the amplitude of CCh-dependent depolarization was significantly lower than the amplitude of CCh -induced depolarization in the presence of IgG control antibodies (4μg/ml, n=7). We also demonstrated that mPFC pyramidal neurons express type NaV 1.9 channels. Furthermore, application of the inhibitor of G-protein ßγ-subunits (gallein, 20 μM, n=12) reduced CCh -dependent depolarization to 1.7±0.42 mV (n=12). CCh -induced depolarization was not affected by: nicotinic receptor antagonist (mecamylamine hydrochloride, 10 μM, n=5); channel blockers, including Ba2+ ions (200 μM, n=6), tertiapin-Q (300 nM, n=5), apamin (100 nM, n=7), flufenamic acid (200 μM, n=5), 2-APB (200 μM, n=6), SKF 96365 (50 μM, n=5), and ZD 7288 (50 μM, n=8); Na+/Ca++ exchanger blocker, benzamil (20 μM, m=11) and intracellular transduction system blockers, including U 73122 (10 μM, n=5), chelerythrine chloride (5 μM, n=6), SQ 22536 (100 μM, n=5) or H-89 (2 μM, n=5), applied to the extracellular solution.

Conclusion: Activation of M1 muscarinic receptors evokes depolarization of mPFC pyramidal neurons due to activation of Nav 1.9 -like Na+ channels via G-protein ßγ-subunits (in a membrane-delimited mode).

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No evidence for role of extracellular choline-acetyltransferase in generation of gamma oscillations in rat hippocampal slices *in vitro*

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In the central nervous system acetylcholine (ACh) is critically involved in regulation of arousal, attention and REM sleep (Metherate et al., 1987; Steriade et al., 1993). Elevated ACh levels during explorative behavior are likely to induce network oscillations in the theta (θ)- and γ-band (Klinkenberg et al., 2011; Picciotto et al., 2012). Acetylcholine is synthesized from choline by choline-acetyltransferase (ChAT), an enzyme which transfers acetate from acetyl-CoA onto choline. This enzyme is considered to be localized inside cholinergic terminals and serves as a marker of cholinergic neurons (Léránth and Frotscher, 1987). After synthesis, ACh is then transported into vesicles and eventually released. At the postsynaptic site, the enzyme acetylcholineesterase (AChE) terminates the signal transmission by hydrolyzing ACh. Recently it has been suggested that ChAT is also present in the extracellular space thus permitting synthesis of ACh outside neurons (Vijayaraghavan et al., 2013).

To prove this finding, we studied the ACh metabolism and pharmacologically intervened at different steps. We tested for effects of physostigmine alone and physostigmine in combination with ACh on γ-oscillations in hippocampal slices. We found that physostigmine alone could dose-dependently induce γ-oscillations which were strongly augmented by ACh. Surprisingly, the choline uptake inhibitor HC-3 did not prevent generation of γ-oscillations by physostigmine but rather led to a slight augmentation suggesting that ACh synthesis can occur under conditions when choline transport is blocked.
To clarify whether additional ACh can be synthesized by extracellular choline-acetyltransferase (ChAT) we applied (2-benzoylethyl)-trimethylammonium iodide (BETA) which antagonizes the synthesis of ACh by blocking ChAT (Chen et al., 1993; Galea and Estrada, 1991). In another set of experiments we applied acetyl-CoA, a substrate for choline-acetyltransferase to form ACh. While we observed a (slight) reduction of physostigmine-induced γ-oscillations by BETA, there was no evidence for a facilitation of γ-oscillations in presence of acetyl-CoA. Together these findings suggest that there is no evidence for an extracellularly located choline-acetyltransferase.

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Pre- and postsynaptic effects of norepinephrine on neurotransmission between Layer 4 excitatory neurons in rat barrel cortex

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Norepinephrine (NE) plays a prominent role in the regulation of sleep and wakefulness, which impacts animal behaviour. The majority of NE in the neocortex is released from synaptic boutons of locus coeruleus neurons, the axons of which project throughout the entire brain. Although subcortical regions are the primary targets of noradrenergic modulation, neocortical neurons are also strongly affected by NE. However, the exact effects of NE on neurotransmission in the neocortex are so far not entirely clear. In this study we investigated effects of NE on synaptic transmission using whole-cell patch clamp recording from both single and synaptically coupled neurons in the barrel cortex.

Acute rat brain slices were prepared from the neocortex of aged postnatal day (P) 17 to P21 old Wistar rats. The lamination and the barrel cortex were clearly visible at 20-fold magnification of microscope. Whole-cell patch clamp recordings of single and synaptically coupled L4 excitatory neurons were performed. Voltage or current traces were recorded during both control condition and NE application.

NE (100 µM) hyperpolarizes single neuron (on average hyperpolarisation amplitude 8.8±2.0 mV) together with a 15% decrease in the input resistance. Paired recordings showed that 10 µM NE causes 14.04% reduction in the EPSP amplitude, 19.20% reduction in the 20%-80% rise time, and 27.84% reduction in the decay constant. These reductions are statistic significant. 10 µM NE also causes a significant increase in the coefficient of variation of EPSP amplitudes (0.17±0.11 vs. 0.20±0.11). These results suggest that NE decreases membrane resistance, which leads to the reduction of EPSP amplitude, meantime NE reduces release probability which also contributes to the EPSP amplitude reduction. As a result, NE suppresses presynaptic transmission by reducing release probability and suppresses postsynaptic transmission by decreasing membrane resistance of postsynaptic cell.

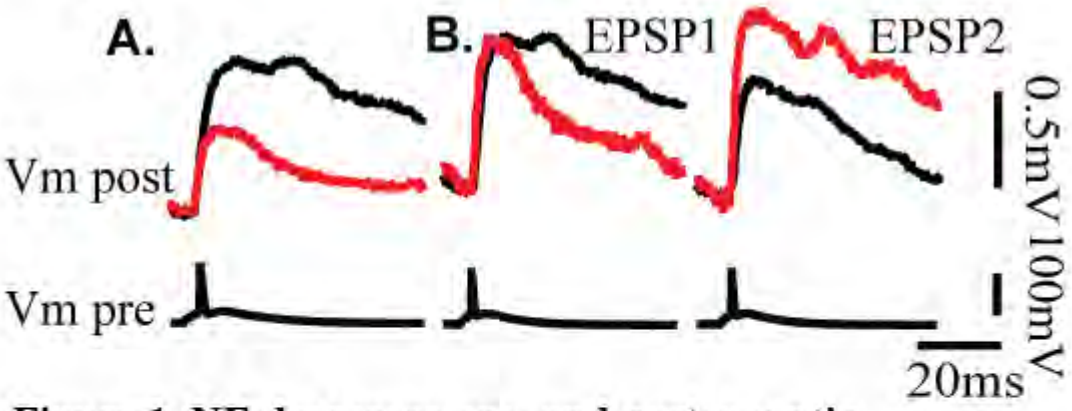

Figure 1. NE decreases pre- and postsynaptic transmission between L4 excitatory neurons

A,NE reduces the amplitude of the evoked EPSPs (red). B, scaling the first amplitude of the EPSPs obtained in NA (red) to the first EPSPs amplitude in control (black) reveals an increase in second EPSP (EPSP2).

Poster Topic

T5: G Protein-linked and other Receptors

T5-1A Activation of μ-opioid receptors inhibits intercalated interneurons and modulates feed-forward inhibition in the mouse amygdala
Lena Goedecke, Peter Blaesse, Michael Bazelot, Marco Capogna, Hans-Christian Pape, and Kay Jüngling

T5-2A Deciphering the neuronal circuit activated by the death-associated odor cadaverine
Milan Dieris, Gaurav Ahuja, Venkatesh Krishna, Sigrun Korsching

T5-1B Modeling and experimental verification of ligand-receptor interaction in a high affinity cadaverine receptor with an unusual bifunctional ligand requirement.
Kanika Sharma, Gaurav Ahuja, Arnd Baumann, Sigrun Korsching

T5-2B Molecular mechanisms of olfactory detection in polyphagous moth *Spodoptera littoralis*: Deorphanization of odourant receptors via the *Drosophila* empty neuron system.
Fredrik Schlyter, William B. Walker , Muhammad Binyameen, Arthur de Fouchier, Claudia Steiner, Christelle Monsempes, Annick Maria, Marie-Christine François, Peter Anderson, Bill S. Hansson, Thomas Chertemps, Nicolas Montagné, Emmanuelle Jacquin-Joly, Mattias Larsson

T5-3B Possible regulation of CB1-Receptor by RGS-proteins in a cell type specific manner
Sebastian Loch, Bernadette Mohr, Krisztina Monory

T5-2C Orexin-CRF Receptor Heteromers in the Ventral Tegmental Area as Targets
Gemma Navarro Brugal, Cesar Quiroz, David Aguinaga, Estefania Moreno, Antoni Cortes, Vicent Casado, Josefa Mallol, Rafael Franco, Carme LLuis, Sergi Ferre, Enric I, Canela

T5-1D The *v1r*-related Ora5 receptor is not expressed in ciliated and microvillous neurons, the major olfactory receptor neuron populations.
Daniel Kowatschew, Shahrzad Bozorg Nia, Yuichiro Oka, Sigrun Korsching

T5-2D Transactivation of TrkB receptor through the Adenosine receptor A2A-R leads to changes in downstream signaling cascades
Stefan Wiese, Teresa Tsai, Dennis Stern, Alice Klausmeyer

Activation of μ-opioid receptors inhibits intercalated interneurons and modulates feed-forward inhibition in the mouse amygdala

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Medial intercalated interneurons (mITCs) function as a relay station between the basolateral complex of the amygdala (BLA) and the centro-medial amygdala (CeM), which is the main output station of the amygdala for conditioned fear responses. mITCs receive excitatory projections from BLA and the prefrontal cortex, and send inhibitory projections to CeM. Hence, mITCs are important for feed-forward inhibition of CeM neurons and further, are thought to act as mediators of fear extinction. An interesting feature of the mITCs is their high expression level of μ-opioid receptors (μORs) (Likhtik et al., 2008; Busti et al., 2011). Employing electrophysiological and pharmacological techniques we characterized the direct effect of μOR activation on mITC neurons and further investigated the influence of μORs on the intra-amygdalar synaptic network.

Current- and voltage-clamp recordings of mITC neurons revealed that the activation of μORs by the specific μOR agonist DAMGO induces a hyperpolarization of mITC neurons due to an outward-directed potassium current. This effect was completely abolished when DAMGO was applied in the presence of the μOR antagonist CTAP. Since the hyperpolarization of mITCs by μOR activation putatively alters the information transmission from BLA via mITCs to CeM, synaptic responses elicited by BLA electrical stimulation were recorded in CeM neurons. These synaptic responses were composed of a monosynaptic evoked excitatory postsynaptic current (eEPSC) and a disynaptic evoked inhibitory postsynaptic current (eIPSC). CeM neurons showed a decrease in the amplitude of the eIPSC component upon μOR activation by DAMGO whereas the eEPSC component was unchanged. Detailed analysis of the different synaptic components revealed that neither excitatory BLA-mITC synapses, nor excitatory BLA-CeM synapses are significantly influenced by DAMGO. This underpinned our hypothesis that the main target of μOR action is a connection of mITCs to CeM. In ongoing experiments, GABAergic transmission onto CeM neurons is elicited by local glutamate uncaging within the mITC cluster. Evoked IPSCs in CeM neurons were suppressed under DAMGO providing first direct evidence of a synaptic connection of mITCs to CeM which is modulated by μORs.

In summary, we show that an activation of μORs inhibits mITC neurons via hyperpolarization, and influences inhibitory but not excitatory synaptic connections of the intra-amygdalar network.

Likhtik E, Popa D, Apergis-Schoute J, Fidacaro GA, Paré D (2008) Amygdala intercalated neurons are required for expression of fear extinction. Nature 454:642-645.

Busti D, Geracitano R, Whittle N, Dalezios Y, Manko M, Kaufmann W, Sätzler K, Singewald N, Capogna M, Ferraguti F (2011) Different fear states engage distinct networks within the intercalated cell clusters of the amygdala. J Neurosci 31(13): 5131-5144.

Deciphering the neuronal circuit activated by the death-associated odor cadaverine

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Carrion smell is deeply aversive for many species. This smell is mainly carried by the diamines cadaverine and putrescine, which are generated by bacterial decarboxylation of the amino acids arginine and lysine during decay. While cadaverine is strongly repugnant to humans and zebrafish, it is attractive for carrion eaters such as certain beetles and carp (Hussain et al., 2013). Trace-amine associated receptor 13c (TAAR13c) recently has been identified as molecular receptor for cadaverine in zebrafish, and stimulation with cadaverine leads to an activation of TAAR13c-expressing olfactory sensory neurons (OSNs) in the olfactory epithelium (Hussain et al., 2013). Zebrafish show a strong aversive behavior in response to cadaverine stimuli. In the heterologous expression system receptor sensitivity lays in the µM range (EC50 = 23 ± 3 µM), similar to the concentration dependence seen in behavioral experiments, where the minimum concentration to elicit a robust aversive response is 10 µM.
In this study we are attempting to decipher the neuronal circuit activated by the death-associated odor cadaverine, from perception of the odor to the specific behavior elicited by it. This includes a characterization of the OSN-type of TAAR13c-expressing cells, the identification of a responsive glomerular area activated by cadaverine in the olfactory bulb as well as the identification of other brain areas involved in the cadaverine-mediated aversive response.
We show that TAAR13c-expressing neurons colocalize with olfactory marker protein (OMP), an accepted marker for ciliated type OSNs, and express Golf, the G protein expected to signal in ciliated neurons. By analysing cadaverine-elicited neuronal activity, we could identify a set of glomeruli in the dorsolateral cluster (dlG) specifically responding to 10 µM cadaverine, which is also the lowest concentration still eliciting aversive behavior. Further experiments aim to extend these findings using in vivo activity mapping of the entire zebrafish brain upon odor stimulation.

Hussain A, Saraiva LR, Ferrero DM, Ahuja G, Krishna VS, Liberles SD, Korsching SI. High-affinity olfactory receptor for the death-associated odor cadaverine. Proc Natl Acad Sci U S A. 2013 Nov 26;110(48):19579-84.

Modeling and experimental verification of ligand-receptor interaction in a high affinity cadaverine receptor with an unusual bifunctional ligand requirement.

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The sense of smell (olfaction) regulates a vast number of essential behaviors. Fish are equipped with only one olfactory organ containing both ciliated and microvillous olfactory sensory neurons that project their axons to the olfactory bulb. They possess four of the five different families of olfactory receptor gene families that are known in vertebrates: odorant (ORs), vomeronasal (V1Rs and V2Rs), and trace amine-associated (TAARs) receptors (Korsching, 2008; Hussain et al., 2009). In the framework of a ligand search for TAAR receptors, we recently identified TAAR13c as a high affinity receptor for cadaverine (Hussain et al., 2013). Structure-activity analysis indicated TAAR13c to be a general diamine sensor, with pronounced selectivity for odd chains of medium length (Hussain et al., 2013). We performed structural modeling together with intra-family sequence alignments, which led to the discovery of a potential candidate cadaverine-binding motif. We are now interested to elucidate the structural dynamics of binding of cadaverine to TAAR13c by structural docking.
The structure for TAAR13c was built by homology and ab initio algorithm. The structure prediction reveals a cleft, which can be assumed as the binding pocket of this receptor. Inside of this potential binding pocket D112 and W269 are located, which belong to the classical aminergic-binding motif. In close proximity and in the same plane there was another aspartate residue found. It is conceivable that both aspartates interact with the positively charged amine groups of the diamines. Based on this, we propose a model of the ligand binding of TAAR13c, whereby D112 and the other aspartate bind the two positively charged amine groups via ionic interaction and W269 might stabilize the non-polar carbon chain. In order to test this hypothetical binding motif, we mutated these positions to amino acids exhibiting a different charge or different side chain lengths. The mutated Taar13c ORFs encoding N terminal Rho tag were cloned in an expression vector for expression in HEK cells. Ligand binding was visualized as change in intracellular Ca2+ concentration using Fluo-4 as calcium indicator dye. Initial results show changes in ligand binding for some of the mutated TAAR receptor variants.

Korsching, (2008) The molecular evolution of teleost olfactory receptor gene families. Results Probl Cell Differ. 2009;47:37-55.
Hussain A, Saraiva LR, Korsching SI, (2009) Positive Darwinian selection and the birth of an olfactory receptor clade in teleosts. Proc. Natl. Acad. Sci, USA 106:4313-8.
Hussain A, Saraiva LR, Ferrero DM, Ahuja G, Krishna VS, Liberles SD, Korsching SI, (2013) High-affinity olfactory receptor for the death-associated odor cadaverine. Proc Natl Acad Sci U S A, 110(48):19579-84.

Molecular mechanisms of olfactory detection in polyphagous moth *Spodoptera littoralis*: Deorphanization of odourant receptors via the *Drosophila* empty neuron system.

Fredrik Schlyter[1], William B. Walker [1], Muhammad Binyameen[1], Arthur de Fouchier[2], Claudia Steiner[1], Christelle Monsempes[1], Annick Maria[3], Marie-Christine François[2], Peter Anderson[1], Bill S. Hansson[4], Thomas Chertemps[3], Nicolas Montagné[3], Emmanuelle Jacquin-Joly[2], Mattias Larsson[1]

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The olfactory sense determines vital steps in insect behaviour, including mate and food search, oviposition site selection and predator/parasitoid avoidance.
The Chemical Ecology research group at the Swedish University of Agricultural Sciences in Alnarp, Sweden and the PISC groups in France (INRA, UPMC), have established the polyphagous noctuid moth, *Spodoptera littoralis* (the Egyptian Cotton Leafworm) as a model for investigation of noctuid olfaction and chemical ecology.
At the molecular level, insect interactions with the olfactory environment are mediated by odorant receptor (OR) proteins, which are functionally expressed in odorant receptor neurons within olfactory appendages, primarily the antennae.
Our aim is to functionally characterise the *S. littoralis* OR genes: “deorphanization”. Individual ORs are expressed in the Empty Neuron system of the fruit fly, *Drosophila melanogaster*, and their response profiles are identified by means of single sensillum electrophysiological recordings. We have also utilized gas chromatography analysis of plant headspace extracts, coupled to single sensillum recordings (GC-SSR) to analyse the tuning of specific ORs to components of ecologically relevant complex odour blends.
Our data demonstrate successful adaptation of these methods to the deorphanization of *S. littoralis* ORs. We find both many earlier well-known ligands as well as new ligands, mostly of plant origin. Dose-response data (corrected for differences in stimuli volatility) show specific responses to many plant volatiles. These results represent an important step in understanding the molecular mechanisms of olfactory mediated behaviours in female *S. littoralis* and other moth herbivores towards host and non-host plants.

Possible regulation of CB1-Receptor by RGS-proteins in a cell type specific manner

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Cannabinoid Receptor 1 (CB1), a very abundant G-Protein coupled receptor responsible for synaptic modulation in various regions of the vertebrate CNS, has been shown to be expressed differentially in glutamatergic vs GABAergic forebrain neurons. Stimulation of the receptor in hippocampal homogenates from conditional CB1 knock-out mice clearly showed a different recruitment of downstream signaling elements in these two cell types. Specifically, we observed a more efficient coupling of CB1 to downstream signaling elements in hippocampal glutamatergic cells. Furthermore, electrophysiological approaches suggested distinguishable mechanics for the signaling in these two cell populations as well. Here we further elucidate CB1-coupled downstream signaling by inquiring possible differences in signal termination mechanisms. To this end, we first analyzed the expression pattern of several Regulator of G-Protein Signaling (RGS) protein subtypes in the adult mouse forebrain with in situ hybridization. RGS proteins interact with GPCRs and facilitate signal termination by enhancing GTP hydrolyzing activity of the G(alpha) protein. Subsequently, we have performed double Fluorescent In-Situ Hybridizations (FISH) in order to determine co -expression of the selected RGS subtypes with CB1. Furthermore, the expression levels of RGS protein subtypes were quantified in the hippocampi of wild type and CB1 deficient mice via qPCR. These experiments will aid us to pinpoint interaction partners of CB1 regarding its signal termination mechanisms.

Orexin-CRF Receptor Heteromers in the Ventral Tegmental Area as Targets

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Release of the neuropeptides corticotrophin releasing factor (CRF) and orexin A in the ventral tegmental area (VTA) play an important role.
In stress induced cocaine seeking behavior. We provide evidence for pharmacologically significant interactions between CRF and orexin A that depend on oligomerization of CRF1 and orexin OX1 receptors.
CRF1R-OX1R heteromers are the conduits of a negative crosstalk between orexin A and CRF as demonstrated in transfected cells and in the VTA, where they significantly modulate dendritic dopamine release. The cocaine target sigmas1 receptor (s1R) also associates with the CRF1R-OX1R heteromer. Cocaine binding to thes1R-CRF1R-OX1R complex promotes a long-term disruption of the orexin A-CRF negative crosstalk. Through this mechanism cocaine sensitizes VTA cells to the excitatory effects of both CRF and orexin-A, thus providing a mechanism by which stress induces cocaine seeking.

The *v1r*-related Ora5 receptor is not expressed in ciliated and microvillous neurons, the major olfactory receptor neuron populations.

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Olfaction (sense of smell) constitutes an essential sense in most species. Organisms use it to search for food, prey and mating partners, and to avoid predators. *Danio rerio* (zebrafish) emerged as an excellent model system for studying vertebrate olfaction due to several technical advantages compared to higher vertebrates, and because it obeys all fundamental dogmas of olfactory coding, such as axonal convergence and monogenic expression. Furthermore, the olfactory epithelium of zebrafish expresses orthologs of most mammalian olfactory receptor families, to wit ORs, ORAs/V1Rs, OlfCs/V2Rs and TAARs. The ORA gene family of fish consists of six highly conserved genes, in stark contrast to the dynamically evolving mammalian V1R families. We were interested to identify the cell types expressing *ora* genes, and have therefore performed co-localization studies with markers for all olfactory sensory neuron populations known in zebrafish. Apart from the major cell types ciliated and microvillous neurons, zebrafish also possess teleost-specific crypt neurons as minor receptor cell population. Recently we have shown that ORA4 is exclusively expressed in crypt neurons (Oka et al., 2012). Currently we attempt to identity the cell type for another member of the *ora* gene family, ORA5. By using double fluorescent *in situ* hybridization, we show that ORA5 is neither expressed in ciliated, microvillous nor in crypt neurons.

Oka Y, Saraiva LR, Korsching SI (2012) Crypt neurons express a single V1R-related ora gene, Chemical Senses 37(3):219-27.

Transactivation of TrkB receptor through the Adenosine receptor A2A-R leads to changes in downstream signaling cascades

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Neurotrophins are potent survival factors for developing and injured neurons. Binding of neurotrophins to Tropomyosin receptor kinase (Trk) receptors results in
receptor auto/transphosphorylation and the activation of downstream pathways like Ras/Ras activated factor (Raf)/Mitogenactivated protein kinase (MAPK) pathway and
phosphoinositide 3-kinase (PI3K)/AKT pathway. The initial thought of these pathways acting as separate cascades has been converted into a model in which both interact at the level of a mitochondrial complex of Raf/AKT/Bcl-2-associated athanogene 1 (Bag-1) and heat shock protein 70 (Hsp70). This complex may be the key regulator of neurotrophin mediated survival and differentiation processes. However, neurotrophins are not used to treat neurodegenerative diseases because of their poor pharmaco-kinetic profile, side effects, and absence of survival properties in clinical trials. Consequently, alternative approaches are gaining importance; one alternative is to use transactivation pathways such as adenosine receptor A2A-R agonist which activate Trk receptor signaling independent of neurotrophins. However, the transactivation kinetic, localization and downstream signaling of transactivated TrkB receptor as well as its influence on motoneuron neurite growth and the mitochondrial protein complex formation is still unsolved. Therefore, the transactivation process of TrkB receptor was investigated in cultured embryonic mouse motoneurons.

Poster Topic

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

T6-1A A multiscale model of *Shaker-type* K_V-channel mutants predicts macroscopic electrophysiological results
Alexander Peyser, Wolfgang Nonner

T6-2A Balancing nociceptive signaling by co-activation of TRESK and TRP channels by lysophosphatidic acid
Sina Kollert, Frank Döring, Erhard Wischmeyer

T6-3A Changes in neural network homeostasis trigger neuropsychiatric symptoms
Jochen C. Meier, Nicola Maggio, Iris Müller, Gürsel Caliskan, Marcus Semtner, Joanna Eller, Ute Häussler, René Jüttner, Tamar Dugladze, Eva Chronowska, Carola A. Haas, Akos Kulik, Tengis Gloveli, Oliver Stork, Uwe Heinemann, Aline Winkelmann

T6-4A Characterization of Calcium Currents in Neonatal and Mature Spiral Ganglion Neurons of $a_2d3^{-/-}$
Friederike Stephani, Wenying Wang, Jutta Engel

T6-5A Chloride transporter, a new therapeutic targets
Mahmoudreza Hadjighassem

T6-6A Complex and sparse coding along the honey bee's olfactory pathway: potential contribution of ionic currents of medial and lateral projection neurons and Kenyon cells
Jan Kropf, Wolfgang Rössler

T6-7A Correlations between neuronal spike trains examined under recreated fluctuating synaptic conductance inputs
Daniele Linaro, Michele Giugliano

T6-8A Deafness by mutation in ATP gated P2X2 channels (DFNA41) is not induced by the dominant negative effect
Yan Zhu

T6-9A DEFECTIVE ESCAPE BEHAVIOUR IN DEAH -BOX RNA HELICASE MUTANTS IMPROVED BY RESTORING GLYCINE RECEPTOR EXPRESSION
Sophie Leacock, Hiromi Hirata, Kazutoyo Ogino, Kenta Yamada, Robert J. Harvey

T6-1B Differential regulation of chloride transporter expression by potassium chloride and ampakine in chicken auditory brainstem *in vitro*

Marcus Joseph Wirth, Lars Roentgen, Hermann Wagner

T6-2B Effects of Epilepsy-associated Ion Channel Mutations on Neuronal Network Activity
Filip Rosa, Sabina Vejzovic, Heidi Löffler, Stephan Theiss, Marcel Dihné, Holger Lerche, Snezana Maljevic

T6-3B Forebrain 5-HT_7 receptors relieve neuropathic pain by reversing dysfunction of dendritic integration
Mirko Santello, Thomas Nevian

T6-4B Functional Characterization of Novel GABA(A) Receptor Mutations Associated with Idiopathic Generalized Epilepsies
Merle Bock, Cristina Niturad, Julia Knaus, Thomas Ott, Timm Danker, Steven Petrou, Yvonne Weber, Holger Lerche, Snezana Maljevic

T6-5B Hyperpolarization-activated cation channels influence synaptic integration in substantia nigra dopamine neurons.
Dominique Engel, Vincent Seutin

T6-6B Impermeant anions, fixed charges, and the driving force of $GABA_A$R-mediated Cl^- currents
Juha Voipio, Kai Kaila

T6-7B *In vivo* tagging of $Ca_V1.3$ calcium channels reveals C-terminal modulation of gating properties and subcellular expression of the full length channel in mouse inner hair cells
Stephanie Eckrich, Anja Scharinger, Dietmar Hecker, Kai Schönig, Dusan Bartsch, Martina J Sinnegger-Brauns, Bernhard Schick, Jutta Engel, Jörg Striessnig

T6-8B Influence of CKAMP44 on AMPA receptor number and function in relay neurons of the lateral geniculate nucleus
Xufeng Chen, Jakob von Engelhardt

T6-9B Intracellular Ca^{2+}/calmodulin modulates Kvß1.1-induced inactivation of Kv channels
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T6-9D Voltage-gated calcium channels in the mouse sciatic nerve
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A multiscale model of *Shaker-type* K_V-channel mutants predicts macroscopic electrophysiological results

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A multiscale physical model of *Shaker*-type K_V channels is used to span from atomic-scale interactions to macroscopic experimental measures such as charge/voltage (QV) and conductance/voltage (GV) relations. The model [1] comprises the experimentally well-characterized voltage sensor (VS) domains described by four replications of an independent continuum electrostatic model under voltage clamp conditions [2, 3] and a hydrophobic gate controlling the flow of ions by a vapor lock mechanism [4], connected by a simple coupling principle derived from known experimental results and trial-and-error. The total Hamiltonian of the system is calculated from the computed configurational energy for each components as a function of applied voltage, VS positions and gate radius, allowing us to produce statistical-mechanical expectation values for macroscopic laboratory observables over the full range of physiological membrane potentials (|V| = 100 mV, in 1 mV steps). The Shaker QV and GV relations seen in Seoh et al. [5] are predicted by this model. With this approach, functional energetic relations can be decomposed in terms of physical components, and thus the effects of modifications in those elements can be quantified. The same model was systematically applied to VS charge mutants (Seoh et al. [5]). The QV and GV relations can be qualitatively predicted and the associated effects on functional domains determined. Additional features such as surface charges become significant for the pathological cases. Our engineering approach clearly elucidates that both normal function and mutant changes are electrostatic in nature.

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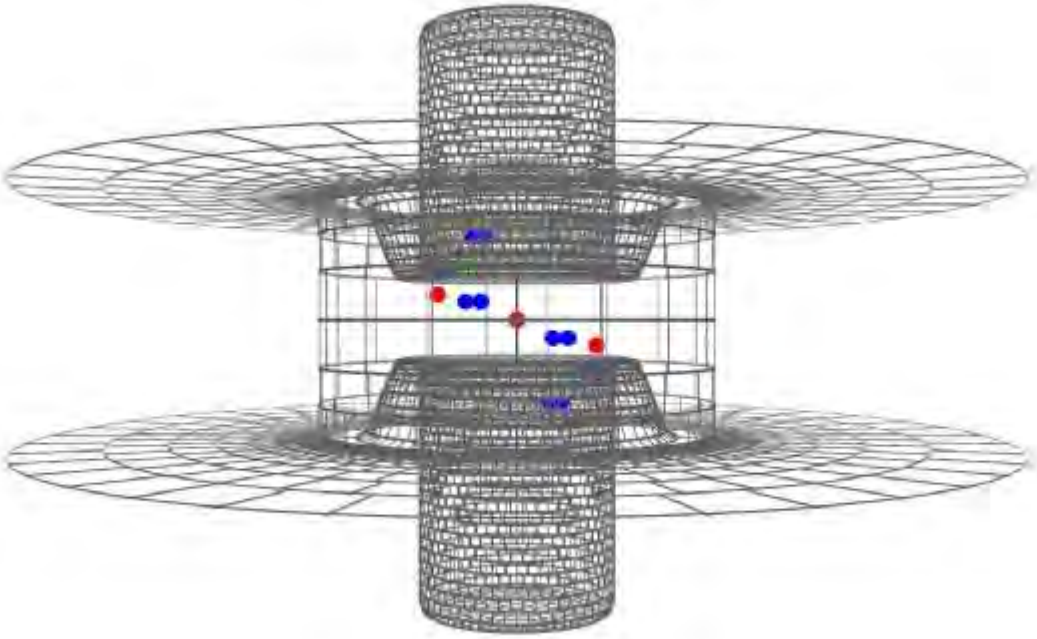

Balancing nociceptive signaling by co-activation of TRESK and TRP channels by lysophosphatidic acid

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In dorsal root ganglia (DRG) neurons the tandem-pore potassium channel TRESK builds the major current component of the standing outward current IKso. A prominent physiological role of TRESK channels has been attributed to pain sensation. During inflammation mediators of pain e.g. biogenic amines or lysophosphatidic acid (LPA) are released and modulate nociceptive signaling by activation of their G-protein coupled receptors. Here we use a newly developed antibody and RT-PCR to show co-expression of TRESK channels and LPA receptors in DRG neurons, respectively. Recombinant co-expression of TRESK channels and LPA2 receptors in Xenopus oocytes revealed an activation of basal K+ currents by 769 ± 20.3% (n=8) upon LPA application (0.5 µM) with an EC50 of 0.2 µM. Using mutant TRESK[PQAVAD] or application of the phospholipase C (PLC) inhibitor U73122 blocked current augmentation by LPA indicating cellular signaling via PLC pathway and calcineurin binding to TRESK channels.

In small diameter DRG neurons nociception results from TRPV1 channel activation by painful stimuli including the inflammatory substance LPA. When we co-express TRESK and TRPV1 channels in Xenopus oocytes cationic inward and outward currents were simultaneously activated by LPA. Under these conditions the reversal potential of ramp recordings was intermediate to recordings from oocytes expressing only the K+ or the unspecific cation channel. Principally this finding demonstrates that TRESK activation by an inflammatory substance dampens noxious excitation induced by the same agent.

DRG-like F11 -cells lacking endogenous TRESK channels generate action potential upon current stimulation. However when TRESK channels were recombinantly expressed in F11 cells, they exhibit a shift of the reversal potential to more negative values, a larger outward current and a loss of voltage-gated sodium currents. Accordingly, depolarising pulses failed to elicit action potentials.

In patch-clamp recordings from primary cultured DRG neurons of WT mice IKso currents increased after application of LPA by 28.7 ± 4.9 % (n=14) whereas under these conditions IKso currents of neurons from TRESK[G339R] (knockout) mice decreased by 10.63 ± 2.4% (n=19).

Under current-clamp conditions LPA application elicited spike trains in DRG neurons, with higher frequency in cell of TRESK[G339R] mice than in cells of WT animals. In addition, upon depolarizing pulses (100 pA) excitability was differentially modulated by LPA in these genotypes. Spike frequency was attenuated from 4.9±1 to 3.4±1 (n=14) spikes/s in TRESK[WT] neurons and augmented from 9.1±2.6 to 12.2±2.9 spikes/s (n=10) in TRESK[G339R] neurons. Hence, in pain sensing neurons excitatory effect of the inflammatory substance LPA is balanced by co-activation of TRESK channels.

Changes in neural network homeostasis trigger neuropsychiatric symptoms

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The mechanisms that regulate the strength of synaptic transmission and intrinsic neuronal excitability are well characterized; however, the mechanisms that promote disease-causing neural network plasticity are poorly defined. We generated mice with targeted neuron type–specific expression of a gain-of-function variant of the neurotransmitter receptor for glycine (GlyR) that is found in hippocampectomies from patients with temporal lobe epilepsy. In this mouse model, targeted expression of gain-of-function GlyR in terminals of glutamatergic cells or in parvalbumin -positive interneurons persistently altered neural network excitability. The increased network excitability associated with gain-of-function GlyR expression in glutamatergic neurons resulted in hypersynchronous network discharges, which provoked cognitive dysfunction and memory deficits without affecting bidirectional synaptic plasticity. In contrast, decreased network excitability due to gain-of-function GlyR expression in parvalbumin-positive interneurons resulted in an anxiety phenotype, but did not affect cognitive performance or discriminative associative memory. Our animal model unveils neuron type–specific effects on cognition, formation of discriminative associative memory, and emotional behavior in vivo. Furthermore, our data identify a presynaptic disease–causing molecular mechanism that impairs homeostatic regulation of neural network excitability and triggers neuropsychiatric symptoms.

Characterization of Calcium Currents in Neonatal and Mature Spiral Ganglion Neurons of $a_2d3^{-/-}$

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Introduction

Spiral ganglion neurons (SGNs) connect hair cells with central auditory neurons and therefore are indispensable for auditory signal transmission. Type I SGNs, which comprise 95 % of all SGNs, are myelinated and make a precise 1 to 1 connection with inner hair cells (IHCs). Auxiliary a_2d subunits of voltage-gated Ca^{2+} channels (VGCC) control the abundance of VGCCs and shape their biophysical properties. Moreover, the auxiliary a_2d3 subunit plays a role for the proper structure and morphology of auditory nerve synapses (Pirone et al., J Neurosci 2014). To investigate the function of the a_2d3 subunit for VGCCs in SGN, we analyzed Ca^{2+} currents in SGNs isolated from $a_2d3^{-/-}$ and wild type (WT) mice.

Methods

Patch-clamp recordings of Ca^{2+} currents were performed on enzymatically dissociated spiral ganglion neurons isolated from neonatal (P5) and mature (P20) mice (cf. Lv et al., J Neurosci 2012). SGNs were cultured for 2 days.

Results

Large voltage -activated K^+ currents of SGNs were blocked by TEA (30 mM), 4-AP (15 mM) and linopirdine (100 µM) in the bath and by 110 mM Cs^+ in the pipette solution. Large voltage-activated inward Na^+ currents were fully suppressed by extracellular NMDG (110 mM). Whole-cell Ca^{2+} currents were reduced in $a_2d3^{-/-}$ SGNs from both, the apical and the basal cochlea at both ages. The contribution of L-type currents was assessed by superfusing SGNs with 10 µM nimodipine. Nimodipine blocked about 30 % of the Ca^{2+} currents in WT SGNs compared with 40 % block in $a_2d3^{-/-}$ SGNs.
The contribution of P/Q-type currents was assessed using 1 µM ω-agatoxin IVA. Preliminary results show that the contribution of P/Q-type currents in WT apical SGNs amounted to 58 %.

Conclusion

Neonatal as well as mature SGN of $a_2d3^{-/-}$ mice showed reduced Ca^{2+} currents compared with WT SGNs. The differential contribution of the different subtypes of Ca^{2+} currents remains to be determined.

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Chloride transporter, a new therapeutic targets

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Alterations in the balance of K-Na-2Cl cotransporter (NKCC1) and Na-Cl cotransporter (KCC2) activity may cause epolarizing effect of gaba-aminobutyric Acid (GABA), and contribute to epileptogenesis in human temporal lobe epilepsy. NKCC1 facilitates accumulation of chloride inside neurons and favors depolarizing responses to GABA. In the current pilot study we provide the first documented look at efficacy of bumetanide, a specific NKCC1 antagonist, on reduction of seizure frequency in adult patients with temporal lobe epilepsy. According to our results, seizure frequency was reduced considerably in these patients.

Complex and sparse coding along the honey bee's olfactory pathway: potential contribution of ionic currents of medial and lateral projection neurons and Kenyon cells

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The olfactory system of the honey bee displays an intriguing pattern of convergence and divergence between the individual neuronal types within the olfactory pathway: about 60000 olfactory sensory neurons (OSN) convey information from the antennae on approximately 900 projection neurons (PN) in the antennal lobe, the first relay center for olfactory information in the brain. The high synaptic convergence of OSNs on much less PNs requires that PNs employ complex responses to odor stimulation with relatively high spontaneous and response frequencies. This allows PNs to transmit all information reliably. Up to 4000 local interneurons (LN) interconnect between OSNs and PNs, yet the exact synaptic connectivity is still unknown. PNs then project via two tracts, the medial (mALT) and the lateral antennal lobe tract (lALT) to the mushroom bodies (MB) and the lateral horn. This system of two parallel tracts of PNs running to the MBs is unique to Hymenoptera and was termed the dual olfactory pathway. Both tracts transmit information from a similar set of odors, yet m-ALT PNS respond more odor specific and have lower response and spontaneous action potential (AP) frequencies (Brill et al. 2013 J Neurosci). In the MB calyces, PNs form synaptic complexes with ~183000 Kenyon cells (KC), the MB intrinsic neurons. KCs do not only receive olfactory input but also gustatory and visual information. Thus, they are likely to act as coincidence detectors within the olfactory systems and even across different sensory modalities. The MBs, therefore, are regarded as the centers for sensory integration and learning and memory. Using Ca^{2+}-imaging KCs have been shown to respond in a very phasic and sparse fashion to odor stimulation (Szyszka et al. 2005 J Neurophysiol). This is most likely due to the divergent/convergent circuitry between PNs and KCs and allows that KCs act as coincidence detectors. In the present study, we aimed to identify intrinsic neuronal properties supporting the complex odor response patterns of PNs and the sparse coding properties of KCs. For PNs, we could identify Na^+ currents and diverse K^+ currents depending on voltage and Na^+ or Ca^{2+}. This set of currents is relatively similar, for example, to currents observed in locust DUM neurons that were shown to support spiking activity (Wicher et al. 2006 J Neurophysiol). L- and m-ALT PNs showed no significant differences. In KCs, we found a very prominent transient K^+ current with A-type current characteristics. Furthermore, we identified a persistent K^+ current with N-shaped IV characteristics indicating that it is a purely Ca^{2+} dependent K^+ channel. The large voltage dependent transient K^+ current is likely to hyperpolarize KCs right after activation, thus providing a strong basis for phasic response properties supporting coincidence detection and sparse coding by KCs. For l- and m-ALT PNs we conclude that the differential coding properties must be determined by differential sensory input (Kropf et al. 2014 Cell Tissue Res) and/or local AL synaptic interactions.

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Correlations between neuronal spike trains examined under recreated fluctuating synaptic conductance inputs

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Neurons embedded in the same cortical microcircuit show in vivo a high degree of functional similarity, both in their sub-threshold membrane potential fluctuations and in the correlations between their output spike trains. Previous in vitro works have shown that, by constraining artificially the fraction of common inputs received by pairs of pyramidal neurons, the correlation between neuronal output spike trains increases with the mean firing rate. In light of these results, we consider a similar approach with the aim of exploring how correlations between neural spike trains are affected by more realistic conductance-driven synaptic inputs.

We consider unconnected pairs of cells and, using the dynamic clamp configuration, we inject correlated conductances, mimicking common presynaptic populations.

We first compute output spike correlations at a constant firing frequency and variable levels of input correlation: as expected, spike correlations increase for increasing input correlations. Then, we fix the degree of correlation in the inputs and systematically vary the spiking frequency by changing the balance between excitatory and inhibitory conductances.

We find that conductance inputs produce a decorrelation of the total injected current that is frequency dependent and that constitutes an upper bound for the correlations in the output spike trains. This result holds both in cases of low and high firing rate variability, which mimic the presence of two (AMPA and GABAa) or three (AMPA, GABAa and NMDA) conductances, respectively.

We propose that such decorrelation is largely attributable to the presence of ionic currents that are activated in the after-hyperpolarization phase that follows the action potential and to test this hypothesis and dissect the contribution of individual currents, we use pharmacological manipulations to abolish spike-triggered currents. To further elucidate the contribution of ionic currents in shaping the correlation input-output relationship, we record from GABAergic interneurons, which are fitted with a significantly different set of ionic currents than those found in principal neurons.

Additionally, using widely established computational models, we investigate in silico the role of the spike initiation mechanisms (e.g., the slope of the membrane potential at spike onset) and of intrinsic membrane currents, such as slow potassium currents responsible for spike frequency adaptation.

Deafness by mutation in ATP gated P2X2 channels (DFNA41) is not induced by the dominant negative effect

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The P2X2 receptor is an ATP-gated ligand (ionotropic) ionic channel and plays important roles in many physiological and pathological processes. Recently, it has been found that p.V60L (c.178G-T) and p.G353R (c.1057G-C) mutations in P2X2 receptors can induce autosomal dominant nonsyndromic hearing loss (DFNA41). However, the underlying alterations in channel properties and the functional consequences of deafness-associated mutations are not well determined. In this study, the effect of deafness P2X2 mutants on wild-type (WT) P2X2 channel activity and function was investigated. P2X2 WT and deafness mutants p.V60L and p.G353R were cloned and transfected into HEK 293 cells. ATP-evoked responses were measured by patch clamp recording. Both p.V60L and p.G353R deafness mutants targeted to the plasma membrane but lacked the response to ATP stimulation. However, co-transfection with WT P2X2 could restore the ATP-evoked currents, as predicted by modeling with no dominant negative effect rather than by modeling with dominant negative effect. Gating analyses of the p.V60L mutation , p.G353R mutation and WT P2X2 were performed. The data demonstrate that both p.V60L and p.G353R dominant deafness mutations have no negative effect on WT P2X2 and are loss-of-function mutations. This finding provides insight into developing therapeutic interventions for this nonsyndromic deafness.

DEFECTIVE ESCAPE BEHAVIOUR IN DEAH-BOX RNA HELICASE MUTANTS IMPROVED BY RESTORING GLYCINE RECEPTOR EXPRESSION

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We report the isolation and characterisation of a novel zebrafish mutant that exhibits an abnormal dorsal bend in the beginning of tactile-evoked escape swimming. Similar behavioural defects were observed in zebrafish embryos treated with strychnine, an antagonist of glycine receptors (GlyRs), suggesting that the abnormal motor response in mutants is attributable to a deficit in glycinergic synaptic transmission. Unexpectedly, positional cloning resulted in the identification of a missense mutation (p.L489P) in dhx37, encoding the DEAH-box RNA helicase Dhx37. RNA helicases regulate RNA metabolism, but their substrate specificity and in vivo function remain largely unknown. In the dhx37 mutant, we found that GlyR a1, a3, and a4a subunit transcript levels were decreased and mis-spliced. Consistent with this finding, over-expression of GlyR a1, a3 or a4a subunits in Dhx37-deficient embryos restored normal behaviour. Conversely, antisense-mediated knockdown of multiple GlyR a subunits in wild-type embryos was required to re-capitulate the Dhx37 phenotype. These results indicate that Dhx37 is required for the biogenesis of a subset of GlyR a subunit mRNAs, thereby regulating glycinergic synaptic transmission and associated motor behaviours. To our knowledge, this is the first identification of pathologically relevant substrates for a RNA helicase. Our study also suggests that mutations in human DHX37 may cause startle disease/hyperekplexia.

Differential regulation of chloride transporter expression by potassium chloride and ampakine in chicken auditory brainstem *in vitro*

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Neurons in the auditory brainstem of chicken maintain an elevated chloride reversal potential even after hatching. The GABAergic input to *Nucleus laminaris* (NL) and *Nucleus magnocellularis* (NM) from the *Nucleus olivaris superior* (SON) thus elicits a depolarisation, but is nevertheless inhibitory due to current shunting and activation of low-voltage activated potassium channels. The physiological relevance of this effect is to increase the accuracy of sound localization. Important regulators of chloride homeostasis are the transporters of the SLC12 family. It is likely that a differential activity of the inward directed sodium-potassium-chloride-cotransporter 1 (NKCC1) and the outward directed potassium-chloride-cotransporter 2 (KCC2) is responsible for the maintenance of the elevated reversal potential.

Our previous studies *in vivo* showed an expression of the inward transporter NKCC1 and the outward transporter KCC2 in neurons of NM and NL. The temporal profiles of expression suggested a correlation with synaptic activity.

To analyze the regulation of the expression of chloride transporters, organotypic cultures have been prepared by the roller-tube technique. Auditory brainstem was explanted at embryonic day 10 and harvested after 7 days *in vitro*. During the time *in vitro* either 25 mM potassium chloride or 300µM CX546 (ampakine) were added to the culture media to enhance synaptic activity. SDS PAGE and Western blot with chemiluminescent immunodetection were performed to analyze the expression of NKCC1 and KCC2 and actin. Expression of NKCC1 and KCC2 was normalized to actin expression.

Our preliminary results point to an increase of NKCC1 expression both under KCL and CX546. KCC2 expression seems to increase under KCL but the results are inconclusive for CX546. It is possible that the positive modulation of glutamatergic transmission by ampakine is not sufficient to reliably drive the KCC2 expression.

We conclude from our biochemical data, that an activity-dependent component exists in the regulation of chloride transporter expression.

Effects of Epilepsy-associated Ion Channel Mutations on Neuronal Network Activity

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The M-current is a non-inactivating outward potassium current mediated by Kv7.2 and Kv7.3 channels, which are encoded by *KCNQ2* and *KCNQ3* genes. Mutations found in the *KCNQ2* have been predominantly associated with benign familial neonatal seizures (BFNS) and recently also with the so-called KCNQ2-related epileptic encephalopathy (KCNQ2-EE). Whereas BFNS presents with transiently occurring seizures and generally mild features, KCNQ2-EE is a severe disease encompassing early onset pharmacoresistant seizures and psychomotor retardation. Functional investigation in non-neuronal expression systems revealed that BFNS mutations mainly caused by haploinsufficiency, and dominant-negative effect seems to be the prevailing effect of KCNQ2-EE mutations. We set out to investigate in vitro characteristics, development and modulation of neuronal networks obtained from E17 embryos of the *Kcnq2* knock-out mouse line. Primary hippocampal neuronal cultures of three different Kcnq2 genotypes (WT,+/-, -/-) were plated on Multi Electrode Array (MEA) dishes and the spontaneous neuronal activity, with and without the Kv7.2 opener retigabine, recorded on 10, 17 and 24 days in vitro (DIV) in all 3 genotypes. Surprisingly, we found no difference in Number of Spikes/min or Number of Bursts/min, which are parameters perceived as mostly associated with increased excitability and thereby epilepsy. Instead, we observed that the developmental changes of burst duration, expressed as Burst Duration DIV17/Burst Duration DIV 10, depend on the particular genotype and are significantly different between WT on one side and +/- and -/- cultures on the other. Upon application of 10 µm retigabine, shortening of Burst Duration was similar for WT and +/- networks, in contrast to substantial prolonging seen in -/- cultures. In conclusion, in the MEA setup Burst Duration appears to be the parameter mostly affected by expression levels of Kv7.2, which confirms in vitro the hampering role of Kv7.2 currents on the repetitive firing. We will further analyze the effects of the KCNQ2-EE mutations on the network activity of these cultures. These neuronal networks can further be applied to evaluate if different compounds may counteract the consequences of the moderate or complete loss of the Kv7.2 channel activity related to BFNS and EE, respectively, and enable improved treatment strategies.

Forebrain 5-HT$_7$ receptors relieve neuropathic pain by reversing dysfunction of dendritic integration

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Neuropathic pain is caused by long-term modifications of neuronal function in the peripheral nervous system, the spinal cord as well as supraspinal areas. Although functional changes in the forebrain are thought to contribute to the development of persistent pain, their significance and precise subcellular nature remain unexplored. Using somatic and dendritic whole-cell patch- clamp recordings from neurons in the anterior cingulate cortex, we discovered that sciatic nerve injury caused an activity-dependent dysfunction of hyperpolarization-activated cyclic nucleotide- regulated (HCN) channels in the dendrites of layer 5 pyramidal neurons resulting in enhanced dendritic integration of excitatory postsynaptic inputs and increased neuronal firing. Specific activation of the serotonin receptor type 7 (5-HT$_7$R) alleviated the lesion-induced pathology by increasing HCN channel function, restoring normal dendritic integration and reducing mechanical pain hypersensitivity in nerve-injured animals in vivo. Thus, serotoninergic modulation of HCN channels at the forebrain level can control neuropathic pain and represents a promising therapeutical target.

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Functional Characterization of Novel GABA(A) Receptor Mutations Associated with Idiopathic Generalized Epilepsies

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Several mutations affecting genes encoding alpha-1 or gamma-2 subunit of the GABA(A) receptor have been associated with idiopathic generalized epilepsies (IGE). Within the EPICURE Consortium, 66 ion channel genes were screened in 95 index patients of families with IGE. Of the 78 detected variants, 9 were found in genes encoding different GABA(A) receptor subunits. Two of these mutations, found in two families with absence seizures, affect the GABRA5 gene encoding the alpha-5 subunit. To assess the pathogenicity of these variants and understand better the role of GABA(A) receptors in epileptogenesis, we functionally analyzed these variants using Xenopus laevis oocyte expression system. The mutations were introduced into the GABRA5 cDNAs using site-directed mutagenesis and the in vitro transcription was used to generate corresponding cRNA, which was injected in the Xenopus laevis oocytes. Recordings were performed 3 days after injection using automated two-electrode voltage-clamp recording technique. Functional analysis provided for M1I and S238R mutation revealed a dramatically reduced GABA-induced response compared to the WT receptors. GABA(A) receptors containing alpha-5 subunits are primarily expressed in the hippocampus and localized to extrasynaptic regions of pyramidal neurons where they mediate the tonic inhibition. Therefore GABRA5 mutations might indicate a novel disinhibition pathomechanism in epileptogenesis. To further evaluate this hypothesis, we generated a mouse carrying the S238R mutation in the mouse Gabra5 gene. The initial characterization of the generated mouse line revealed normal life span and unaltered behaviour. Mouse lines carrying mutations in GABRA genes usually present with absence like seizures. Therefore, we are currently performing the long-time video-EEG monitoring to assess the occurrence of spontaneous seizures in this mouse line, and a comprehensive neurophysiological analysis using in vitro (in primary neuronal cultures) and ex vivo (in acute brain slices) approaches is underway.

Hyperpolarization-activated cation channels influence synaptic integration in substantia nigra dopamine neurons.

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Hyperpolarization-activated channels (*I*h or HCN channels) are widely expressed in principal neurons and in some interneurons. Their contribution to neuronal signaling includes the modulation of various parameters such as passive and active membrane properties, intrinsic resonance, synaptic integration and synaptic plasticity. The loss of specific subunits of this channel enhances cortical excitability and epileptogenesis. One of the most characteristic function of *I*h is to influence synaptic integration along dendrites by shortening the width of postsynaptic potentials (PSPs) and dampening the summation of trains of input. Herewith, PSPs arriving at the soma have similar width, regardless of their origin. Interestingly, this effect has been correlated to an increasing density of Ih from the soma to the distal dendrites in cortical and hippocampal pyramidal neurons. In substantia nigra (SN) dopamine neurons, the presence of *I*h is recognized, but its distribution along the somatodendritic axis and its function in synaptic integration are fully unknown. In contrast to pyramidal neurons, the axon of dopamine neurons often originates from a dendrite dividing the dendritic compartement of these neurons in axon- and non-axon bearing dendrites. In addition, dopamine neurons express different *I*h channel subunits in comparison to pyramidal neurons. Given these specific characteristics of dopamine neurons, it is important to examine whether a gradient of *I*h exists and how this distribution affects synaptic integration in dopamine neurons.

We used cell-attached patch-clamp recordings to map the distribution of *I*h in dopamine neurons along its somatodendritic axis and showed that Ih exhibits a nonuniform density along the somatodendritic axis. Using simultaneous whole-cell somatic and dendritic recordings we determined the effect of the current on single and multiple synaptic inputs, and observed that *I*h shortens PSP duration and minimizes PSP summation.

Impermeant anions, fixed charges, and the driving force of $GABA_AR$-mediated Cl^- currents

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The key role of the cation-chloride cotransporters (CCCs) KCC2 and NKCC1 in neuronal Cl^- regulation and in setting the driving force (DF) of $GABA_A$ receptor-mediated Cl^- currents has been demonstrated using electrophysiological, pharmacological and genetic approaches (Kaila et al., 2014). However, it has been recently suggested that, rather than CCCs, immobile extracellular and intracellular anions determine the DF and polarity of GABAergic currents (Glykys et al., 2014; Delpire and Staley, 2014). The mechanisms by which immobile anions were suggested to generate a driving force for Cl^- currents include electrostatic effects on local intracellular or extracellular Cl^- concentration and thereupon on the CCCs; passive Donnan mechanisms; and Cl^- transport by water-transporting CCCs (Glykys et al., 2014; Delpire and Staley, 2014). However, generating and maintaining ionic currents across neuronal membranes is not possible without energy, and passive immobile anions neither do work nor consume energy (Voipio et al., 2014). Local electric fields of immobile anions can shift the concentrations of mobile ion species in aqueous microdomains from which binding of ions to CCCs takes place, but there is no effect on the free energy of ion transport by CCCs. Donnan mechanisms working across Cl^- permeable neuronal membranes in the absence of active Cl^- transport will result in a thermodynamic equilibrium of Cl^- distribution across the plasma membrane, and there will be no driving force for Cl^- currents. Intracellular gradients in the density of immobile charges may generate intracellular spatial gradients of electrical potential along which freely mobile cytosolic ions equilibrate. This would result in identical shifts of membrane potential and the equilibrium potential of the ions, and there would be no change in the transmembrane driving forces of ionic currents. Thus the proposed mechanism (Glykys et al., 2014; Delpire and Staley, 2014) cannot account for observations such as the presence of depolarizing and hyperpolarizing GABAergic Cl^- currents in the axon initial segment and dendrites, respectively (Szabadics et al., 2006; Khirug et al., 2008). Finally, the available experimental evidence is indirect and does not provide support for the idea that KCC2 expressed in CNS neurons would transport ~500 water molecules along with each K^+ and Cl^- ion. We conclude that the ideas on the role of immobile anions in determining the polarity of channel-mediated Cl^- currents conflict with basic thermodynamic principles and with the overwhelming amount of evidence demonstrating the crucial role of CCC-mediated ion transport in neuronal Cl^- regulation.

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In vivo tagging of $Ca_V1.3$ calcium channels reveals C-terminal modulation of gating properties and subcellular expression of the full length channel in mouse inner hair cells

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Ca^{2+} currents through $Ca_V1.3$ Ca^{2+} channels are essential for synaptic transmission of inner hair cells (IHCs). In general, gating of $Ca_V1.3$ channels is under control of (tissue-specific) C-terminal alternative splicing within the $Ca_V1.3$ a1 subunit. Long $Ca_V1.3$ isoforms exhibit a C-terminal modulatory mechanism (CTM) in which binding of a proximal (PCRD) to a distal (DCRD) C-terminal domain forms an inhibitory module that competes with CaM binding, thereby attenuating Ca^{2+}-dependent inactivation (CDI). IHCs express transcripts of a long and a short C-terminal $Ca_V1.3$ splice variant as shown by RT-PCR. The spliced channels either include (long variant, $Ca_V1.3_L$) or exclude (short variants) the DCRD that is required for CTM. The role of the CTM for IHC Ca^{2+} currents was investigated in mice in which the CTM was disrupted by partial replacement of the distal C-terminal regulatory domain (DCRD) with an HA tag ($Ca_V1.3_L$-$DCRD^{HA/HA}$ mice).

The localization of HA-tagged $Ca_V1.3$ channels in IHCs was determined by immunolabeling. Specific anti-HA immunolabeling was present at *all* ribbon synapses of $Ca_V1.3_L$-$DCRD^{HA/HA}$ IHCs. Patch-clamp recordings of mature IHCs around postnatal day 20 revealed that CDI but not voltage-dependent inactivation (VDI) was significantly reduced rather than enhanced in $Ca_V1.3_L$-$DCRD^{HA/HA}$ IHCs following 300 ms depolarizations to V_{max}. Amplitudes of both Ca^{2+} and Ba^{2+} currents (I_{Ca}, I_{Ba}) were increased by 33% and 40%, respectively, whereas voltage dependences of I_{Ca} and I_{Ba} activation were normal. The observed changes in IHC Ca^{2+} current properties did neither affect auditory brainstem response (ABR) hearing thresholds nor distortion products of otoacoustic emissions (DPOAE). However, amplitudes of ABR waves III (superior olivary complex) and IV (nuclei of the lateral lemniscus) were reduced, suggesting that $Ca_V1.3_L$ is important for development and/or mature function of central auditory neurons. Our data demonstrate that the long $Ca_V1.3$ isoform is an intrinsic component of $Ca_V1.3$ clusters at all IHC ribbon synapses and that its C-terminal modulatory domain is required for normal CDI and I_{Ca} amplitude. We hypothesize that CaBPs are more effective than CaM in modulating CDI of $Ca_V1.3$-43S in WT IHCs. Disruption of the DCRD of $Ca_V1.3_L$ in $DCRD^{HA/HA}$ IHCs may enable better binding of CaBPs to the unmasked PCRD, thereby reducing CDI.

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Influence of CKAMP44 on AMPA receptor number and function in relay neurons of the lateral geniculate nucleus

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Lateral geniculate nucleus relay neurons receive excitatory inputs from retinal ganglion cells via retinogeniculate synapses and from cortical neurons via corticogeniculate synapses. There is a developmental increase in AMPA receptor (AMPAR)-mediated transmission during the first 3 postnatal weeks. However, the underlying mechanism of this process is poorly understood.
We investigated how the AMPAR auxiliary protein CKAMP44 influences AMPAR-mediated currents in relay neurons by using wildtype and CKAMP44$^{-/-}$ mice. AMPAR-mediated currents were decreased in P5-P7 and P9-P11 CKAMP44$^{-/-}$ mice indicating that CKAMP44 helps to insert AMPARs into synapses. However, in P23-P33 CKAMP44$^{-/-}$ mice mEPSCs frequency was reduced without a change in amplitude. Since the AMPA/NMDA ratio was also reduced, these results suggest that CKAMP44 deletion increases the number of silent synapses. Indeed, minimal stimulation experiments showed that the proportion of silent retinogeniculate fibers increased in CKAMP44 $^{-/-}$ mice. We previously showed that CKAMP44 modulates AMPAR gating. Thus, AMPARs that interact with CKAMP44 display a very slow recovery from desensitization. Since it had been shown that desensitization influences short-term plasticity (STP) in retinogeniculate synapses, we tested if this influence depends on CKAMP44. Indeed, EPSCs in retinogeniculate synapses showed less short-term depression in CKAMP44-/- mice. In contrast, deletion of CKAMP44 did not affect STP in corticogeniculate synapses, consistent with no influence of desensitization on plasticity in this synapse type. Moreover, in CKAMP44$^{-/-}$ mice, EPSP amplitudes evoked by 10 retinogeniculate axon stimulations at 50 Hz increased, whereas they decreased in wildtype mice. In addition, relay neurons of wildtype mice were more likely to fire at the beginning of the stimulation train, whereas the firing probability was high towards the end of the stimulus train in CKAMP44$^{-/-}$ mice. Thus, CKAMP44 plays an important role in how retinal and cortical inputs are conveyed onto relay cells and influences synaptic AMPAR number in both synapse types, by modulating short-term plasticity in retinogeniculate synapses. Finally, CKAMP44 controls the strength and timing of relay neuron activity when stimulating retinogeniculate axons at frequencies that are observed in vivo.

Intracellular Ca^{2+}/calmodulin modulates Kvß1.1-induced inactivation of Kv channels

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Voltage-gated K^+ channels of the Kv1 subfamily are targets of auxiliary Kvß subunits. Upon coassembly, Kvß1.1 induces inactivation of Kv1.1 channels, which normally only exhibit non-inactivating delayed-rectifier characteristics, by means of its flexible N-terminal ball domain that occludes the ion-conducting pore. Fine-tuned regulation of the speed and degree of Kv channel inactivation is a relevant physiological process for controlling cellular excitability, such as for spike frequency adaptation. Therefore, various cellular processes affect Kvß1.1-induced inactivation. Among them, as demonstrated by Jow et al. (2004, PNAS, 43: 15535–15540), an increase in the intracellular Ca^{2+} concentration counteracts inactivation and, hence, may constitute a negative feedback to limit cellular excitation. However, the underlying mechanisms remained to be elucidated.

Studying Kv1.1/Kvß1.1 channels in macro-patches from Xenopus oocytes we found that the Ca^{2+}-binding protein calmodulin (CaM) is required to mediate the effect of Ca^{2+} on inactivation. An in-silico identified CaM-binding motif in the Kvß1.1 N-terminal domain was functionally evaluated. Disruption of this motif by a triple arginine mutation (R37/41/48N) or a double phenylalanine mutation (F40/53S), prevented Ca^{2+}-dependent CaM binding to the Kvß1.1 protein in vitro and both mutations eliminated the influence of Ca^{2+}/CaM on Kvß1.1-induced inactivation in Xenopus oocytes and in mammalian cells.

Kvß1.1-induced inactivation can also be impaired by application of a substrate for the Kvß-subunit's enzymatic activity as an aldoketoreductase, as previously shown by Pan et al. (2011, PNAS 108: 5885-5890). This phenomenon relies on an immobilization of the N-terminal ball domain via electrostatic interactions involving R37 and R48. Expectedly, Kvß1.1 mutation R37/41/48N eliminated the impact of the substate 4-cyanobenzaldehyde (4-CY) on inactivation. However, mutant F40/53S did not affect the sensitivity of inactivation with respect to 4-CY. Vice versa, a mutation removing electrostatic interaction of the ball domain with the Kvß core domain (E349K) – a consequence of the enzymatic activity – did not abolish the Ca^{2+}/CaM-mediated loss of inactivation.

It can be concluded that inactivation of Kv channels induced by the Kvß1.1 subunit gains its Ca^{2+} sensitivity by Ca^{2+}-dependent binding of CaM to the ß-subunit's N-terminus and that this mechanism, which couples Kv channel activity to the state of cellular excitation, is independent of the Kvß subunit's enzymatic activity.

Persistent discharges in dentate gyrus perisoma-inhibiting interneurons require HCN channel activation

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Parvalbumin (PV)-expressing perisoma-inhibiting interneurons (PIIs) of the dentate gyrus (DG) integrate rapidly correlated synaptic inputs and generate short-duration action potentials that propagate along the axon to their output synapses, supporting fast and phasic inhibitory signaling onto their target cells. In this work, using whole-cell patch clamp recordings of morphologically identified basket and axo-axonic cells, we show that PV-PIIs in rat and mouse DG can integrate their spiking activity and enter into a persistent firing mode characterized by long-lasting trains of action potentials at ~50 Hz in the absence of additional inputs. Persistent discharges resemble antidromic action potentials, do not occur in axonless fast-spiking PV interneurons and can be stopped only by hyperpolarization of the axonal compartment using halorhodopsin, demonstrating that persistent firing emerges in the distal axon. Persistent firing in DG PIIs markedly depends on hyperpolarization-activated cyclic nucleotide-gated channels (HCNCs) that are found in the axonal compartment, as it was effectively blocked by ZD7288 and Ivabradine. Detailed computational single cell PIIs models revealed that HCNC-mediated conductances can actively contribute to persistent firing during conditions of a shift in their voltage activation curve to more depolarized potentials. Persistent firing properties are also modulated by intracellular Ca2+-levels and somatic membrane potential. Paired recordings from PIIs and their target granule cells (GCs) show that PF supports strong inhibitory output signaling. Thus, PF may emerge during conditions of intense activation of the network, thereby providing silencing to the circuitry and the maintenance of sparse activity in the DG.

Intracellular sodium changes mediated by the electrogenic sodium-bicarbonate co-transporter NBCe1 in mouse cortical astrocytes

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The electrogenic sodium bicarbonate co-transporter isoform 1 (NBCe1, SLC4A4) is one of the major transporters for Na+ and HCO3- in many epithelial cells. In the brain, NBCe1 is predominantly expressed in astrocytes operating with 1 Na+: 2 HCO3- stoichiometry and has been reported to be an important regulator of intracellular pH (Deitmer and Chesler, 2009). In this study, NBCe1-mediated changes in intracellular sodium, [Na+]i, in primary cortical astrocyte cultures of wild type (WT, C57BL/6) and of NBCe1 gene -deficient mice (NBCe1 -KO) were studied by calibrated live -cell imaging by confocal microscopy with the Na+-sensitive fluorescent indicator Asante Sodium Green 2–AM (ANG-2 AM). Changing from HEPES -buffered, nominally CO2/ HCO3- free, to CO2/ HCO3- -buffered saline resulted in an increase in the [Na+]i by ~7 mM in WT astrocytes. This increase was reduced or even reversed in NBCe1-KO astrocytes. Imposing an intracellular acid load by a weak acid (40 mM butyrate) to challenge the NBCe1 activity also led to significant increase in [Na+]i and rate of sodium rise in CO2/HCO3- saline in WT as compared to NBCe1-KO astrocytes. Removal of Na+ or K+ abolished the [Na+]i transient observed during application of CO2/ HCO3-, but removal of extracellular Cl- did not affect CO2/ HCO3- -mediated sodium transient. These observations imply that NBCe1 plays an essential role for the rise in [Na+]i when challenged by intracellular acidification in mouse cortical astrocytes. We conclude that the changes in [Na+]i recorded are largely dependent on the Na+/K+ pump and on NBCe1.

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Mechanisms of KCC2-mediated neuroprotection

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We have previously shown that expression of the glycine receptor (GlyR) a3K^{185L} RNA variant is up-regulated in the sclerotic hippocampus of temporal lobe epilepsy patients. Furthermore, chronic activation of GlyR a3K^{185L} by micromolar glycine impedes neuronal action potential firing through tonic shunt inhibition and induces neurodegeneration of primary hippocampal neurons. The choride transporter KCC2 was shown to exhibit neuroprotective effects in the GlyR a3K^{185L} model of neurodegeneration as well as in the NMDA model of cell death. However, the mechanisms of KCC2-dependent neuroprotection are under debate. We now performed a series of electrophysiological and immunohistochemical experiments to study the mechanisms of KCC2-dependent neuroprotection. Our results show that the neuroprotective action of KCC2 does neither involve rescue of passive membrane properties nor changes in the intracellular Cl^- or Ca^{2+} concentrations. They also rule out changes in action potential generation, GABAergic network activity and NO signaling as possible junctions between neuroprotection and KCC2 expression. Rather, our analyses of a series of truncated KCC2 variants reveal that the neuroprotective effects of KCC2 can be attributed to a protein structural function.

Motoneuron function in Drosophila is shaped by cacophony (Ca_V2) calcium channel post-transcriptional modifications

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Motoneurons are not merely passing through the information they are getting through pre-synaptic partners. Instead they are actively shaping motor output. Somatodendritically localized voltage gated calcium channels (VGCCs) play major roles in mediating excitability at postsynaptic sites. If placed close to synaptic input sites VGCCs may, for example, contribute significantly to boosting synaptic input. This in turn enables the motoneuron to amplify its inputs, making it more likely to reach threshold at the spike initiating zone. The Drosophila Ca_V2 homolog *cacophony* mediates both low (LVA) and high voltage activated (HVA) somatodendritic calcium channels which, strategically expressed throughout the neuron, open up a variety of possibilities to shape synaptic input. The Drosophila *cacophony* gene consists of 34 exons, some of which are alternatively spliced, as well as multiple A-I pre-mRNA editing sites. In addition, cacophony associates with accessory subunits, such as a_2d and ß-subunits. All of these mechanisms may contribute to post-transcriptional modification and expression of *cacophony* which may result in different cacophony ion channels as well as different localization throughout the neuron. Here we probe cacophony channel function for input integration depending on post-transcriptional modification. Using the Drosophila tool box we express single genetically engineered cacophony splice variants possessing alternatively spliced exons that localize to domains such as the voltage sensor or accessory subunit binding domains in an otherwise *cacophony* null mutant background. To assess cacophony calcium channel function we employ *in situ* whole cell patch clamp as well as calcium imaging. As a model we use a well-studied motoneuron, MN5, that innervates two of the six wing depressor muscle fibers and thereby contributes to wing down stroke which is involved in two essential behaviors: flight and courtship. We show that cacophony calcium channels are expressed in MN5 dendrites where they enhance synaptic depolarization upon activation of nicotinic acetylcholine receptors. Furthermore, accessory subunits are needed to mediate LVA and HVA cacophony channels. Additionally, expression of specific cacophony splice variants results in altered calcium currents and altered excitability whereas the absence of A-I pre-mRNA editing merely shifts activation voltages of both LVA and HVA cacophony calcium currents. Overall, post-transcriptional modifications as well as accessory subunits contribute to the normal functioning of cacophony VGCCs in MN5. This now enables us to study the consequences for behavior.

Multiple Ca^{2+} channel dependent components in growth-hormone secretion from male rat anterior pituitary somatotrophs

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Voltage-gated Ca2+ influx (VGCI) through Ca2+ channels plays a key role in the secretion of pituitary hormones. The involvement of L-type Ca2+ channels in this VGCI is well established, yet, much less is known about the involvement of non-L-type Ca2+ channels. In this study we examined: 1. whether non-L-type Ca2+ channels exist in the membrane of pituitary cells. 2. Whether non-L- Ca2+channels regulates growth-hormone (GH) secretion. 3. Whether Ca2+ channels in the membrane of pituitary cells are distributed among raft and nonraft membrane domains. Whole-cell recordings from dissociated rat anterior pituitary (AP) cells, and the use of specific Ca2+ channel blockers; ω-agatoxin-IVA, ω-conotoxin GVIA and SNX-482, to block P/Q-, N-and R-type Ca2+ channels, respectively, revealed a fraction of non-L-type VGCI that might reach ~46%. Western blotting identified immunblots for; a1C, a1D, a1A, a1B and a1E subunits, corresponding to Cav1.2, Cav1.3, Cav2.1, Cav2.2, and Cav2.3 channels. Additionally, RT-PCR identified transcripts for a1C, a1D, a1A and a1B subunits. Transcripts for a1E were non-specific and transcripts for a1S were not detected at all. Taken together these results clearly demonstrate the co-existence of L-type (Cav1.2 and Cav1.3) and non L-type (Cav2.1, Cav2.2 and Cav2.3) Ca2+ channels in the membrane of AP cells (Tzour et al 2013). We next examined whether these non-L-type channels regulate GH secretion. To this end the same Ca2+ channel blockers were used to block K+-stimulated GH secretion from dissociated AP cells. Our results revealed that non-L-type Ca2+ channels contribute ~50% to K+-stimulated GH secretion, similar to the contribution of these channels to the total VGCI (~46%). Whether Ca2+ channels are segregated among different membrane compartments was further investigated in flotation assays using Nycodenz gradients. Western blotting of gradient fractions revealed that Cav1.2 and Cav1.3 channels were distributed among light and heavy gradients fractions, i.e., among raft and non raft membrane domains. Cav2.1 channels were mostly localized in light gradient fractions, i.e., in raft membrane domains whereas Cav2.2 and Cav2.3 channels were mostly localized in heavy gradient fractions, i.e., in nonraft membrane domains (Tzour et al 2013). In summary, our results demonstrate multiple pathways for VGCI through Ca2+ channels in the membrane of native AP cells, which are well correlated with multiple Ca2+ channel dependent components in GH secretion. Compartmentalization of these channels among raft and nonraft membrane domains may underlie their differential regulation by different signalling pathways, under different physiological conditions.

Tzour et al (2013). Multiple pathways for high voltage-activated Ca(2+) influx in anterior pituitary lactotrophs and somatotrophs. J Neuroendocrinology 25, 76–86).

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Mutations in *GABRA3* cause X-linked idiopathic epilepsy

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Two novel mutations affecting *GABRA3* gene encoding alpha-3 subunit of the $GABA_A$ receptor family have been associated with idiopathic generalized epilepsy (IGE) and X-linked epilepsy. To assess the pathogenicity of these variants and better understand the role of $GABA_A$ receptors in epileptogenesis, we set out to functionally analyze these variants. Using site-directed mutagenesis, detected mutations were introduced into the cDNAs encoding affected human $GABA_A$ receptor subunit and studied using *Xenopus laevis* oocyte expression system and automated two-electrode voltage-clamp recording technique. Electrophysiological analysis of both mutations showed a significant decrease of GABA-induced currents in comparison with the wild-type (WT) alpha-3 subunit of the $GABA_A$ receptor. These results indicate for both mutations a complete loss of function suggesting *GABRA3* as a potential novel epilepsy gene.
Recently, it was shown that mice, in which the benzodiazepine binding to alpha-3 subunit-containing $GABA_A$ receptor is disrupted, exhibit an increase incidence of spontaneous spike and wave discharges, a characteristic of absence seizures. Therefore, the alpha-3 subunit of the $GABA_A$ receptor may play an essential role in preserving the normal oscillatory activity in the thalamo-cortical network. In fact, it is known that the alpha-3 subunit of the $GABA_A$ receptor is the predominant subunit expressed in the thalamic reticular nucleus (nRT), which is important to maintain the inhibition and a normal functionality within the thalamo-cortical circuit. Further studies of detected alpha-3 subunit variants are necessary to define their contribution to epileptogenesis.

Neocortical neurons possess two distinct persistent sodium currents with different voltage dependence and different underlying mechanism of generation

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The central role of voltage-gated Na^+ channels in generation and propagation of action potentials (APs) make them key to neuronal excitability. In addition to the well-described, fast-inactivating component of the Na^+ current, neocortical neurons also exhibit a slowly inactivating, persistent Na^+ current (INaP), that plays a role in determining AP threshold, in repetitive firing and in synaptic integration. The mechanisms underlying INaP remain unclear. We previously showed in single channel and whole cell recordings that INaP in pyramidal neurons is primarily generated in the axon initial segment (AIS). This was recently confirmed by imaging Na^+ influx during prolonged sub-threshold depolarization as well as during slow voltage ramps. Imaging experiments also suggest the presence of somatic INaP with right-shifted voltage dependence. In whole-cell voltage clamp at 22^{o}C, voltage ramps reveal two distinct persistent Na^+ currents, each with a distinct voltage-dependence; upon warming to 35^{o}C, the distinction between the currents is lost. Simultaneous Na^+ imaging reveals a somatic INaP with an I-V relationship that is shifted by about 20 mV in the depolarizing direction as compared to the axonal INaP. The voltage dependence of the Na flux in the AIS and in the soma suggests that the axonal INaP is generated by "window current", as predicted by the Hodgkin Huxley formalism, whereas the somatic INaP reflects a periodic failure of individual channels to inactivate ("modal gating"). This conclusion is supported by non-stationary noise analysis of the INaP.

Physiology and ion channel expression of axons of amygdala projection neurons

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Axons are the principal elements of neuronal signal generation and propagation in the brain. Despite this central function, our knowledge of axonal physiology originates mainly from classical studies of peripheral and cranial nerves, which is typically generalized to the CNS. However, there is a growing body of evidence demonstrating that CNS axons are highly diverse between various brain areas with remarkable functional properties that influence neuronal output and signal transmission (Debanne et al., 2011). Most of these studies were performed in the cortex, hippocampus, cerebellum or auditory brain stem, whereas virtually nothing is known about the physiological function of axons of amygdala projection neurons - pyramidal-like neurons which are critically involved in emotional learning and processing. Here we use a combination of direct axonal electrophysiological recordings and two-photon Ca2+ imaging to characterize the features of axonal action potential initiation, transmission and activity-dependent Ca2+ signaling in basolateral amygdala projection neurons. In addition, we investigated their axonal ion channel distribution and myelination pattern as well as potential mechanisms of axonal plasticity using immunohistochemistry and confocal imaging in combination with Pavlovian fear conditioning. These data will not only increase the knowledge of the physiological variety of CNS axon function, but also provide further insight into the functional properties of amygdala projection neurons, which are potential drug targets for the treatment of anxiety disorders.

Debanne et al.; Axon physiology; 2011; Physiol Rev.; 91(2): 555-602

Reciprocal modulation of $Ca_V2.3$ voltage-gated Ca^{2+} channels by copper(II) ions and kainic acid.

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Kainic acid (KA) is a non-proteinogenic amino acid and potent agonist at non-NMDA ionotropic glutamate receptors (iGluRs) commonly used to induce seizures and excitotoxicity in animal models of epilepsy. $Ca_V2.3$ voltage-gated Ca^{2+} channels have been implicated in the pathogenesis of KA-induced seizures, but signaling pathways leading to their activation remain to be elucidated. A high-affinity extracellular metal binding site on the domain I gating module of these channels endows them with an exceptional sensitivity to endogenous Cu^{2+} and Zn^{2+}, both of which are present in the brain at resting concentrations sufficient for tonic inhibition. Based on whole-cell patch clamp recordings in cells stably transfected with human $Ca_V2.3$ & $ß_3$ channel subunits, we describe an *in vitro* mechanism of KA-induced changes in $Ca_V2.3$ channel gating, which is iGluR-independent and involves reversal of Cu^{2+}-induced tonic suppression. Using electroretinographic (ERG) recordings from the isolated and perfused bovine retina under scotopic conditions, we exemplify how such a mechanism can interfere with normal transmission in neuronal systems. Micromolar KA selectively stimulated $Ca_V2.3$ activation gating in stably transfected cells in the presence but not absence of low nanomolar extracellular Cu^{2+} (50 nM nominal concentration), and its effects were qualitatively and quantitatively reproduced by Cu^{2+} chelation with artificial amino acid tricine. To avoid interference by activation of iGluRs, KA trace metal chelation in the bovine retina was simulated with tricine, which in the presence of 100 nM nominal Cu^{2+} produced significant suppression of the b-wave amplitude by reducing a late component. We have previously demonstrated that the same b-wave component is enhanced by reducing GABAergic reciprocal signaling with Zn^{2+}, Ni^{2+} or genetic ablation of $Ca_V2.3$ channels, suggesting that Cu^{2+} chelation acted by stimulating $Ca_V2.3$ mediated GABA release. Although the relevance for KA-induced ictogenesis and neurotoxicity remains to be elucidated, our findings indicate that chelation of endogenous Cu^{2+} could potently sensitize $Ca_V2.3$ channels and contribute to iGluR mediated effects of excitatory amino acids *in vivo*.

Regulation of intracellular H^+ from alkalosis in cortical astrocytes is mediated by the electrogenic sodium-bicarbonate cotransporter NBCe1

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The regulation of pH_i/H^+_i from intracellular alkalosis (acid loading) is generally attributed to Cl^--coupled bicarbonate transporters like anion exchangers (AE's). The electrogenic sodium-bicarbonate cotransporter (NBCe1, SLC4 A4) is a major acid/base transporter in glial cells, particularly in astrocytes and known to operate with a stoichiometry of $1Na^+:2HCO_3^-$. The equilibrium potential of NBCe1 (E_{NBC} -70 to -80 mV) is close to the resting membrane potential of astrocytes. Therefore, depending upon the ion distribution and membrane potential of astrocytes, NBCe1 can function as an acid extruder and acid loader by mediating inwardly or outwardly-directed bicarbonate transport, respectively. In the present study we have investigated the contribution of NBCe1-mediated, outwardly-directed sodium bicarbonate cotransport to the regulation of H^+_i from an acute alkalosis in primary cultured mouse cortical astrocytes culture of wild-type and NBCe1-KO mouse, and have employed the pH-sensitive fluoresce probe BCECF-AM and confocal microscopy to measure intracellular $[H^+]$,Our results suggest that outwardly-directed NBCe1 is the major regulator of H^+_i from an acute alkalosis induced by the removal of either 5%CO_2/26 mM HCO_3^- or 40 mM butyrate. A minor contribution of chloride bicarbonate exchange was evident only when there was a large outwardly-directed bicarbonate gradient during alkalosis. Attributable to the high bicarbonate sensitivity of NBCe1, its contribution was found to be dominant even in the nominal absence of CO_2/HCO_3^- (HEPES -buffered saline), when the intra - and extracellular concentrations of bicarbonate are very low (< 1 mM). In brain tissue, cells are closely packed; therefore any small change in the flux of ion transport in these cells can significantly modulate the concentration of these ions in the extracellular space of the brain. Therefore we postulate that the outwardly-directed sodium bicarbonate transport by NBCe1 not only regulates the pHi of the astrocytes from alkalosis, but also helps to stabilize extracellular pH in the brain.

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Ryanodine receptor activation induces long-term plasticity of spine calcium dynamics.

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Dendritic spines compartmentalize signalling at excitatory synapses. Upon action potential firing, the majority of spines are subject to global backpropagating action potential (bAP) mediated calcium transients. Here, we demonstrate that bAPs are electrochemically coupled to calcium release from intracellular stores. We describe a new function of ryanodine receptor (RyRs) mediated spine intracellular calcium release, the activity-dependent long-term enhancement of the bAP-calcium transient. This form of plasticity is highly compartmentalized and independent of dendritic calcium regulation. Further, this functional state change depends exclusively on bAPs travelling antidromically into dendrites and spines, without requirement for concomitant synaptic transmission. Induction, but not expression, of bAP calcium transient enhancement is a spine-specific function of the RyR calcium-nanodomain. We describe a new form of spine calcium transient plasticity that constitutes a storage mechanism of neuronal suprathreshold activity patterns

Selective ablation of ionotropic glutamate receptor subunits 2 and 4 in horizontal cells of the mouse retina

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Horizontal cells (HC) receive glutamatergic input from photoreceptors through ionotropic glutamate receptors (GluR) located on their dendrites in the outer plexiform layer (OPL) of the retina. These AMPA-sensitive receptors are composed of four subunits (GluR1-4) in homo- or heteromeric fashion causing a depolarization of HC in the absence of light. HC provide GABA -mediated inhibitory feedback to photoreceptors and thereby enable localized gain and contrast control.

Our group has previously established a transgenic mouse line (GluR4fl/fl:Cx57+/Cre) in which receptor subunit GluR4 was selectively ablated in HC through the Cre-loxP mechanism (Ströh et. al, 2013) to study the effects on physiological properties of HC. Here we report on two new transgenic mouse lines in which subunit GluR2 (GluR2fl/fl:Cx57+/Cre) and both subunits, GluR2 and 4 (Glur2fl/fl/GluR4fl/fl:Cx57+/Cre), have been ablated in this cell type.

To test for successful ablation we ran Western blots of retina total homogenates using antibodies directed against GluR2 and GluR4 (Millipore, Darmstadt, Germany). Intensity of protein bands was quantified with ImageJ (NIH, USA) and GluR signals were normalized against the house-keeping protein a-tubulin (Sigma, St. Louis, USA). Furthermore, we examined retinal sections histologically, using a Leica DMRE confocal microscope (Leica, Wetzlar, Germany). GluR2/4 -immunoreactive (IR) plaques in the OPL were counted and analyzed with ImageJ and processed in Excel (Microsoft, Redmond, USA).

Western blot analysis showed a significant reduction of both GluR2 and GluR4 protein expression in all Cre-mice compared to the wild -type. While reduction of both proteins was nearly identical in Glur2fl/fl/GluR4fl/fl:Cx57+/Cre double knock-outs (GluR2: 74.2±21.2%, p=0.005; GluR4: 70.5±19.1%, p=0.009; n=5), GluR2 levels in GluR2fl/fl:Cx57+/Cre mice showed a greater reduction than GluR4 levels (GluR2: 58.2±23.4%, p=0.0003; GluR4: 72.95±24.1%, p=0.002; n=5). Therefore, the ablation of the GluR2 gene alone also lead to a significant reduction of GluR4 expression. Interestingly, the additional ablation of the GluR4 gene in the Glur2fl/fl/GluR4fl/fl:Cx57+/Cre double knock-outs did not lower the expression level of this subunit any further.

Histological analysis of retinal slices presents a comparable picture: GluR4 -IR plaques in the OPL are reduced significantly in both, number and size, in all GluR2fl/fl:Cx57+/Cre (count: 56.7±4.8%, area: 47.9±8.5%, n=2) and all Glur2fl/fl/GluR4fl/fl:Cx57+/Cre mice (count: 32.8±32.1%, area: 25.9±28.1%, n=3). GluR2-IR plaques in the OPL are reduced in number and size in two out of three Glur2fl/fl/GluR4fl/fl:Cx57+/Cre double knock-outs (count: 33.4±18.2%, area: 22.6±14.6%) and one GluR2fl/fl:Cx57+/Cre mouse (count: 63.2%, area: 51.5%). All numbers indicate percentage of wild-type levels.

In summary, we provide evidence that the ablation of GluR2 and GluR2/4 in HC has been successful in our new transgenic mouse lines. Furthermore, expression of both subunits seems to be linked in a way that has yet to be explored.

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Several potassium conductances modulate action potential kinetics and discharge patterns in GFP-expressing interneurons (GIN) in the mouse cingulate cortex.

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Classification of interneurons is based on morphological, electrophysiological and neurochemical features and so far more than 20 distinct classes of GABAergic interneurons have been described. Action potential kinetics and discharge behavior are considered to be reliable classification criteria. This study was performed to investigate the modulation of single action potential kinetics and firing patterns in interneurons by potassium conductances. We used a transgenic mouse line where eGFP is expressed under the control of the human GAD1 promoter [1]. In an earlier study, we have analyzed the neurochemical, morphological and basic electrophysiological properties of these neurons. In the study presented here we examined the actions of different potassium channel blockers on action potential kinetics and discharge patterns. We mainly focused on three types of potassium conductances: on two voltage-dependent potassium currents named I_A (fast transient outward current) and I_D (delayed inward rectifier), as well as on calcium-activated potassium currents of the BK type. All experiments were performed on slice preparations *in vitro*. Action potential kinetics and discharge patterns were investigated in current-clamp mode; activation and deactivation properties of potassium currents in voltage-clamp mode.

The neurons' subthreshold current-voltage relationship was characterized by inward rectification in both, depolarizing and hyperpolarizing direction and it remained largely unaffected by inhibition of I_A, I_D and BK currents. The amplitude and duration of current-induced single action potentials were found to be 80 – 90 mV and 0.7 – 1 ms, respectively. Inhibition of I_A by 4-aminopyridine (4-AP, 50 µM) led to a prolongation of the action potential duration and a block of the fast afterhyperpolarization. A similar effect was found in the presence of the I_D blocker tetraethylammonium (TEA, 0.3 – 10 mM). The BK channel blocker apamin (100 nM) on the other hand resulted in a shortening of the action potential duration without affecting afterhyperpolarizations.

TEA significantly reduced outward currents activating around -40 mV and unmasked a 4-AP-sensitive, rapidly-inactivating fast outward current that could not be detected under control conditions. Application of 4-AP alone on the other hand, reduced the initial fast component of the total outward current and, in addition, led to an unmasking of a slow TEA-sensitive current. Application of apamin in turn resulted in an increase in the TEA-sensitive potassium outward current.

Firing patterns in GIN were investigated by injecting long-lasting (1-4 s) suprathreshold depolarizing current steps. We observed four different discharge patterns: (1) repetitive firing with a high initial frequency, a steadily declining interspike interval (so-called regular spiking pattern with adaptation), (2) phasic firing pattern with strong accommodation, (3) irregular firing pattern with clusters of three or more 'regularly' occurring action potentials separated by interspike intervals of variable durations, and (4) bursting discharge pattern with two to five action potentials at threshold. Both, 4-AP and TEA modulated the spike discharge in all neurons investigated independent of the control pattern.

In summary, the firing behavior of GIN seems to be determined by the spatio-temporal interaction of different potassium conductances.

[1] Oliva AA et al., J. Neurosci. 20: 3354, 2000

STIM1 controls neuronal Ca^{2+} signaling and mGluR1/TRPC3-dependent synaptic transmission in cerebellar Purkinje cells

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The metabotropic glutamate receptor type 1 (mGluR1) is highly expressed in Purkinje cells (PCs) of the mammalian cerebellum. At parallel fiber-PC synapses, activation of mGluR1 evokes a complex synaptic response consisting of IP_3 receptor-dependent Ca^{2+} release from endoplasmic reticulum (ER) Ca^{2+} stores and a slow depolarizing potential involving the transient receptor potential (TRPC) channel subunit TRPC3. A link of mGluR1 to its downstream effectors may involve the ER Ca^{2+} sensor stromal interaction molecule 1 (STIM1), which is known to interact with TRPC channels in non-excitable cells.
Here, we investigated the functional role of STIM1 in mouse cerebellar PCs. Using quantitative single cell RT-PCR and immunostaining we demonstrated that STIM1 is ten times more abundant than its homolog STIM2 in PCs. In a newly generated knockout ($STIM1^{pko}$) mouse line the Purkinje cell-specific deletion of *Stim1* caused impairments in cerebellar motor behavior. Next, we analyzed PCs in acute cerebellar slices using whole-cell recordings in combination with confocal Ca^{2+} imaging. In $STIM1^{pko}$ mice, ER Ca^{2+} release was tested with local application of agonists of mGluR1 and ryanodine receptors, as well as with focal UV-hydrolysis of caged IP_3 and found to be strongly attenuated. In control mice, Ca^{2+} stores were always filled at resting membrane potential. Prolonged perfusion of slices with nominally Ca^{2+} free solutions emptied Ca^{2+} stores and abolished TRPC3-mediated currents evoked by the mGluR-specific agonist dihydroxyphenylglycine (DHPG). By contrast, activity-mediated Ca^{2+} entry through voltage-gated Ca^{2+} channels (VGCCs) transiently overcharged ER Ca^{2+} stores. In $STIM1^{pko}$ mice, both mGluR1-dependent synaptic potentials and DHPG-evoked TRPC3-mediated inward currents were absent in PCs at resting membrane potential. However, activation of VGCCs transiently filled ER Ca^{2+} stores and rescued, for the filling period, TRPC3-mediated currents, indicating its dependence on intracellular Ca^{2+} (Hartmann et al., Neuron, 2014).
STIM1 in non-excitable cells regulates both cytosolic and ER luminal Ca^{2+} levels by activating Ca^{2+}-permeable Orai- and TRPC channels in the plasma membrane. We tested a possible involvement of TRPC3 and other TRPC subunits for store refilling in PCs. We found that spontaneous refilling of emptied ER Ca^{2+} stores persists in TRPC-HeptaKO mice, which lack each of the seven TRPC subunits. Furthermore, we tested how TRPC3 gating depends on the filling state of ER Ca^{2+} stores. In control mice, we found that DHPG-evoked TRPC3-mediated inward currents are not altered when ER Ca^{2+} stores are emptied by blocking SERCA pumps using cyclopiazonic acid (CPA). Moreover, opening of VGCCs by short depolarizing pulses in $STIM1^{pko}$ mice allows the transient activation of TRPC3 by application of DHPG when store filling was prevented by the presence of CPA in the bath. In the same experiment, however, inclusion of 25 mM BAPTA into the pipette solution completely prevented both depolarization-evoked Ca^{2+} entry and the DHPG-evoked TRPC3-mediated inward current. Together, these results establish that STIM1 is a powerful regulator of the ER store Ca^{2+} content and functions as a messenger that couples mGluR1 and TRPC3 in cerebellar PCs through the regulation of cytosolic Ca^{2+}.

Surface mobility of a2d-subunits within the neuronal membrane

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High voltage gated calcium channels (VGCC) are composed of several subunits, the pore forming alpha1-subunit, the intracellular beta-subunit and the mostly extra-cellular located alpha2delta-subunits. Only the complex of all three subunits has been reported to resemble similar kinetic properties as calcium channels in excitable membranes, suggesting a very stable stoichiometry between all three subunits. In addition the activation of VGCC is generally known to be a local transient increase of intracellular calcium that can trigger many processes as vesicular release or kinase activation. However, investigations of the structure of alpha2delta-subunits propose that those subunits are only anchored to the membrane by a GPI-anchor (Davis et al. 2010), which suggest a very labile localization within the cellular membrane. Hence we were wonder, whether a lipid anchored subunit is firmly linked to the pore forming alpha1-subunit of calcium channels or might only a transient interacting partner. We used extracellular HA-tagged alpha2delta-subunits that did not alter the trafficking and kinetic properties of the calcium channel. Surface expressed alpha2delta-subunits were labelled with quantum dots and enabled us to explore the surface diffusion properties of alpha2delta-subunits by single particle tracking (SPT). We explored the surface dynamic in two systems, HEK-cells and primary hippocampal neurons, which gave us the opportunity to either investigate single channel subunit combinations in isolation or to monitor the diffusive properties in the background of the complex environment of the neuronal membrane. Comparing the diffusion properties of alpha2delta -subunits with a GPI -anchored GFP strongly support the lipid based anchoring of these subunits in the membrane but speak against a stable connection to the poor forming alpha1-subunit. Within the neuronal membrane we focused in different membrane compartments as the dendrite, axon and synapse. Here alpha2delta subunits showed highest mobility within the axon and transient confinement or immobilization within the synapse. Increasing the release probability of the synapses by increasing the Ca2+/Mg2+ ratio from 2/2 to 5/1 lead to a stronger confinement of alpha2delta-subunits, whereas blocking network activity by TTX under the same conditions prevented changes in alpha2delta-subunit mobility. Analyzing kinetic channel properties in the absence and presence of alpha2delta subunits in HEK cells indicated an alteration of inactivation time course of VGCC. This might suggest that the dynamic interaction of channel subunits in the neuronal membrane might be used to tune VGCC kinetic and thus functional impact for example in transmitter release.

Transient receptor potential melastatin-3 (TRPM3)-induced activation of AP-1 requires Ca^{2+} ions and the transcription factors c-Jun, ATF2, and TCF

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Transient receptor potential melastatin-3 (TRPM3) channels are non-selective cation channels. The analysis of TRPM3-deficient mice revealed that TRPM3 is a nociceptor channel involved in the detection of noxious heat in the somatosensory system. Recently, we showed that stimulation of TRPM3 channels with the neurosteroid pregnenolone sulfate upregulates activator protein (AP)- 1 regulated gene transcription. Here, we have analyzed the signaling pathway leading to increased transcription of an AP-1-responsive reporter gene following stimulation of TRPM3 channels. The results show that the signaling pathway requires an influx of Ca^{2+} ions into the cells and a rise in the intracellular Ca^{2+} levels. The upregulation of AP-1 was attenuated in cells that had been preincubated with a protein kinase C (PKC) inhibitor or that overexpressed MAP kinase phosphatase (MKP)-1, indicating that Ca^{2+} ions prolong the signaling cascade via activation of PKC and MAP kinases. On the transcriptional level, expression of a dominant-negative mutant of the basic region leucine zipper (bZIP) protein c-Jun, a major constituent of the AP-1 transcription factor complex, or expression of a c-Jun-specific small-hairpin (sh) RNA attenuated pregnenolone sulfate-induced AP-1 activation. In addition, stimulation of TRPM3 channels increased the transcriptional activation potential of the bZIP protein ATF2. Inhibition of ATF2 target gene expression via expression of a dominant-negative mutant of ATF2 or expression of an ATF2-specific shRNA interfered with TRPM3-mediated stimulation of AP-1. Moreover, we show that a dominant-negative mutant of the ternary complex factor (TCF) Elk-1 attenuated the upregulation of AP-1 following stimulation of TRPM3 channels. Thus, c-Jun, ATF2 and TCFs are required to connect the intracellular signaling cascade elicited by activation of TRPM3 channels with enhanced transcription of AP-1 regulated genes. We conclude that pregnenolone sulfate-induced TRPM3 channel activation changes the gene expression pattern of the cells by activating transcription of c-Jun, ATF2 and TCF controlled genes.

TRPV1 regulates innervation in the hippocampus

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The presence of the TRPV1 channel and its splice isoforms in the central nervous system (CNS), especially in the hippocampus, has been a subject of debate. Using biochemical, electrophysiological, and immunohisto- and cytochemical methods we have identified the presence of TRPV1 in hippocampal neurons and its VR5 splice isoform. Surprisingly VR5 expression persists in TRPV1 -/- mice. We have also found that TRPV1 and VR5 splice isoform are differentially expressed in subsets of cells. We detected a subpopulation of inhibitory interneurons that express high levels of TRPV1, are reelin-positive, and express high levels of NGF and BDNF. In dissociated hippocampal cultures, these TRPV1-expressing neurons also have denser innervation compared to surrounding neurons. Activation of native TRPV1 with capsaicin increased excitatory innervation of these high TRPV1-expressing neurons, with no change in inhibitory innervation. Conversely, blockade of TRPV1 channels decreased excitatory innervation (with no change in inhibitory innervation) on these neurons. Transfection of cultured hippocampal neurons with TRPV1 before synapse formation, on DIV2, resulted in a similar effect on innervation: the number and strength of excitatory terminals formed on TRPV1 -transfected neurons increased compared to control, but the number and strength of inhibitory terminals decreased. These effects were reversed when the channel was blocked with a TRPV1 specific antagonist. Conversely, if TRPV1 was transfected after the main period of synaptogenesis, at DIV10, a reduction in both inhibitory and excitatory presynaptic strength was observed. Both neuronal activity and NGF appear to control TRPV1 expression, since treating cultures with bicuculline, forskolin, or NGF increased TRPV1 expression. Both neurons with high endogenous levels of TRPV1 and TRPV1-transfected neurons had higher levels of BDNF compared to surrounding neurons. Because BDNF can affect presynaptic strength and act in a retrograde manner, we hypothesized that increased innervation of TRPV1-expressing neurons may be caused by increased BDNF in these neurons. To test this, we treated TRPV1-transfected neurons with a BDNF scavenger and found that the TRPV1-induced increase in excitatory synaptic strength was blocked. In summary, we found that post-synaptic TRPV1 alters innervation, and specifically alters the ratio of excitatory to inhibitory inputs, on a subpopulation of inhibitory interneurons in the hippocampus, which might explain the reported effects of TRPV1 on synaptic plasticity. Further research is needed to determine the endogenous ligands present in the CNS that activate TRPV1 to cause changes in innervation and synaptic strength.

Tyramine functions as a neuromodulator of *Drosophila* larval motoneurons

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In many species motor behavior is modulated by biogenic amines. In spinal cord for example, the gain of motor output is strongly enhanced by descending serotonergic drive from brainstem. Similarly, invertebrate motor behaviors, such as insect flight and walking, or crawling in *C.elegans* or *Drosophila* larvae are under modulatory control of biogenic amines. In multiple invertebrates, interactions of the amines octopamine and tyramine have been demonstrated to be essential for both the initiation and the maintenance of locomotor behaviors. However, the cellular targets and molecular mechanisms of tyramine and octopamine interaction remain largely unknown. This study combines the genetic power of *Drosophila* with patch clamp recordings from identified motoneurons to pinpoint possible effects of tyramine and octopamine on crawling motoneurons.

Crawling motoneurons projecting to larval *Drosophila* body wall muscles can be distinguished by morphological and physiological criteria into those with small boutons (Is motoneurons) that innervate multiple muscles of one segment and those with big boutons (Ib motoneurons) that innervate only one muscle. Our data show that tyramine exerts differential effects directly onto these motoneurons. Bath application of tyramine (10^{-5} M in standard saline) reversibly decreases the firing frequencies of Is motoneurons as induced by somatic ramp or square pulse currents injections. It also reversibly increases the delay to the first action potential of each burst. This decrease in Is motoneuron excitability is in accord with decreased locomotor activity of larvae with genetically increased tyramine levels. Voltage clamp recordings reveal that tyramine increases transient potassium current amplitude. We now combine genetic and pharmacological manipulation to identify the molecular basis of this increase in transient potassium current. Interestingly, Ib motoneurons show the opposite response in that they show increased firing rates. In summary, this study reveals the first direct effects of the biogenic amine tyramine on *Drosophila* central neurons, and that different types of motoneurons respond differently to the same biogenic amine. Since motor behavior is affected by the concerted interplay of tyramine and octopamine, we will next address also the effects of octopamine and analyze both in the context of ongoing central pattern generation by in situ patch clamp recordings during crawling.

Voltage-gated calcium channels in the mouse sciatic nerve

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Voltage-gated calcium channels (VGCCs) are known to mediate several cellular functions, one of them being fast vesicular release at neuronal synapses. Recently, it has been demonstrated that VGCCs also mediate vesicular release of neurotransmitter at neuron-glia synapses in central nervous system white matter.

In my study, I aimed to set up calcium imaging in mouse live sciatic nerve slices, in order to investigate whether calcium transients mediated by VGCCs also occur along the peripheral axons. So far, calcium transients investigated in different peripheral nerves have been triggered by trains of stimulation and recorded at low time resolution, which may lead to uncertainties in determining the real transient amplitude. In my study, I applied a single stimulation pulse and recorded with a time resolution of 2 ms. Because resting calcium concentration and activity-dependent changes in peripheral nerve axons have not been quantitatively determined, I tested both the low affinity calcium indicator Magnesium Green AM and the high affinity calcium indicator Oregon Green BAPTA-1 AM (OGB-1 AM). Due to higher signal-to-noise ratio, I chose the high affinity indicator. However, calcium imaging experiments of sciatic axons loaded with OGB-1 AM and perfused with a solution containing a low calcium concentration point out that the calcium transients' amplitude varies sublinearly with the extracellular calcium concentration, making small changes in calcium influx difficult to report. Combining calcium imaging with OGB-1 AM and pharmacology, I was able to show that action potentials propagating along sciatic nerve axons trigger calcium transients in these axons, which are mediated by VGCCs. My experiments also suggest that these transients are partially mediated by N-type and L-type calcium channels. Calcium entry through the VGCCs present along peripheral axons may be involved in activation of potassium channels responsible for repolarization of the action potential, fast axonal transport, or neurotransmitter release along these axons. N-type VGCC-mediated exocytosis could contribute to axonal-glia communication, which can be important for myelination, axonal growth or regeneration.

Poster Topic

T7: Synaptic Transmission, Pre- and Postsynaptic organization

T7-1A A point mutation abolishes the targeting of Mover to presynaptic terminals.
Asha Kiran Akula, Saheeb Ahmed, Camin Dean, Thomas Dresbach

T7-2A Actions of fluoxetine (Prozac) on behavior and neuronal function
Sandra Larissa Elena Blümich, Kyle Ritter, Zana R. Majeed, Jonathan Robinson, Eugen Brailoiu, Robin L. Cooper

T7-3A ADF/cofilin in synapse physiology and mouse behavior
Marco Rust, Andreas Görlich, Anika-Maria Zimmermann, Michael Wolf, Marco Sassoè-Pognetto, Christine Gurniak, Eckhard Friauf, Walter Witke

T7-4A Alteration in dendritic spine morphology and synaptic receptor composition in RICH2 knock-out mice
Tasnuva Sarowar, Stefanie Grabrucker, Juergen Bockmann, Tobias M. Boeckers, Andreas M. Grabrucker

T7-5A BDNF recruitment to optogenetically activated regions and its transfer to neighbouring cells
Markus A. Stahlberg, Karl Deisseroth, Stefan W. Hell, Camin Dean

T7-6A Calcium channel mobility within the presynaptic membrane
Martin Heine, Romy Schneider, Eric Hosy, Johannes Kohl, Yulia Klueva, Daniel Choquet, Ulrich Thomas, Andreas Voigt

T7-7A Complexins 3 and 4 act as fusion clamp and regulate vesicle availability at photoreceptor ribbon synapses in mouse retina
Johann Helmut Brandstätter, Anna Sendelbeck, Andreas Feigenspan, Michaela Fuchs, Norbert Babai, Kerstin Reim

T7-8A Deletion of the tryptophan-rich basic protein Wrb causes progressive hearing impairment in mice
Tina Pangrsic, Iliana Panou, Christian Vogl, Gulnara Yamanbaeva, Carolin Wichmann, Artur Indzhykulian, Shuh-Yow Lin, Sonja Wojcik, Nicola Strenzke, David Corey, Tobias Moser

T7-9A Depolarizing GABA orchestrates inhibition in developing mouse neocortex *in vivo*
Knut Kirmse, Michael Kummer, Yury Kovalchuk, Otto W. Witte, Olga Garaschuk, Knut Holthoff

T7-10A Diffusion of Sodium Signals in Spiny Dendrites
Christian Kleinhans, Karl W. Kafitz, Christine R. Rose

T7-1B Diffusion versus extrusion: mechanisms for recovery from sodium loads in mouse CA1 pyramidal neurons
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A point mutation abolishes the targeting of Mover to presynaptic terminals.

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Synapses are asymmetric cell-cell contacts. Targeting of synaptic vesicles to presynaptic sites is one of the most intricate examples of polarized trafficking and selective accumulation. Surprisingly little is known about amino acid sequences or structural requirements mediating presynaptic targeting of synaptic vesicles proteins. Here we have characterized presynaptic targeting of Mover / TPRGL / SVAP30, a 266 amino acid protein associated with synaptic vesicles as a peripheral membrane protein. We used expression of GFP-tagged versions of Mover in cultured hippocampal neurons as a screening system, testing which recombinant variants of Mover undergo presynaptic targeting. We found that deleting either of 4 predicted phosphorylation sites did not impair targeting. By contrast, introducing a phenylalanine to arginine mutation into a c-terminal area of Mover completely abolished presynaptic targeting, leading to a diffuse distribution of the mutant protein. To delineate the domain mediating targeting we expressed several deletion constructs. However, neither an n-terminal region, a c- terminal region nor a central region allowed for targeting. These deletion constructs also failed to dimerize, as revealed in co-immunoprecipitation assays. A yeast-2-hybrid assay and co-immunoprecipitation corroborated the strong tendency of Mover to undergo homomeric interaction. These data suggest that Mover forms dimers or oligomers as a prerequisite for a phenylalanine residue to become exposed and mediate association with components of the axonal trafficking machinery or with synaptic vesicles directly.

Actions of fluoxetine (Prozac) on behavior and neuronal function

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Fluoxetine (Prozac) is a commonly used medication for altering mood (e.g., depression, obsessive-compulsive disorders, appetite, learning, and cognition). The therapeutic action is related blocking the reuptake of serotonin in the presynaptic nerve terminals. Usually Prozac does not bear severe side effects which explains its broad use; however, high levels of fluoxetine can lead to serious health problems or even death. In 1999 5% of acute intoxications lead to a fatal outcome. If more precise mechanisms of acute action with high levels of fluoxetine can be determined then effective treatment may be possible to counteract the pathological conditions. In examining the potential actions of fluoxetine in altering neuronal activity, during development within the larval Drosophila CNS, we noted acute and chronic actions on behavior and neural responses with high doses. We pursued this observation with 100µM exposure in this current study. Drosophila melanogaster is not only a genetically tractable organisms but many of the physiological functions at a cellular level are conserved and have be shown to be similar to those found in mammals. We showed that evoked transmission at the NMJ not only in Drosophila larvae but also at crayfish NMJ is blocked but also there is an increase in rate of spontaneous quantal events in both model preparations. The Drosophila larval heart was also used as a model and rapidly stopped when exposed to 100µM. Examining fluoxetine on mammalian glutamatergic neurons with Fura 2 (Ca2+ indicator) in culture indicated Ca2+ release from ER as a potential mechanism to explain some of the observations but not all.

ADF/cofilin in synapse physiology and mouse behavior

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Actin depolymerizing proteins of the ADF/cofilin family are essential for actin dynamics, which is critical for synaptic function. Two ADF/cofilin family members, namely ADF and n-cofilin, are highly abundant in the brain where they are present in excitatory synapses. Previous studies demonstrated the relevance of n-cofilin for dendritic spine morphology, synaptic plasticity, associative learning and anxiety. However, they also suggested overlapping functions for ADF and n-cofilin. To test whether both proteins have redundant function, we genetically removed ADF together with n-cofilin from synapses. In double mutant mice, synaptic actin dynamics was impaired and more severely affected than in single mutants. The resulting cytoskeletal defects heavily affected the organization, mobilization, and exocytosis of synaptic vesicles. Impaired striatal glutamate release caused behavioral abnormalities in double mutants that were not present in single mutants, including hyper-locomotion that was reversed by the attention deficit/hyperactivity disorder (ADHD) medication methylphenidate. Our data demonstrate that ADF and n-cofilin have overlapping synaptic function and highlight the relevance of synaptic actin dynamics for presynaptic physiology and behavior.

Alteration in dendritic spine morphology and synaptic receptor composition in RICH2 knock-out mice

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Small GTPases of the Rho family play important role in neuronal growth and development, in particular in neurite formation, axonal guidance and branching, dendrite formation and spine morphogenesis. Various types of mental retardation have been associated with a defect in the Rho GTPase signaling pathways. Therefore, a functional study of key players of this pathway, their interconnection, and other molecules that control the activity of these GTPases is important given their clinical implication.
On the basis of a proteomic screen, a brain specific RhoGAP protein (RICH2/RhoSAP) was identified which interacts with the post-synaptic scaffolding protein Shank3. This protein has a N-BAR domain, a RhoGAP domain and a C-terminal PDZ domain. In vivo data suggests that RICH2 acts as a RhoGAP protein for Rho GTPase Rac1 which has a role in spine morphogenesis and glutamatergic receptor clustering. On the basis of these observations, we have generated a mouse model lacking RICH2 to further investigate the mechanism used by Rho GTPases to exert their activity on synaptic plasticity in terms of membrane vesicle trafficking and actin remodeling.
Our data shows that expression of RICH2 is brain region specific and especially high in hippocampus and cerebellum. We detected a change in the brain volume in RICH2 knock-out mice. Moreover an alteration in spine morphology and spine area could be detected using Golgi staining. We have also detected an alteration in glutamatergic receptor composition using both western blot and immunohistochemistry. Phenotypically the knock out mouse show impaired motor learning.
Taken together, our results point towards an effect of increased Rho GTPase activity via decreased GAP activity. Moreover, the interaction with Shank3 might provide a link of RICH2 to synaptic pathways associated with neuropsychiatric diseases.

BDNF recruitment to optogenetically activated regions and its transfer to neighbouring cells

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Neuronal networks form the basis of information processing in the brain, leading to complex phenomena, including learning and memory. These phenomena rely on the ability of the brain to modify the strength of individual synapses in response to environmental stimuli and changes in neuronal activity. Brain-derived neurotrophic factor (BDNF) has been implicated in potentiating synaptic strength during synaptic plasticity, for example during long-term potentiation (LTP). But whether BDNF is recruited specifically to active synapses to modulate their function has not been directly demonstrated. In addition, it is debated whether BDNF signals in a pre - to postsynaptic direction, or in the reverse direction, from post to presynaptic sites (or even between neurons and astrocytes). However many of these functions are controversially discussed and certainly not clearly understood in detail.
To test if BDNF-harboring vesicles are specifically recruited to sites of increased activity, we use optogenetics to achieve focal stimulation of small regions and single synaptic sites in hippocampal neurons. Neurons co-transfected with BDNF-RFP and channelrhodopsin variants were focally illuminated with either a single wavelength in a center spot to activate channelrhodopsin, or (in a novel STED-inspired illumination approach) with one wavelength in a center spot to activate channels, and a surrounding donut illuminated by a different wavelength of light, to close channels. Ideally this should prevent light scatter and diffusion of activated channels, and thus create a highly focal region of activation, approaching the size of a single synapse or even smaller. Recruitment of BDNF-RFP vesicles to activated sites was then tested. To investigate whether recruited BDNF vesicles undergo exocytosis at these sites we transfected neurons with an orange pHluorin variant, mOrange2, fused to BDNF and examined exocytosis dependent fluorescence increases in response to focal activation. In addition, using a reporter construct co-expressing BDNF-RFP and cytosolic CFP, we observed transfer of BDNF-RFP to neighboring cells, including both neurons and astrocytes. We are investigating the extent of transfer and whether it is activity or cell-type dependent, by pharmacological and optogenetic control of culture activity followed by visualization of BDNF-RFP in distinct cell types. In addition, by injecting AAV BDNF-RFP-P2A-ECFP into either CA3 or CA1 regions of the hippocampus in organotypic cultures, we are investigating if transfer of BDNF-RFP occurs in the pre to postsynaptic, or post to presynaptic direction, in an intact circuit where BDNF is required for LTP. Together these experiments will answer key questions regarding the specificity of recruitment and transfer of BDNF between cells to affect circuit function and synaptic plasticity.

Calcium channel mobility within the presynaptic membrane

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Ca2+-triggered neurotransmitter release from synaptic vesicles (SVs) is crucial to neural signalling. The functional and positional coupling between presynaptic voltage-dependent calcium channels (VDCCs) and docked SVs is a critical determinant for the probability of release. Distances from <10 to >100 nm have been reported for different types of synapses and are named as nano- or microdomain coupling. By exploring release probabilities of different synapses the positioning of calcium channels has been proposed to be a synapse specific feature. Here we explored VDCC surface dynamics at hippocampal synapses using superresolution microscopy. Tracking channels by the use of single particle tracking photoactivation localisation microscopy (sptPALM) we observed that the majority of channels are mobile. Depending on the expression of CaV2.1 or CaV2.2 we are able to dominate the majority of synapses of the transfected neuron with the tagged calcium channel. Hence, depending on the isoform we can either replace the endogenous channel population with CaV2.1 or dominate the synapse with CaV2.2. Independent of the isoform we express ~60% of VDCCs are mobile but nonetheless confined to the presynaptic membrane compartment. Outside synapses about 80% of channels are mobile. Strong depolarisation with 40 mM KCl or long action potential trains (40 AP, 20 Hz) reduce channel mobility, predominant for CaV2.2. Intracellular Ca2+ chelation by EGTA or BABTA reduced VDCC mobility while accelerating syntaxin-1A. Increasing surface expression of VDCCs by overexpressing subunit a2d1 led not to changes in channel mobility but to enlarged active zones rather than higher channel density. We propose that synaptic VDCC mobility supports channel cooperativity and tunes neurotransmitter release by equalizing fusion probabilities of docked SVs. In addition the stimulation history might tune channel dynamic within the presynaptic zone and hence contribute to the release probability of a particular synapse.

Complexins 3 and 4 act as fusion clamp and regulate vesicle availability at photoreceptor ribbon synapses in mouse retina

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Photoreceptor cells transmit light signals over a wide range of intensities, release neurotransmitter tonically, and respond to changes in light intensities with graded changes in release. To accomplish these tasks, photoreceptor cells possess a highly specialized type of chemical synapse, the ribbon synapse. Faithful transmission of light signals at ribbon synapses requires the availability of a large pool of releasable vesicles and mechanisms that facilitate evoked release and prevent spontaneous release to optimize signal-to-noise ratio and extend the dynamic range in response to stimuli. Complexins (Cplxs), small cytosolic proteins that bind to SNARE complexes, are associated with both facilitating Ca^{2+}-triggered evoked release and clamping spontaneous release. In preceding studies, we showed that Cplx3 and Cplx4 are present at photoreceptor ribbon synapses, and that the knockout of the two (Cplx3/4 DKO) affected ribbon synaptic release moderately (Reim et al., 2005; 2009).

In the present study, we extended our analysis of Cplx3 and Cplx4 function at photoreceptor ribbon synapses, and performed patch-clamp recordings on retinal horizontal cells (Hc) from Cplx3/4 DKO and wild-type (WT) control mice. We recorded spontaneous and light evoked Hc responses. At a holding potential of –60 mV, Hc displayed spontaneous excitatory postsynaptic currents (spEPSCs), which were caused by release of glutamate from presynaptic cone photoreceptors. The frequency of spEPSCs in Cplx3/4 DKO mice was 108.8 ± 8.2 Hz (n = 7), which was significantly higher than spontaneous activity measured under the same conditions in WT mice (45.2 ± 2.7 Hz, n = 6).
In light experiments, Hc of Cpx3/4 DKO mice responded to photopic full-field illumination with a transient outward current, which declined monoexponentially to a steady state during the stimulus. The peak current amplitude was 327 ± 41 pA (n = 16), and it decayed to 48 ± 8 pA (n = 12) with a time constant of 239.1 ± 64.4 ms (n = 16). In comparison, in wild-type mice the same light stimulus elicited a tonic outward current with an amplitude of 19 ± 9 pA. All values are mean ± s.d.
Synaptic vesicle distribution (electron microscopy) at photoreceptor ribbons of light and dark adapted WT and Cplx3/4 DKO mice differed significantly between the two genotypes. At WT photoreceptor ribbons, synaptic vesicles were distributed uniformly along the ribbon in the dark, while in the light, the base of the ribbon was almost free of synaptic vesicles. Such differences could not be detected in Cplx3/4 DKO mice. In the light and dark, comparable numbers of synaptic vesicles were distributed uniformly along the ribbon.

From the results, we conclude that the Cplxs 3 and 4 function as a fusion clamp at photoreceptor ribbon synapses, most likely by regulating the availability of synaptic vesicles for release in a light dependent manner.

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Deletion of the tryptophan-rich basic protein Wrb causes progressive hearing impairment in mice

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Tryptophan-rich basic protein Wrb has recently been proposed to mediate the insertion of tail-anchored (TA) proteins into the ER-membrane. A mutation in the gene coding for a mammalian Wrb homolog causes hearing, vestibular and visual deficits in zebra fish (named the pinball wizard mutant line). Here, we have investigated how the deletion of Wrb affects hearing and balance in mice. Our results demonstrate a progressive hearing loss that cannot be explained by impaired cochlear amplification by outer hair cells, reduced mechanotransduction or perturbed synaptic calcium currents. We found normal formation, maintenance and function of the hair bundles. Patch-clamp recordings from inner hair cells (IHCs) revealed normal synaptic calcium currents, but a perturbation of synaptic exocytosis. Immunohistochemistry demonstrated a progressive loss of ribbon synapses. Moreover, ribbon synapses further displayed unusual accumulation of large vesicles and subplasmalemmal cisternae alongside reduced numbers of ribbon-associated vesicles as revealed by transmission electron microscopy. Finally, in the IHCs lacking Wrb we found a reduction in the protein levels of the Wrb-interacting partner TRC40 and their likely target, otoferlin, a predicted TA protein. Otoferlin is required for calcium-regulated neurotransmitter release at the IHC ribbon synapse. Based on these findings, we hypothesize that the deletion of the Wrb gene perturbs the membrane insertion of otoferlin, thereby affecting IHC presynaptic function.

Depolarizing GABA orchestrates inhibition in developing mouse neocortex *in vivo*

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Whereas γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the adult brain, a large body of *in vitro* evidence suggests that GABAergic transmission is partially excitatory during early development. The latter is supposed to result from a comparatively high intracellular chloride concentration in immature neurons that renders $GABA_A$-receptor mediated responses depolarizing. At present, however, the mode of GABA action in the intact developing mammalian brain remains controversial. We here combine two -photon Ca^{2+} imaging and electrophysiological techniques in spontaneously breathing, head-fixed neonatal mice to address the mode of GABA action *in vivo*. We provide evidence that GABA depolarizes a substantial fraction of immature neurons in the upper cortical plate. Our data further reveal that GABA spatiotemporally constrains the generation of spontaneous network activity in the occipital cortex. Thus, our data identify GABA as a dual depolarizing-inhibitory neurotransmitter in the immature neocortex *in vivo*.

Diffusion of Sodium Signals in Spiny Dendrites

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At glutamatergic synapses, opening of postsynaptic ligand-gated ion channels following release of glutamate induces sodium increases in spines and dendrites, acting as localized sources, sodium is spreading from. Sodium elevations diminish the driving force for sodium influx and sodium-dependent transport and will thus affect basic neuronal properties. Despite its central role, essential parameters for diffusion of the highly mobile, mostly non-buffered sodium ions into and particularly along neuronal processes are largely unknown. Likewise, it is still in question whether spines might compartmentalize sodium due to a restricted diffusion through their narrow spine necks that connect them to the adjacent dendrite.

Here, we studied biophysical features of sodium diffusion in CA1 pyramidal cells in acute mouse hippocampal slices employing multiphoton imaging with the sodium-sensitive dye SBFI and whole-cell patch-clamp. Local sodium influx into dendrites and spines was elicited by UV laser photolysis of caged glutamate or by direct electroporation.

Both stimulation techniques induced inward currents, which, at peak amplitudes of 400-500 pA and total charges between 150-200 pC, showed decay-time constants of ~1 s. They were accompanied by dendritic sodium signals that exhibited monoexponential decay with time constants of around 10 s. Peak amplitudes were largest close to the stimulation site and depended on the diameter of the dendrite for a given stimulus (e. g. peak sodium increase was ~5 mM in primary dendrites at 15-30 µm distance from the soma). Signal amplitudes decayed with increasing distance from the stimulation site and subsided within 50 µm along the dendrite. Propagation speed of these signals was approximately 11 µm/s, and diffusion constants calculated from one-dimensional diffusion equation amounted to 160 $\mu m^2/s$, which is considerably slower than in aqueous solution (~950 $\mu m^2/s$). Furthermore, we found a notable delay between sodium peaks in dendrites and spines. Our data indicate that intracellular diffusion of sodium in and between different cellular structures is hindered by tortuosity and/or local binding and sodium export through the Na^+/K^+-ATPase. Moreover, they suggest that spine necks represent a significant barrier for diffusion of sodium between spines and dendrites, supporting the notion of a compartmentalization of sodium signals in spines.

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Diffusion versus extrusion: mechanisms for recovery from sodium loads in mouse CA1 pyramidal neurons

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In the brain, sodium ions (Na^+) do not only represent basic charge carriers for the generation of action potentials and excitatory synaptic currents. The inwardly directed sodium gradient also provides the energy for many secondary active transport processes, the activity of which is altered upon increases in intracellular sodium. Sodium entering active neurons has thus to be extruded efficiently to guarantee proper function of these processes. In the present study, we analyzed the mechanisms for recovery from intracellular sodium loads in different cellular compartments of principal neurons in situ.

To this end, we performed quantitative widefield imaging in somata and dendrites of CA1 hippocampal neurons of acute mouse hippocampal tissue slices, combined with patch-clamp recordings. The dye SBFI (sodium-binding benzofuran isophalate) was used as a sodium indicator. To evaluate if addition of SBFI introduced a distortion of intracellular sodium signals due buffering of sodium, neurons were loaded with different concentrations of SBFI (0.1 - 1 mM) and sodium signals induced by focal pressure application of glutamate. Normalization of glutamate-induced sodium transients to the accompanying membrane currents revealed that sodium signals were not influenced by the dye concentration used, indicating that SBFI introduces no buffering under our experimental conditions. In slices loaded with the membrane-permeable form of SBFI (SBFI-AM), analysis of the recovery from glutamate-induced sodium signals revealed a maximum sodium extrusion rate of 0.139 ± 0.006 mM/s for cell bodies and of 1.97 ± 0.21 mM/s for primary dendrites. In the presence of ouabain, which blocks the Na^+/K^+-ATPase, the extrusion rate in the soma was reduced to almost negligible values. However, blocking of Na^+/K^+-ATPase had only minor effects on the recovery from sodium increases extrusion in primary dendrites, and the extrusion rate obtained was 1.93 ± 0.63 mM/s. In summary, these data thus indicate that the Na^+/K^+-ATPase is the main mechanism responsible for the removal of sodium in the soma. In dendrites, however, the recovery from local sodium transients is largely mediated by lateral diffusion and not by activation of Na^+/K^+-ATPase. These results suggest that sodium increases in cellular microdomains as generated during excitatory synaptic activity will not per se cause a local increase in ATP consumption nor energy metabolism.

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Dynamic Fidelity Control to the Central Auditory System: Synergistic glycine/GABAergic Inhibition in the Cochlear Nucleus

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GABA and glycine are the major inhibitory transmitters that attune neuronal activity in the CNS of mammals. The respective transmitters are mostly spatially separated, i.e. synaptic inhibition in the forebrain areas is mediated by GABA, while glycine is predominantly utilized in the brainstem. Accordingly, inhibition in auditory brainstem circuits is largely mediated by glycine, but there are few auditory synapses employing both transmitters in maturity. Little is known about physiological advantages of such a two-transmitter inhibitory mechanism. We explored the benefit of engaging both glycine and GABA with inhibition at the endbulb of Held-spherical bushy cell synapse in the auditory brainstem of juvenile Mongolian gerbils. This model synapse enables selective in vivo activation of excitatory and inhibitory neuronal inputs through systemic sound stimulation and precise analysis of the input (endbulb of Held) - output (spherical bushy cell) function. The combination of in vivo and slice electrophysiology revealed that the dynamic AP inhibition in spherical bushy cells closely matches the inhibitory conductance profile determined by the glycine-R and GABAA-R. The slow and potent glycinergic component dominates the inhibitory conductance, thereby primarily accounting for its high-pass filter properties. GABAergic transmission enhances the inhibitory strength and shapes its duration in an activity-dependent manner, thus increasing the inhibitory potency to suppress the excitation through the endbulb of Held. Slow transmitter clearance from the synaptic cleft caused prolonged receptor binding and, in the case of glycine, spillover to nearby synaptic or extrasynaptic receptors. The GABAergic component appears not to derive from extrasynaptic receptors, but it rather prolonged the decay by contributing to the asynchronous vesicle release. Finally, in silico modelling provides a strong link between in vivo and slice data by simulating the interactions between the endbulb- and the synergistic glycine-GABA-conductances during in vivo-like spontaneous and sound evoked activities.

Establishment of appropriate methods for the identification of putative interaction partners of glycine transporter 2

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An important inhibitory neurotransmitter in the central nervous system of mammals is glycine. Glycinergic neurotransmission is terminated by glycine transporters (GlyTs). One isoform, GlyT2, is localized in presynaptic membranes of neurons. It takes up glycine from the synaptic cleft into presynaptic terminals, thereby maintaining glycine concentrations. GlyT2 have received growing attention as potential target for treatment of pain. Since protein-protein interactions can determine regulation of GlyT2, it is mandatory to investigate its interactome. Until today, seven interaction partners of GlyT2 have been identified: calnexin, syntaxin-1, Na^+/K^+-ATPase subunit a1, a2, and a3, syntenin-1 and unc-33-like protein 6. Yet a comprehensive analysis has not been performed thus far. The aim of this study is the establishment of a sophisticated proteomic approach for the identification of the interactome of GlyT2. To do so, co-immunoprecipitations (co-IPs) of GlyT2 and GST-Pull-Downs of GlyT2 C- and N-termini were optimized. As a prerequisite, a suitable GlyT2 antibody for co-IPs was launched. GlyT2 knockout animals as well as unspecific IgGs were defined as negative controls for co-IPs. In addition, two detergent buffer systems were used, with either mild (0.9% Octyl-ß-D-glucopyranoside - 50% solubilization) or more stringent (1% Triton-X-100 - 100% solubilization) solubilization conditions, to investigate weak and strong interactions. Furthermore, the expression of GST fusion proteins was established and the Pull-Down with GST itself was determined as negative control for Pull-Downs with GST-GlyT2 C- or N-terminus. Interactors, which bound to GlyT2, its C- or N-terminus during co-IPs or GST-Pull-Downs, were precipitated, separated on acrylamide gel electrophoresis and identified by mass spectrometry or western blot. First, GlyT2 co-IPs under stringent solubilization conditions revealed immunoprecipitation of GlyT2 and the Na^+/K^+-ATPase subunit a3 via western blots. In a mass spectrometric analysis of GlyT2 co-IPs and GST-Pull-Downs of the GlyT2 C- or N-terminus, 460 different proteins were identified with twice the amount of exclusive unique peptide counts compared to controls. 264 proteins were identified in the co-IPs of GlyT2 and 225 in GST-Pull-Downs of GlyT2 C- and N-termini. Among these, five already known interaction partners of GlyT2, calnexin, syntaxin-1, Na^+/K^+-ATPase subunit a1, a2 and a3, were identified. Because unc-33-like protein 6 interacts with GlyT2 only when phosphatase inhibitors are present during the co-IP its absence was expected. In addition, protein kinase C, clathrin and dynamin were detected. All three have been described to be involved in GlyT2 trafficking. Together, these results provide proof of concept for a complementary proteomic approach with co-IPs and GST-Pull-Downs for the identification of novel interaction partners of GlyT2.

Homeostatic changes of neuronal excitability in the somatosensory cortex of mice following traumatic brain injury

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Aims: Traumatic brain injury (TBI) affects neuronal activity and synaptic transmission in the surviving tissue surrounding the lesion. The aim of the present study was to characterize in detail the temporal and spatial profile of these alterations in the first week following TBI.

Methods: TBI was induced in the sensorimotor cortex of young-adult, anaesthetized mice (P18-P23) using a controlled cortical impact (CCI) lesion model. To analyze functional alterations, we used acute brain slices from TBI-treated and sham-operated animals. Spontaneous neuronal firing and extracellular evoked local field potential (LFP) were recorded in both the hemisphere ipsilateral and contralateral to the lesion with a 32-channel Multi-Electrode Array (MEA). Whole-cell patch-clamp recordings were performed in the same areas of both cortices.

Results: Extracellular electrophysiological recordings revealed an increased spontaneous neuronal activity during the first week post-TBI, in both cortical hemispheres. Interestingly, the time course of this hyperexcitability varied between the two hemispheres. In the cortex ipsilateral to the lesion, hyperexcitability developed progressively during the first week post-TBI. Conversely, the contralateral hemisphere showed a rapid peak of hyperactivity at 1 day post-lesion, which disappeared in the following days. Patch-clamp recordings further revealed that this early hyperexcitability in the undamaged cortex cannot be explained by changes in the intrinsic membrane properties of the active neurons, but rather by changes in synaptic transmission. Accompanying this acute hyperexcitable state, we found a contralesional cortex-specific alteration in the horizontal spreading of the evoked LFP suggesting processes of functional reorganization in the contralesional cortex as early as 24 hours post-injury.

Conclusions: TBI in sensorimotor cortex of mice leads to an increase in the neuronal spontaneous activity, which occurred as early as 24 hours post-injury in the contralateral hemisphere. This hyperexcitable state was accompanied by alterations of synaptic transmission in the same hemisphere. The observed enhanced activity in the cortical areas contralateral to the lesion could represent a global homeostatic mechanism to compensate for the loss of the homotopic brain structures.

Homeostatic regulation of function in specific subtypes of GABAergic interneurons following focal cortical lesions

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Proper brain function requires neuronal networks to operate within a well-defined range of activity. In line with this, a number of pathological conditions such as epilepsy or brain injuries induced by cortical trauma are characterized by an increase in neuronal activity. Interestingly, it has already been shown that changes in inhibitory transmission play a key role in the development of this hyperexcitable state. However it is still unknown, whether specific subpopulations of inhibitory interneurons contribute differentially to such pathological condition. Therefore, in the present study we investigated functional alterations of two subtypes of GABAergic interneurons following cortical lesion induced-hyperexcitability. Focal cortical unilateral lesions with a diameter of 1 mm were induced in vivo in the visual cortex of anaesthetized wild type or GFP-GAD67 mice at the age of 21 days. After a survival time of 3-6 days post-lesion we prepared acute slices from the hemisphere ipsilateral to the lesion. Initially we functionally mapped the vicinity of the lesion by recording spontaneous neuronal firing with a multielectrode array (MEA). Neuronal hyperexcitability was observed at 1 mm distance from the border of the injury when compared to sham-operated animals. Next, we performed whole-cell patch clamp recordings from visually identified GFP-labeled GABAergic interneurons in GFP-GAD67 mice. Interestingly, we found opposite alterations in the excitability of non fast-spiking (Non Fs) and fast-spiking (Fs) interneurons in acute cortical slices from injured animals. Non Fs interneurons displayed a depolarized membrane potential and a higher frequency of spontaneous excitatory postsynaptic currents (sEPSCs). In contrast, Fs interneurons showed a reduced sEPSCs amplitude. We propose that the observed downscaling of excitatory synapses targeting Fs interneurons may prevent the recruitment of this specific population of interneurons to the hyperexcitable network. On the one hand this mechanism may seriously affect neuronal network function and exacerbate hyperexcitability but on the other hand it may be important to protect this particular vulnerable population of GABAergic neurons from excitotoxicity.

Imaging exocytosis and Ca^{2+} influx at individual inner hair cell ribbon synapses

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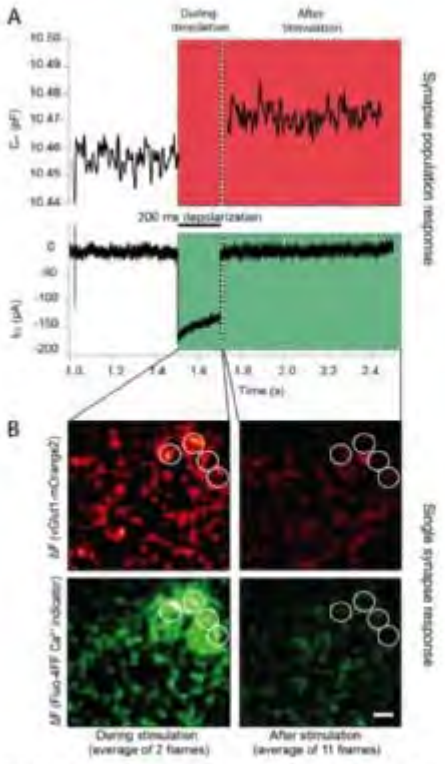
Indefatigable sound encoding at highly specialized inner hair cell (IHC) ribbon synapses is mediated by Ca^{2+} influx-evoked glutamate release. Based on the functional diversity of spiral ganglion neuron (SGN) spiking and the heterogeneity of Ca^{2+} influx at individual IHC active zones (AZs), we hypothesize that presynaptic heterogeneity is a key-mechanism enabling encoding of sound intensities over the whole audible range. However, beyond Ca^{2+} signaling so far functional differences among the synapses have not yet been studied and a causal link of the heterogeneity of Ca^{2+} influx to SGN response diversity remains to be demonstrated.

We aim to elucidate how presynaptic Ca^{2+} signals relate to exocytosis in order to test whether IHCs segregate auditory information via functionally heterogeneous presynaptic AZs and thus decompose auditory information into functionally diverse neuronal pathways to the brain. Simultaneous imaging of Ca^{2+} signals through small molecule dye Ca^{2+} indicators and exocytosis through the genetically encoded, pH sensitive fluorophore mOrange2 provides an optical readout of Ca^{2+} triggered exocytosis enabling to test whether the strength and spatial and temporal properties of synaptic Ca^{2+} flux and exocytosis relate linearly.

In this work, I specifically targeted the exocytosis reporter vGlut1-mOrange2 to IHC synaptic vesicles by employing viral mediated gene transfer with adeno-associated virus packed into capsid proteins of serotype 1 and 2 (AAV1/2). Immunohistochemistry revealed successful expression of vGlut1-mOrange2 in IHCs, with similar subcellular localization as the endogenous vesicular protein vGlut3. Furthermore, I collected preliminary data on imaging of exocytosis at fluorescently labeled synaptic ribbons, which indicate that vGlut1 -mOrange2 safely reports synaptic vesicle exocytosis, because its fluorescent hotspots co -localize with the ribbon peptide signal and the fluorescence signal intensity increase of vGlut1-mOrange2 fluorescence occurs specifically during IHC stimulation. To measure the relative contributions of individual AZs to the whole-cell response I finally combined mOrange2 imaging with that of synaptic Ca^{2+} influx.



(A) Whole cell membrane capacitance changes (C_m) and Ca^{2+} current (I_{Ca}) of a representative AAV1/2 vGlut1-mOrange2 transduced IHC during 200 ms depolarization to -17 mV.
(B) Corresponding confocal sections (XY scans) show changes of mOrange2 and Fluo-4FF fluorescence intensities (ΔF) during and after stimulation (indicated by boxes in A). Fluo-4FF was applied via the patch pipette (400 µM). Hotspots of mOrange2 fluorescence (white circles) reporting exocytosis and Fluo-4FF fluorescence (white circles) reporting Ca^{2+} influx co-localize and represent activity of single active zones.

Local postsynaptic sodium channel activation in dendritic spines of olfactory bulb granule cells

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Recent findings imply that spines can operate both as chemical and electrical compartments in which voltge-gated conductances can be activated locally. Here we provide evidence for postsynaptic voltage dependent sodium channel (Na_v) activation within dendritic spines via their impact on local calcium entry and postsynaptic depolarization.

The olfactory bulb (OB) granule cell (GC)/mitral cell reciprocal synapse is a special dendrodendritic connection. On the GC side, it accommodates a glutamatergic postsynapse and a GABAergic presynapse within the same spine. By means of two-photon glutamate uncaging (TPU) we could directly stimulate individual GC spines in acute OB brain slices from juvenile Wistar rats (P11 – 18) while reading out local calcium signals as $(\Delta F/F)_{TPU}$ via two-photon imaging and recording uncaging-evoked EPSPs (uEPSPs) at the soma.

Physiological TPU stimulation levels were assessed by comparing $(\Delta F/F)_{TPU}$ and uEPSPs with previously recorded synaptic data. TPU-evoked Ca^{2+} transients were strictly localized to single spine heads and also otherwise showed similar properties, as well as uEPSPs.

Blocking Na_vs with 500 nM TTX resulted in a strong reduction of $(\Delta F/F)_{TPU}$ in most spines (to 0.62 ± 0.24 of control, mean ± S.D., n = 35). uEPSP amplitudes were only slightly decreased (0.89 ± 0.30 of control, $P < 0.05$) whereas the rise time became substantially slower (1.24 ± 0.37 of control, $P < 0.01$). Notably, the magnitude of the blocking effect of TTX on $(\Delta F/F)_{TPU}$ was highly correlated with the magnitude of the TTX-induced increase in rise time across experiments ($r = -0.68$, $P < 0.001$). We hypothesized that the extra depolarization provided by Na_vs boosts Ca^{2+} entry mainly via high voltage activated calcium channels (HVACCs). Therefore we blocked N/P/Q type Ca^{2+} channels with ω-conotoxin MVIIC (CTX), which decreased $(\Delta F/F)_{TPU}$ to 0.7 ± 0.21 of control (n= 24). CTX applied after TTX reduced $(\Delta F/F)_{TPU}$ only marginally (to 0.88 ± 0.22 of TTX-only condition, n = 14). The reverse experiment (TTX after CTX) yielded 0.97 ± 0.22 vs. CTX-only (n = 8). Thus HVACC activation is the main source of Na_v-induced Ca^{2+} entry.

The blockade of other known postsynaptic calcium sources (NMDA receptors, internal stores and low voltage activated T-type calcium channels) did not occlude the effects of TTX. In turn, blockade of NMDARs post TTX application still had a considerable effect on $(\Delta F/F)_{TPU}$ (to 0.20 ± 0.08 of the value in TTX, n = 9), indicating that NMDAR-mediated Ca^{2+} entry is not boosted by Na_vs. Pharmacological blockade of various types of K^+ channels did not significantly change $(\Delta F/F)_{TPU}$ or uEPSPs and also did not occlude effects of TTX. Therefore the differential effects of TTX on EPSP rise time and EPSP size

are probably not due to an interference of TTX with K^+ channel activation. Rather they result from the passive filtering exerted by the spine neck, which could also be replicated in a NEURON model.

Our results strongly suggest that Na_Vs contribute to local postsynaptic Ca^{2+} entry through HVACC activation, adding yet another crucial postsynaptic conductance to the GC spine's tool kit for its operation as an independent microcircuit. The observed acceleration of EPSPs by Navs may contribute to precise timing of GC output, e.g. in the context of fast sensory-evoked network oscillations.

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Localization and quantification of the presynaptic protein Bruchpilot in the honeybee central brain and in subcompartments of mushroom body lip boutons

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Synaptic plasticity is essential for information processing, learning, and memory. Modifying the number of synapses or the amount of transmitter released at presynaptic sites are ways to alter the communication between neurons. One protein which is thought to contribute to synaptic plasticity is Bruchpilot (BRP). In Drosophila, BRP was shown to be localized at active zones of presynapses. Reducing the BRP level resulted in decreased neurotransmitter release, mislocalized Ca^{2+} channels and impaired anesthesia-resistant memory. This led to the hypothesis that BRP is involved in concentrating Ca^{2+} channels near synaptic vesicles, thereby regulating the efficacy of synaptic transmission. In fact, the local amounts of the BRP scaffold protein seem to directly scale with the release probability of a given synapse.
Honeybee workers display age-related division of labor. Typically, worker bees perform in-hive duties for the first 2-3 weeks of adult life before they start to forage. This age-dependent behavioral plasticity is associated with structural changes in neuropils involved in the processing of sensory information such as the mushroom bodies and the antennal lobes. A good example are the synaptic complexes (microglomeruli) in the mushroom body calyces where presynaptic boutons and active zones are increased in size respectively in number in forager bees compared to nurse bees.
In this study, we used a newly developed antibody against fruit fly BRP (Anti-BRPlast200) to investigate for the first time the localization of *Apis mellifera* BRP (AmBRP) in the honeybee brain. In addition, western blot analysis and immunohistochemistry were used to quantify the relative amount of AmBRP in the central brain and in mushroom body microglomeruli, examining an age-dependent alteration of AmBRP levels.
From our results, we conclude that AmBRP is localized at presynaptic sites in the honeybee brain. The relative amount of AmBRP varied in an age-dependent manner in the central brain as well as in boutons of the mushroom body lip. This indicates either a change in the number of presynapses or in the synaptic efficacy at single presynapses. Both would point towards age-dependent alterations in the transmission of information in the bee brain, especially in the mushroom body lip, a region where olfactory information is processed.

Mechanisms of synaptic vesicle release studied at single active zones

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The synapse between inner hair cell (IHC) and type I spiral ganglion neuron (SGN) encodes auditory signals at high rate for long periods of time. Spiking of each unbranched SGN is solely driven by a single IHC active zone. To meet the functional demand, IHC active zone needs to be equipped with highly efficient release machinery. Deciphering the release mechanism is the key to understand how hearing happens. With whole-cell patch-clamp recordings directly at the minute postsynaptic terminal of SGNs, one can monitor vesicle release from a single presynaptic active zone by measuring the excitatory postsynaptic currents (EPSCs) flowing through AMPA receptors. At IHC synapses, spontaneous EPSCs show large size and shape variability, which led to the hypothesis of synchronized multivesicular release(Glowatzki and Fuchs). EPSCs can be classified into two categories, mono- and multiphasic EPSCs, according to their waveform. The multivesicular hypothesis explains mono- and multiphasic EPSCs by synchronous and asynchronous release of multiple vesicles, respectively (Glowatzki and Fuchs). We have recently provided
evidence for uniquantal release through a dynamic fusion pore, which can also explain mono- and multiphasic EPSCs well (Chapochnikov et al.). However, the debate is not yet fully resolved. We will present further work aimed at settling the question and at determining the basic unit of synaptic transmission at this special synapse to provide a platform for further quantitative analysis.

miRNA-96 alters information transfer at a central auditory synapse

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MicroRNAs (miRNAs) are small non-coding RNAs involved in the regulation of gene expression. The miRNA-96 mouse mutant Diminuendo *(Dmdo/Dmdo)* shows deafness associated with arrested cochlear hair cell development. miRNA-96 is also expressed in the brainstem but the consequences of its mutation for the central auditory system are unclear. To elucidate the impact on central processing, we investigated changes in information transfer in the medial nucleus of the trapezoid body (MNTB) in the auditory brainstem.

We find by electrophysiological and morphological comparison of homozygous *Dmdo/Dmdo* mutants and their wild type littermates at postnatal day P24 -25 that the size of postsynaptic MNTB somata was reduced in mutant mice. The input resistance was enhanced, whereas no change in resting membrane potential was apparent. Thus, the mutation induces changes in the passive membrane properties in central neurons. The action potential generation and firing pattern were analyzed to evaluate the postsynaptic influence on information processing. To compare the action potential parameters in mutant and control mice, short EPSC-approximating current pulses were injected to elicit supra-threshold responses. Both the current and the voltage threshold were significantly reduced in mutant mice. The firing pattern was quantified from the response to injections of long current steps. MNTB neurons in control mice responded predominantly with onset action potentials while the majority of cells from mutant mice displayed continuous firing and had lower current thresholds. Morphometric analysis of confocal serial sections of SV2 immunolabeled presynaptic calyces indicated a deterioration of the donut-like substructures in mutant compared to wild type animals. To probe for accompanying changes in synaptic transmission in *Dmdo/Dmdo* mutants, miniature EPSCs were quantified. Consistent with a morphological phenotype, mEPSCs had a larger amplitude and a lower frequency in mutant mice, yet maintained similar kinetics. Furthermore, initial fiber stimulation experiments provide evidence for an enhanced short term depression in *Dmdo/Dmdo* mutants.

Taken together, the observed differences in passive and active properties of MNTB neurons could compensate for the lack of normal synaptic activity in mutant mice, indicating a homeostatic balancing to maintain the overall excitability in this neuronal circuit. Alternatively, the mutant mice reflect a developmental premature state, consistent with the view of arrested cochlear development in *Dmdo/Dmdo* mutant mice.

Modulation of neurotransmitter release by endogenous amyloid beta in physiological concentration involves molecular remodeling of presynaptic release apparatus

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Excessive accumulation and aggregation of amyloid beta (Aß) is linked to neuronal and synaptic pathology of Alzheimer's disease (AD). Apart of being involved in this pathological process, there are growing evidences suggesting a physiological role of secreted Aß in synaptic functional plasticity, which is critical for normal brain function (Abramov et al., 2009; Puzzo et al., 2011). The aim of the presented study was to approach the mechanism, by which endogenous Aß regulates release from presynapses. We used rat cortical neurons as convenient cellular model accessible for modulation of extracellular concentrations of endogenously secreted Aß and allowing pharmacological manipulations and consecutive immunocytochemical and functional analysis. By interference with natural production and clearance of endogenously secreted Aß we confirmed its ability of bidirectional control of presynaptic release, which was also seen upon addition of picomolar and high nanomolar amounts of recombinant Aß. This change in presynaptic efficacy was connected with rapid changes in synaptic recruitment of presynaptic scaffolds, synaptic vesicle proteins and functional coupling of Cav2.2-containing VDCCs with release sites. Furthermore, our data clearly demonstrated that Aß -driven presynaptic potentiation requires functional presynaptic a7 nicotinic acetylcholine receptors and Cav2.2 type of VDCCs. Taken together, our study provides new mechanistic insights into Aß synaptic function in normal, physiological conditions, which could help to better understand the effects of its chronic elevation on synaptic failure likely causing the cognitive decline in AD and to design more effective and safe AD therapy.

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Mover: A New Player in Synaptic Strength

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Mover is a vertebrate-specific novel synaptic vesicle phospho-protein. Knock-down of Mover at the calyx of Held, increased the rate of vesicle reloading after synaptic depression, as well as the calcium sensitivity and probability of release. Mover was shown to bind to Bassoon, also a vertebrate-specific presynaptic protein. Disruption of Bassoon leads to an increase in release probability and a reduction of synaptic Mover levels, whereas other synaptic proteins keep their levels unchanged. The authors speculate that Mover could be an effector of Bassoon and that its downregulation contributed to the observed increase in release probability. Unpublished data from our research group suggest that Mover also binds to Calmodulin and preliminary data suggest that a specific point mutation in Mover prevents Calmodulin binding.

Mover is differentially expressed among synapses. It is strongly expressed in the hippocampal mossy fiber to pyramidal cell synapse in the CA3, but weakly present in cerebellar mossy fiber to granule cell synapse. Despite being morphologically similar, these synapses exhibit distinct functional characteristics. While hippocampal mossy fibers exhibit low release probability, cerebellar mossy fibers exhibit high release probability. Such observations support the notion that Mover may dictate synaptic strength.

Shedding light onto the role of this new participant in the synaptic machinery would help us comprehend three fundamental questions: a) how is release probability regulated? b) how is synaptic heterogeneity accomplished? c) are there special signatures of vertebrate synapses?

Mover has the potential to be a key effector of important synaptic proteins such as the vertebrate-specific Bassoon and the highly conserved Calmodulin. Investigation of the role of Mover will bring us one step closer to unraveling the mystery of what regulates synaptic strength in health and disease. Transmission dysregulation has been implicated in disorders such as schizophrenia, autism and epilepsy. Accordingly, Mover is strongly upregulated in anterior cingulate cortex brains of schizophrenic patients (Clark, Dedova, Cordwell, & Matsumoto, 2006), indicating the importance of a better understanding of this protein.

In this study, we have used a Mover knockout mouse line in combination with imaging and whole-cell voltage clamp to understand the role of this protein in synaptic transmission.

Nano-architecture of presynaptic P/Q-type calcium channels in parvalbumin-expressing hippocampal basket cells

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Parvalbumin (PV)- expressing basket cells are crucial in shaping the synchronous firing output of hippocampal pyramidal cells, which requires the timely -precise release of GABA from their axon terminals. This process is depending on calcium influx through high-voltage-activated calcium channels which are modulated by metabotropic receptors, e.g. the GABAB receptor which is a heterodimer composed of GABAB1 and GABAB2. Therefore, we examined the ultrastructural distribution of P/Q-type (Cav2.1) calcium channels and their spatial relationship to GABAB receptors in PV-expressing basket cells in the CA1 area of the hippocampus using SDS-digested freeze-fracture replica-labeling and quantitative immunoelectron-microscopy. The boutons of PV cells were identified using the muscarinic acetylcholine receptor subtype 2 (M2) which is known to be strongly expressed by this type of neuron. Accordingly, M2 positive axon terminals were strongly labeled for Cav2.1 with an average of 18 particles per bouton. Immunogold particles were concentrated within the putative active zone showing a clustered distribution. Quantitative analysis further revealed that the clustering of Cav2.1 was highly significant compared to generated random control particle distributions. Interestingly, we found a strong colocalization of GABAB receptors with Cav2.1 proteins. About 85% of the immunogold particles labeling the GABAB1 subunit were located within a distance of 100 nm from the calcium channel. In conclusion, our results provide evidence that in PV-expressing axon terminals Cav2.1 channels form clusters and confined to the putative release site consistent with the proposed tight coupling of Cav2.1 channels and calcium sensors of exocytosis in this cell type. Moreover, the strong co-localization with the GABAB1 protein suggests that the Cav2.1 channel is modulated by GABAB receptors.

New genetic tools for the analysis of GABA and glycine co-transmitting neurons

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Co-transmission of two or more classical neurotransmitters is a phenomenon in the CNS that offers a wide spectrum of signalling variations and therefore contributes to the high complexity of the neuronal network. Contradicting Dale's principle, the co-release of more than one transmitter from a single neuron has recently been found in many different brain regions indicating the importance of co-transmission in the CNS. Specifically, co-release of the two fast inhibitory neurotransmitters GABA and glycine is often observed in the mouse brainstem and spinal cord. The neurotransmitter phenotype is highly plastic during development, in the context of disease and during changes of environmental conditions. However, a developmental and functional in-vivo analysis of neurons co-transmitting GABA and glycine is challenging and time-consuming because of the difficulty to unequivocally detect and identify co-transmitting neurons. To overcome these limitations we developed new genetic tools for visualisation of GABA and glycine co-transmitting neurons in-vivo. Additionally, the time-controlled irreversible genetic labelling of these neurons by using the split-CreERT2 system allows investigating the developmental fate of neurons that co-release GABA and glycine as well as identifying their projecting areas.

Novel functions of c-Jun N-terminal kinases in neurons

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The c-Jun N-terminal kinases (JNKs) are stress-activated serine-threonine kinases that have recently been linked to various neurological disorders. In patients with intellectual disability (ID), we detected *de novo* truncations in the CNS-expressed *MAPK10/JNK3* gene, highlighting an important role for JNK3 in human brain development. These truncated proteins cannot phosphorylate the classical JNK target c-Jun and showed a lower binding affinity for known JNK scaffold proteins, suggesting that the patient phenotype potentially arises from partial loss of JNK3 function.

To further elucidate the function of JNK3 in the brain, we searched for neuronal interaction partners and novel phosphorylation targets. We identified several novel JNK3 interaction partners, including the synaptic membrane-associated guanylate kinase (MAGUK) PDZ-domain proteins SAP102 (involved in ID) and PSD-95, the Shank proteins (involved in autism), as well as other neuronal scaffolding proteins. JNK3, like JNK1 phosphorylates PSD-95 *in vitro*, whereas disease-associated mutant JNK3 proteins do not. We conclude that reduced JNK3 activity has potentially deleterious effects on neuronal function via altered regulation of a set of post-synaptic proteins.

We are currently investigating the molecular properties of these interactions, focussing in particular on the MAGUK family of JNK binding partners. Using phospho-specific antibodies, we are investigating the influence of JNK-mediated phosphorylation on the subcellular localisation of selected endogenous post-synaptic scaffold proteins in hippocampal rat neurons. We will also use viral-mediated gene transfer of tagged phospho-mimicking / phospho-deficient expression constructs and subsequent FRAP experiments to explore how the mobility of these novel JNK targets is regulated. Given the location of JNK docking and phosphorylation of these post-synaptic scaffold proteins, specific protein-protein interactions and subsequent signalling may also be affected by JNK.

We will further examine how JNK phosphorylation of MAGUK family proteins influences their binding to selected neuronal proteins. Our data on novel synaptic JNK targets, together with the fact that JNK3 has been implicated in neurodevelopmental disorders, provide the impetus for further studies on novel functions of JNK3 in neurons.

Novel group of neuron bound extracellular metallopeptidases (NEMPs) located in the synaptic area

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Proteolytic enzymes in addition to catabolism perform a control of vital processes. This statement applies in full measure to the brain extracellular proteolytic enzymes: matrix proteases, ADAM, neurolysin etc. It should be noted that all known brain extracellular proteolytic enzymes are narrowly specific.

We have discovered that isolated from mammalian brains axonal endings of neurons (synaptosomes) exhibit not very specific peptidase activity against peptides of different composition. Mild treatment of highly purified synaptosomes with nonionic detergent Triton X-100 (0.1%, 0oC, 30 min) releases a group of attached to the outer side of synaptosomal plasma membrane metallopeptidases (1,10-phenantrolin inhibits their activitie). They were named NEMPs (Neuronal Ecto MetalloPeptidases). The peptidases were partially separated by means of gel electrophoresis under non -denaturizing conditions and characterized according to the type of activity. Altogether four NEMPs were found:

1. Carboxypeptidase (NEMP1)
2. Aminopeptidase (NEMP2)
3. Endopeptidase A (NEMP3)
4. Endopeptidase B (NEMP4)

The exopeptidases (NEMP1 and NEMP2) can split dipeptides, but not dipeptide ßala-his (carnosine). Therefore carnosine present at the C-end, at the N-end or at the both ends of a peptide protect it from NEMP1, from NEMP2 or from the both, correspondingly. Instead of carnosine, present in its composition ßalanine can be used for the peptide ends protection. Some specific properties of certain NEMPs were detected. NEMP1 is composed of rather short polypeptides, which tend to form multimers, which are in dynamic equilibrium,. A specific feature of NEMP3 is its activation by carnosine, ßalanine and some other substances. Being activated, NEMP3 splits peptides predominantly near proline, alanine, phenylalanine, lysine and arginine residues. The data obtained in this ongoing research will be useful for engineering of therapeutic peptides reliably protected from degradation in brain intercellular medium, in particular, in the area of synapses, where NEMPs are located.

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Physiological role of Nlgn2 in anxiety processing circuitry.

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Nlgn2 is a synaptic cell adhesion molecule that is thought to be involved in the development and function of inhibitory synapses. Deletion of Nlgn2 leads to alterations in structure and function of inhibitory synapses *in vivo*. Behavioral studies show that alterations in Nlgn2 levels affect a very specific set of behaviors in mice, in particular those related to anxiety processing. Moreover, there is growing evidence that lack of Nlgn2 does not affect all types of inhibitory synapses equally, but is more critical for some synapses that are yet to be characterized. Using Nlgn2 KO mice, we aim to characterize the circuitry in basal amygdala that is involved in anxiety processing in these mice, and study the role of Nlgn2 at this circuitry at molecular, anatomical and behavioral levels. Combining behavior testing, multi labeling immunohistochemistry and confocal microscopy we show that Nlgn2 deletion affects composition of inhibitory post synaptic sites in basal amygdala without changing the total number of inhibitory synapses. Furthermore, exposure to stress results in enhanced activation of pyramidal cells but not inhibitory interneurons in this structure. Based on these findings we propose a model in which Nlgn2 deletion leads to impairment of specific inhibitory circuits in basal amygdala causing an increased behavioral response to anxiogenic stimulus. Our results may contribute to understanding of anxiety phenotype in Nlgn2 KO mice and expand current knowledge on physiological role of Nlgn2 in inhibitory transmission in the brain.

Potential basis for local feedback from horizontal cells to cones in the mouse retina

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Cone photoreceptors (cones) relay light-evoked signals to different types of bipolar cells which then feed into the ganglion cells, the output neurons of the retina. In the mouse retina, a single type of GABAergic interneuron, the horizontal cell (HC), modulates the glutamatergic output from cone axon terminals by parallel feedback mechanisms (Kemmler et al., J Neurosci 2014). However, it is still unclear how this feedback is generated in HC dendrites; in particular, how local and global dendritic HC signals shape the feedback at the level of the individual HC-to-cone synapse.

To understand how cone input is processed in HC dendrites, we measured light-evoked calcium signals in HC dendrites using two-photon calcium imaging. The mouse retina contains two types of cones: “true” S-cones (Haverkamp et al., J Neurosci 2005), which exclusively express the short wavelength-sensitive (S)-opsin, and M-cones, most of which co-express the medium wavelength-sensitive (M)-opsin and the S-opsin. This co-expression is set up as dorso-ventral gradient: the dorsal retina is dominated by mostly M-opsin expressing cones, while the ventral retina is dominated by almost exclusively S-opsin expressing cones (Baden, Schubert et al., Neuron 2013). Mouse HCs sample from all cones within reach. Therefore it is unclear, if chromatic information from individual cones remains “isolated” within local dendritic regions of a HC, or if it spreads across an HC’s entire dendritic tree or even in the electrically coupled HC network. To address this question, we recorded calcium signals in HC processes at the subcellular level using a transgenic mouse line that selectively expresses a fluorescence calcium sensor in HCs (Wei et al., J Neurosci 2012).

We focussed on calcium signals measured in HC dendritic compartments directly opposite of cone axon terminals. Consistent with the dorso-ventral opsin gradient, we found that light responses recorded in such HC compartments were dominated by the M-opsin in the dorsal retina, whereas in the ventral retina, responses were dominated by the S-opsin. However, in many cases, responses measured in neighbouring HC compartments varied in their chromatic preference, suggesting that the calcium signal in the postsynaptic HC compartment preserves the chromatic signature of the presynaptic cone(s). Such a local processing of chromatic signals in postsynaptic structures in the HC was unexpected regarding the strong electrical coupling of the HC network, and hints at a cellular basis for local, cone-specific HC feedback.

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Pre- and postsynaptic functions of the voltage-dependent Ca^{2+}-channel DmCa1D (Ca_V1) in *Drosophila*

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Voltage-gated calcium-channels (VGCCs) are indispensable for neuronal information processing. The different functions mediated by VGCCs are dependent on their conductance, activation- and inactivation kinetics and their location in different compartments. These include exocytosis of synaptic vesicles, generating of plateau potentials, influence on action potential-shape and firing frequency of neurons as well as postsynaptic potential amplification in dendrites. Consequently dysfunctions of VGCCs cause several neurologic diseases. In vertebrates 10 genes encode VGCCs and fall into three families, namely Ca_V1, Ca_V2 and Ca_V3. In *Drosophila* only 3 genes exist, DmCa1D (Ca_V1), DmCa1A (Ca_V2) and DmCa1G (Ca_V3), each of which is homologous to one vertebrate family. This study combines genetic with anatomical and physiological tools to address DmCa1D function in *Drosophila* motoneurons. DmCa1D is the homolog to vertebrate L-type calcium channels.

Analysis with an antibody specific for DmCa1D showed localization of this channel in motoneuron somata, clustered localization in dendrites, continuous axonal localization, and presynaptic localization to periactive zones. We currently address DmCa1D function in these different compartments by combining physiological and genetic tools. Our data indicate that axonal localization decreases firing frequencies in response to somatic current injection and during ongoing locomotion via activation of calcium activated potassium channels. By contrast, dendritic localization seems to amplify synaptic drive to motoneuron dendrites during locomotion. Therefore, DmCa1D channels seem to exert distinctly different functions in the axonal and dendritic compartments of *Drosophila* motoneurons. The respective consequences for larval crawling motor behavior are currently under investigation. Moreover, preliminary data suggest a role of DmCa1D channels localized to the periactive zone in activity dependent synaptic vesicle recycling. However, these data currently rely on RNAi experiments with insufficient knockdown magnitude. Therefore, we have now created null mutant mosaic animals (MARCM) to fully address the multiple functions of DmCa1D channels in different neuronal compartments. We expect these data to reveal fundamental insight into the roles of L-type like calcium channels in synaptic vesicle endocytosis and motor behavior.

Regulation of PSD-95 complex assembly

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The postsynaptic scaffold protein PSD-95 is an essential regulator of synaptic protein networks and synaptic transmission. We are interested in the molecular mechanisms by which PSD-95-family MAGUK proteins assemble and disassemble multiprotein complexes and thereby regulate synaptic plasticity. For this purpose we have developed a cell-based assay that takes advantage of established ligand / PDZ domain interactions for investigating scaffold protein complex formation. This assay allows for quantitative analysis of PDZ domain mediated protein clustering using bimolecular fluorescence complementation (BiFC): Two non-fluorescent fragments of a fluorescent protein (EYFP) are fused to C-terminal PDZ ligand sequences to generate split-EYFP probes that sense for PDZ domain binding grooves of adjacent (interacting) scaffold proteins. When these probes are brought into proximity by sequence-specific interaction with the PDZ domains of a multiprotein scaffold, a functional fluorescent EYFP molecule refolds and can easily be detected. We have used this system to examine the properties of PSD-95 variants and thereby delineated regions of importance for PSD-95 multimerization. Further analysis suggested that PSD-95 multimerization can be triggered by the binding of monomeric PDZ ligands to PSD-95 PDZ domains, suggesting that PDZ-ligand interactions may influence multiple aspects of PSD-95 mediated multiprotein complex formation. Thus our study provides a basis for future investigations into the nature of ligand-mediated PSD-95 multimerization in post-synaptic receptor clustering.

RIM proteins at the photoreceptor ribbon synapse

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RIMs (Rab3-interacting molecules) are essential components of the active zones (AZ) of chemical synapses, known to interact with many synaptic vesicle and AZ proteins. Due to their various interactions they are suggested to play important roles in the vesicle priming process and in the synchronization of release during exocytosis by tethering and modulation of Ca^{2+} channels. The RIM protein family is composed of seven structurally divergent members encoded by four genes – two a-RIMs (RIM1a and 2a), two ß-RIMs (RIM1ß and 2ß) and three γ-RIMs (RIM2γ, 3γ and 4γ) – which mediate to some extent redundant functions. In our studies we aim to identify the localization and possible function of the RIM members present at the highly specialized photoreceptor ribbon synapse.
Comparing the expression profiles of the longest isoforms RIM1a/ß and RIM2a/ß in cortex and retina with RT-PCR, we detected all RIM1/2 variants in cortex and only RIM1a and RIM2a/ß in retina. Each a-isoform showed a tissue-specific expression pattern of its splice-variants. In isolated photoreceptor cells RIM2a was the only long RIM-isoform expressed. Immunocytochemical analyses localized RIM2a at the arciform density of the photoreceptor ribbon complex. Consistent with the PCR results, no immunoreactivity was found for RIM1 in photoreceptors. To gain insight into the role of RIM2a at the photoreceptor ribbon complex, we examined wild -type and RIM2a -Knockout (KO) mice with immunocytochemical stainings and electroretinographic recordings (ERG). Measurements of the arciform density length in retinal whole mount preparations (ubMunc13-2 staining) showed a slight reduction in the RIM2a-KO mice. However, ERG recordings did not differ significantly between the two genotypes and also RIM2-stainings were virtually identical. This mild phenotype might be explained by a hitherto undescribed RIM2a splice-variant, which we identified by RT-PCR.
This novel RIM2a splice-variant is not affected by the RIM2a-KO. It lacks most of the binding sites for Rab3a and Munc13, pointing towards a minor role of RIM2a in vesicle priming at the photoreceptor ribbon synapse and a more important role in the organization of the active zone in photoreceptor cells. In the future, studying the Ca^{2+} channel kinetics in the RIM2a-KO photoreceptor cells might reveal whether there are a more subtle changes in synaptic function which are not detectable in the ERG recordings. Moreover, since the antibodies do not distinguish between the alpha- and beta isoforms, we also plan to analyse whether a compensatory up-regulation of the RIM2ß-isoform might account for the mild synaptic phenotype in the RIM2a-KO.

Role of Bassoon in the regulation of neurotransmitter release

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The regulation of neurotransmitter release from presynaptic boutons is crucial for the functioning of chemical synapses. The release takes place at the active zone (AZ), which is a region of the presynaptic plasma membrane characterized by the presence of an electron-dense structure called the cytomatrix at the active zone (CAZ). This cytoskeletal matrix are composed of multidomain scaffold proteins (RIM, RIM-BP, Munc-13, CAST/ELKS, Liprins a, Piccolo and Bassoon), which act as functional and structural organizers of the AZ and regulators of synaptic vesicle exocytosis2. The role of Bassoon (Bsn) in presynaptic function has been investigated in several studies1, 3, which suggest that this protein does not have an essential role in synapse formation but rather contributes to the plasticity of neurotransmitter release. In order to understand the mechanism of this Bassoon function, we characterized presynaptic composition and function in primary hippocampal neurons derived from mice lacking Bsn expression. We observed decrease in the synaptic abundance of most CAZ proteins in Bsn lacking synapses and in line with our previous studies1,3,4 defects in synaptic vesicle release. To disect this phenotype we used the synaptophysin-pHluorin-based reporter technique allowing investigation of release characteristics and analysis of synaptic vesicle pools. We found that ready-releasable pool (RRP) and recycling pool (RP) were reduced and proportion of resting vesicles that do not participate in release was increased in the absence of Bsn. Using phamacological intervention we identified involvement of CDK5 and PKA-dependent signaling in this process. Moreover, we tested involvement of UPS -dependent protein degradation, which was recently shown to be target of regulation by Bassoon and its homologue Piccolo5. Together our study provides new inside into mechanistical understaning of Bassoon function at presynapse.

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Synchronous epileptic activity between CA1 and CA3 hippocampus during postsynaptic blockade of glutamate and GABA receptors.

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Epileptoform discharges are produced by excitations of hundreds to thousands neurons at the same time. While gabaergic and glutamaergic synaptic transmission play a crucial role in synchronization of neuronal net during epileptic seizures, other non-synaptic factors such as extracellular ions fluctuations, field effects and ephaptic interactions between closely apposed neuronal membranes support simultaneous activity of numerous neurons. In this work we compared three models of non-synaptic epilepsy (1. low Ca2+; 2. blocking Ca2+ channels with Cd2+ and 3. using synaptic transmission blockers (CNQX, 10μM; MK-801, 1μM; bicuculline, 10μM)) recorded simultaneously in CA1 and CA3 zones of rat hippocampus. Low-Ca and Cd2+ models of epilepsy are models of mediator release termination, while blockers model is a model of blockade of postsynaptic receptors. To evoke epileptiform activity in synaptic blockers model we increased pH of ACSF to 7.45 and omitted Mg2+ from extracellular solution.
In response to inhibition of synaptic transmission with low-Ca or Cd2+ solutions we observed field bursts of sharp negative population spikes of high frequency (up to 4 Hz), slow waves with spikes superimposed, persistent and intermitted bursts in CA1 as well as in CA3 area of hippocampus. These epileptic discharges remained stable for several hours and were never synchronous between CA1 and CA3 zones. Electrical activity in the blockers model of epileptic discharges started with big amplitude fast waves (0.1Hz, 0.5s) synchronous between CA1 and CA3 zones. This activity lasted 40-60 min. After this transient period, single spikes were predominant manifestation of epileptic activity in this model. Addition of Cd2+ on 20 min to the perfusion solution resulted in elimination of synchronization between CA1 and CA3 zones.
Synaptic transmission blockade caused the higher level of epileptiform activity synchronization between CA1 and CA3 comparing to low Ca2+ and Cd2+ models. Thus we suggest that other than glutamatergic and gabaergic synaptic transmission systems are involved in the synchronization between CA1 and CA3 zones.

The Morphological and Molecular Nature of Synaptic Vesicle Priming at Presynaptic Active Zones

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Synaptic vesicle docking, priming, and fusion at active zones are orchestrated by a complex molecular machinery. We employed hippocampal organotypic slice cultures from mice lacking key presynaptic proteins, cryo-fixation, and three-dimensional electron tomography to study the mechanism of synaptic vesicle docking in the same experimental setting, with high precision, and in a near-native state. We dissected previously indistinguishable, sequential steps in synaptic vesicle active zone recruitment (tethering) and membrane-attachment (docking), and found that vesicle docking requires Munc13/CAPS family priming proteins and all three neuronal SNAREs, but not Synaptotagmin-1 or Complexins. Our data indicate that membrane-attached vesicles comprise the readily-releasable pool of fusion-competent vesicles, and that synaptic vesicle docking, priming, and trans-SNARE complex assembly are the respective morphological, functional, and molecular manifestations of the same process, which operates downstream of vesicle tethering by active zone components.

Tight coupling between Ca^{2+} sensors of exocytosis and presynaptic Ca^{2+} channels at inner hair cell ribbon synapses

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The spatial organization of presynaptic Ca^{2+} channels and readily releasable pool vesicles is a key determinant of vesicle fusion dynamics at the presynaptic active zone. In this way, the active zone topography markedly shapes neural information processing and transfer by synapses. Two limiting, idealized cases of the release face topography have been postulated in the field of synaptic physiology [1]. In the so-called Ca^{2+} nanodomain coupling regime, fusion of each vesicle at the active zone is induced by one directly associated Ca^{2+} channel. In the so-called Ca^{2+} microdomain coupling regime, the vesicle fusion is induced by Ca^{2+} contributed by many channels that are, on average, located more distant from the Ca^{2+} sensors of exocytosis.

Here, we present results of mathematical modeling of active zone topographies and presynaptic Ca^{2+} dynamics of auditory inner hair cell (IHC) synapses constrained by data from patch clamp recordings as well as STED and electron microscopy.

Our STED microscopy data indicated that Cav1.3 Ca^{2+} channels of IHC presynaptic active zones formed stripe shaped clusters (~100 nm on ~400 nm) in the plasma membrane just below the synaptic ribbon. The electron microscopy revealed that the presynaptic Ca^{2+} channel clusters were surrounded by ~15 readily releasable pool vesicles in rest. Modeling of exocytosis to reproduce the membrane capacitance increments in response to short depolarizing pulses at different fractions of Ca^{2+} channels blocked by a dihydropyridine blocker isradipine revealed that each release site was effectively driven by one or, at most, a few effective Ca^{2+} channels on average. Modeling of exocytosis to reproduce the membrane capacitance increments in response to short depolarizing voltage pulses in IHC dialyzed with different concentrations of the fast and slow Ca^{2+} chelators BAPTA and EGTA suggested a short distance, 10 – 30 nm, between the Ca^{2+} source and Ca^{2+} sensors of exocytosis.

Altogether, our results indicated that fusion of an average vesicle at presynaptic active zones of mature IHCs is driven by one to a few Ca^{2+} channels tightly coupled to the Ca^{2+} sensors of exocytosis. The short distance between the Ca^{2+} sensors and the associated Ca^{2+} channels has several consequences for temporal evolution of $[Ca^{2+}]$ at the sensors. First, for presynaptic active zone topographies compatible with our experimental and modeling results, $[Ca^{2+}]$ build up and decay at the Ca^{2+} sensors is predicted to be nearly instantaneous upon opening and closing of the Ca^{2+} channels contributing most of the Ca^{2+}. Second, the tight coupling is predicted to make vesicle release at the active zone more resistant to accumulating global Ca^{2+} due to relatively high local $[Ca^{2+}]$ at the Ca^{2+} sensors produced by the near-most Ca^{2+} channels.

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Type of inhibitory transmitters distinguishes responses to anoxia in fragile and non-fragile motor neurons in rats

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Motor neurons innervating skeletal muscle are markedly vulnerable to deficiency in energy supply, such as occurring during hypoxia, anoxia, metabolic stress and mitochondrial failure. This leads to pathological consequences with selective motor neuron death, as shown in amyotrophic lateral sclerosis (ALS). ALS is a fatal neurodegenerative disorder characterized by the selective loss of motor neurons in the brainstem and spinal cord. Clinical studies have indicated that there is a distinct region-dependent difference in the vulnerability of motor neurons. For example, the motor neurons in the facial and hypoglossal nuclei (VII and XII, respectively) are more fragile than those in the oculomotor nucleus (III). To understand the mechanism underlying the differential fragility of the neurons in different motor nuclei, we compared the effects of chemical anoxia on the membrane currents and postsynaptic currents in different motor nuclei. The membrane currents were recorded from neurons in III, VII and XII in brain slices of juvenile Wistar rats by using whole-cell recording in the presence of tetrodotoxin. In these three nuclei, NaCN induced a continuous inward current accompanied by a significant increase in the spontaneous action potential-independent synaptic inputs. Whereas the NaCN-induced increase in the synaptic input frequency in XII and VII was abolished by strychnine but not by picrotoxin, it was unaffected by strychnine but was abolished by picrotoxin in III. Blocking ionotropic glutamate receptors in any of the three motor nuclei did not affect the NaCN-induced release facilitation. These results suggest that inhibitory transmitters distinguishes responses to anoxia in fragile and non-fragile motor neurons, furthermore, anoxia selectively facilitates glycine release in the ALS-vulnerable motor neurons. The region-dependent differences in the neurotransmitters involved in the anoxia-triggered release facilitation might provide a basis for the selective vulnerability of motor neurons in the neurodegeneration associated with ALS.

Ultrastructural determination of dynamic vesicle pools at inner hair cell ribbon synapses

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Encoding auditory signals into a neuronal code during the process of hearing, in mammals is mediated through the ribbon synapses of the inner hair cells (IHCs) and spiral ganglion neurons. Acoustic information is transmitted with high temporal precision over long periods of time requiring high rates of transient and sustained release. These specialized synapses comprise a synaptic ribbon capable of tethering dozens of synaptic vesicles and a presynaptic density that anchors the ribbon to the membrane. Morphologically, synaptic vesicles are also tethered to the active zone membrane, which, functionally, might represent the readily releasable vesicle pool. Neither the proteins mediating vesicle tethering to the ribbon nor to the active zone membrane at IHC ribbon synapses are identified so far. Moreover, an understanding of the mechanisms underlying exocytosis and endocytosis in IHCs in order to maintain the high release rates is just starting to emerge.
We investigate, which proteins contribute to the release machinery to coordinate synaptic vesicle release and recycling. To approach this, we study mutants of active zone proteins that show impairment in function, taking advantage of recent advances in electron microscopy technology such as high-pressure freezing/freeze-substitution (HPF/FS) and electron-tomography.
Here we will focus on two proteins: (i) otoferlin, a C2-domain protein critical in vesicle fusion (Roux et al., 2006) and vesicle replenishment (Pangrsic et al., 2010) and (ii) the clathrin adapter protein AP-2 that in IHCs promotes efficient vesicle replenishment and interacts with otoferlin (Duncker et al., 2013). We will present detailed analyses of vesicle pool changes upon activity, vesicle reformation and also tethering of vesicles on the ultrastructural level in otoferlin and AP-2µ KOs.

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Vertebrate-specific presynaptic protein Mover controls release probability at the calyx of Held

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Mover is a vertebrate-specific presynaptic protein previously discovered in a yeast-two-hybrid screen of bassoon-interacting proteins. Mover is expressed in subsets of excitatory and inhibitory synapses throughout the brain, including hippocampus, cerebellar cortex and brain stem. While a more global expression in all terminals would suggest an essential contribution to synaptic transmission, the pronounced subset-specificity of Mover expression points towards a synapse-specific modulatory function. Biochemical characterization revealed that Mover is attached to the surface of synaptic vesicles and binds to calmodulin. However, the function of this protein remains unknown.
We have previously shown that Mover is expressed in the rat calyx of Held, a giant terminal in the auditory brain stem. To assess the function of Mover, we generated an in vivo knock-down of Mover using adeno-associated virus (AAV) mediated shRNA expression in globular bushy cells of the cochlear nucleus, the projection neurons forming the calyx of Held. 3D Immunohistochemistry revealed a strong reduction of Mover expression in identified single calyces expressing mOrange in cis with the shRNA. Calyceal Mover knock-down did not affect spontaneous synaptic transmission but increased the amplitude of evoked EPSCs. The size of the releasable pool was unaltered while short-term depression was accelerated and enhanced. Direct measurements of presynaptic calcium currents confirmed that these effects are not caused by altered calcium influx, but may be the result of differences in the calcium dependence of synaptic vesicle release. This was further substantiated by presynaptic capacitance measurements, showing that shorter depolarizing pulses were sufficient to evoke maximal release in Mover-depleted calyces compared to control calyces. Finally, by using Ca2+-uncaging to generate defined Ca2+ concentrations in the calyx, we found that Mover knock-down increases the calcium sensitivity of neurotransmitter release.
These findings are in line with a model depicting Mover as a negative regulator of release probability by decreasing the calcium sensitivity of the presynaptic release machinery. The expression of Mover in certain subsets of synapses may thus constitute a novel mechanism to tune the bandwith of synaptic transmission by regulating release probability.

Vesicular replenishment in cochlear inner hair cells operates without Munc13 and CAPS priming proteins

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Cochlear inner hair cells (IHCs) encode sound with high temporal precision, a process that requires tight regulation of presynaptic vesicle release. Recent data show that the molecular composition of IHC active zones differs substantially from conventional glutamatergic synapses. Hence, fundamental steps of the synaptic vesicle cycle – such as vesicular tethering, docking and priming – have to be revisited in these cells. In neurons, neuroendocrine and immune cells, vesicular release critically depends on priming factor proteins of the Munc13 and calcium-dependent activator protein for secretion (CAPS) families. In the present study, we tested whether Munc13 and CAPS proteins also regulate exocytosis in mouse IHCs, in which another C2-domain protein, otoferlin, is critical for priming and fusion of vesicles. We combined auditory systems physiology, IHC patch-clamp recordings and immunohistochemistry in Munc13 and CAPS mouse mutants to probe for potential roles of these proteins in IHC exocytosis. We show that IHC presynaptic calcium currents and exocytosis as well as auditory brainstem responses remained largely unchanged in Munc13 and CAPS deletion mutants. Moreover, we provide evidence for complete absence of Munc13-like proteins from adult IHC ribbon synapses but instead detected local clustering of otoferlin in the presynaptic membrane below the ribbon. In otoferlin mutants, we observed a dramatic reduction of exocytosis and, using electron microscopy, a significant increase in synaptic vesicle tether length, both indicative of impaired vesicular priming and attenuated fusion competence. In conclusion, our data indicate that IHCs do not rely on Munc13-like priming factors but instead use a priming machinery that critically depends on otoferlin.

Vesicular synaptobrevin2/VAMP2 levels guarded by AP180 control efficient neurotransmission

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Neurotransmission in the brain depends on the fusion of synaptic vesicles (SVs) via complex formation between vesicular synaptobrevin 2 (Syb2) and the presynaptic plasma membrane SNAREs syntaxin and SNAP-25. Exocytotic fusion is followed by the retrieval of SV membranes and the reformation of SVs of the correct size and molecular composition. Although Syb2 is the single most abundant SV protein with on average about 70 copies per vesicle, surprisingly only 1-3 Syb2 molecules appear to be sufficient for exocytotic fusion in vitro or in living cells, raising the question of why Syb2 is present in such a high copy number in vivo. Here, we demonstrate that mice lacking AP180, a specific clathrin/ AP-2 associated endocytic sorting adaptor for Syb2, suffer from severely reduced postnatal viability and neurological symptoms including spontaneous epileptic seizures as well as other behavioral abnormalities. AP180 knockout (KO) mice display reduced basal excitatory synaptic transmission, increased paired-pulse facilitation, and a faster rundown of the releasable vesicular pool in response to sustained synaptic stimulation. Recordings of somatic population spikes revealed stronger activity-dependent dysinhibition of hippocampal networks and reduced GABAergic feedback inhibition in AP180 KO mice, indicative of excitatory/ inhibitory imbalance. These functional deficits are explained by use-dependent Syb2 missorting causing its selective repartitioning from vesicular to surface pools and an accumulation of endosome-like vacuoles akin to those observed in Syb2 KO mice. Further reduction of Syb2 expression in the absence of AP180 (AP180-/- / Syb2+/-) exacerbated the AP180 KO phenotype and resulted in perinatal lethality, whereas reduced Syb2 expression in Syb2 heterozygous animals phenocopied loss of AP180 with respect to reduced basal neurotransmission. These data unravel a crucial role of AP180-mediated maintenance of vesicular Syb2 pools for proper neurotransmission and SV reformation in vivo.

a5-GABA$_A$ receptors regulate dendritic integration in CA1 pyramidal neurons

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Alpha5-containing GABAA receptors (a5-GABAR) have been implicated in tonic inhibition, while their contribution to phasic synaptic inhibition remains more controversial. Recently, the a5-selective negative allosteric modulator (NAM) RO4938581 was shown to increase synaptic plasticity and improve spatial learning in the Ts65Dn (TS) mouse model of Down syndrome (Martinez-Cue et al., 2013). Although this treatment has been suggested to rectify the over-inhibition found in Down syndrome, the precise cellular mechanisms remain obscure. Therefore, we investigated the effect of RO4938581 on individual hippocampal neurons in mouse brain slices. Tonic inhibition was evoked by bath-application of 5 µM GABA in the presence of 25 µM AP5 and 10 µM CNQX. Application of the a5-NAM (3 µM) reduced the GABA-dependent tonic currents (~25 pA) by about 25% in both granule cells and CA1 pyramidal neurons. To study phasic inhibition, inhibitory postsynaptic currents (IPSCs) were evoked by stimulating locally in the dendritic layer or close to the soma of CA1 pyramidal neurons. Slow dendritic IPSCs evoked in stratum lacunosum moleculare were significantly reduced to 64.4 ±14.4% after the application of µM a5-NAM. In contrast, there was no reduction of somatic IPSCs evoked in stratum pyramidale. To test the effects of the a5-NAM in TS mice, we measured input-output curves for both, excitatory and inhibitory post-synaptic currents. The ratio of inhibitory to excitatory inputs in CA1 pyramidal cells was not significantly increased in TS mice relative to wt littermates ($p>0.05$; n1=16, n2=12, 2-way ANOVA). Remarkably, excitatory postsynaptic potentials evoked by burst stimulation in stratum radiatum and stratum lacunosum moleculare were increased to 137.0 ±15.8% (n=8) in TS mice after the addition of the a5-NAM (1 µM) similar to wt mice (148.7 ±14.5%, n=9). These results point to increased postsynaptic integration after a5-NAM as the principle mechanism that may underlie enhanced synaptic plasticity in the previous studies.

Synaptic properties of SOM- and CCK-expressing cells in dentate gyrus interneuron networks.

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Hippocampal GABAergic cells are known to be highly heterogeneous, but the functional significance of this diversity is poorly understood. We performed paired recordings in synaptically connected interneurons in slice preparations of the rat and mouse dentate gyrus (DG) to show that morphologically identified interneurons form complex neuronal networks. Synaptic inhibitory interactions exist between cholecystokinin (CCK)-expressing hilar commissural associational path (HICAP) cells and among somatostatin (SOM)-containing hilar perforant path-associated (HIPP) interneurons. Moreover, both interneuron types inhibit parvalbumin (PV)-expressing perisomatic inhibitory basket cells (BCs), whereas BCs and HICAPs rarely target HIPP cells. HICAP and HIPP cells produce slow, weak, and unreliable inhibition onto postsynaptic interneurons. The time course of inhibitory signaling is defined by both the identity of the pre- and postsynaptic cells, it is the slowest in HIPP-HIPP synapses, intermediately slow in HICAP-HICAP synapses, but fast in BC-BC synapses. GABA release at interneuron-interneuron synapses also shows cell type-specific short-term dynamics, ranging from multiple-pulse facilitation at HICAP-HICAP synapses, to biphasic modulation at HIPP-HIPP synapses, and depression at BC-BC synapses. Although dendritic inhibition at HICAP-BC and HIPP-BC synapses appears weak and slow, channelrhodopsin 2-mediated excitation of SOM terminals demonstrates that these synapses effectively control the activity of BCs. They significantly reduce the discharge probability but sharpen the temporal precision of action potential generation. Thus, dendritic inhibition seems to play an important role in determining the activity pattern of GABAergic interneuron populations and thereby control the flow of information through the DG circuitry.

Poster Topic

T8: Synaptic Plasticity, LTP, LTD

T8-1A A high content *in vitro* screen for measuring regulation of synaptic structure/function by small molecules
Judith Rudolph, Kerstin Kellner, Isabell Cardaun, Karsten Kottig, Christina Thiede, Kenneth Young, Heinz von der Kammer

T8-2A A transgenic mouse model (BLEV) for visualization of the differential usage of BDNF exon IV and VI in the living organ
Wibke Singer, Eleonora Passeri, Hyun-Soon Geisler, Da Guo, Florian Mayer, Jing Hu, Verena Bautze, Jörg Strotmann, Ulrike Zimmermann, Lukas Rüttiger, Rama Panford-Walsh, Marlies Knipper

T8-3A Switched to T8-10D

T8-4A Activity dependent modulation of ECM by extracellular proteolysis
Jeet Bahadur singh, M.J.C. Valenzuela, E.D. Gundelfinger, C.I. Seidenbecher, Renato Frischknecht

T8-5A Activity-dependence and target specificity of synapse formation during long-term potentiation
Cvetalina Nikolaeva Coneva, Tobias Bonhoeffer, Tobias Rose

T8-6A Circuit analysis of functionally characterized neurons in the visual cortex of mice
Simon Weiler, Tobias Rose, Mark Hübener, Tobias Bonhoeffer, Volker Scheuss

T8-7A Contribution of calcium-induced calcium release to synaptic plasticity at the mossy fiber synapse
Urban Maier, Alexander Drakew, Michael Frotscher

T8-8A Development and spike-time dependent plasticity of inhibition in a binaural E/I nucleus
Brice Bouhours, Enida Gjoni, Ralf Schneggenburger

T8-9A Different forms of timing-dependent LTP can coexist at CA3-CA1 hippocampal synapses
Elke Edelmann, Efrain Cepeda-Prado, Volkmar Leßmann

T8-10A Differential contribution of matrix metalloproteinases 3 and 2/9 to long-term potentiation of NMDAR-mediated field potentials and LTP in basal vs apical dendrites in mouse CA1 hippocampal region.
Tomasz Wojtowicz, Patrycja Brzdak, Jerzy W. Mozrzymas

T8-1B Dynein light chain - An essential factor for gephyrin clustering
Vanessa Kress, Günter Schwarz

T8-2B Endoplasmic reticulum dynamics and spine structural plasticity are correlated
Alberto Perez Alvarez, Shuting Yin, Wolfgang Wagner, John A Hammer III, Thomas G Oertner

T8-3B Gad65-specific antibody effects on synaptic plasticity in hippocampal organotypic slices
Paula Paci, Laurence Ris

T8-4B Growth factor receptor bound-protein 2 is essential for social long-term recognition memory formation
Judith Camats Perna, Thomas Schlüter, Kristina Langnäse, Thomas Endres, Rita Murau, Volkmar Leßmann, Lars Nitschke, Oliver Stork, Klaus-Dieter Fischer, Mario Engelmann

T8-5B Hippocampal synaptic plasticity can be induced via patterned afferent stimulation of the primary olfactory cortex
Christina Strauch, Denise Manahan-Vaughan

T8-6B Impaired short-term plasticity in the hippocampus of sphingosine-1-phosphate lyase deficient mice
André Deutschmann, Daniel N. Mitroi, Maren Raucamp, Michael Hans, Jochen Walter, Gerhild van Echten-Deckert, Dieter Swandulla

T8-7B Interplay between the dopaminergic system and the extracellular matrix in terms of learning
Jessica Mitlöhner, Constanze Seidenbecher, Renato Frischknecht, Alexander Dityatev

T8-8B Lack of type 1 IFN receptor affects synaptic plasticity in mouse hippocampus
Gayane Grigoryan, Shirin Hosseini, Chintan Chhatbar, Wiebke Arendt, Kristin Michaelsen-Preusse, Ulrich Kalinke, Martin Korte

T8-9B Light-inducible transcriptomic and epigenomic changes underlying brain plasticity in honeybees
Nils Becker, Robert Kucharski, Sylvain Foret, Ryszard Maleszka, Wolfgang Rössler

T8-1C Long-term plasticity and fear learning in adult heterozygous BDNF knockout mice
Susanne Meis, Thomas Endres, Thomas Munsch, Volkmar Lessmann

T8-2C Long-term potentiation in perforant-path dentate gyrus synapses of freely behaving rats requires activation of ß-adrenergic receptors
Niels Hansen, Anne Kemp, Hardy Hagena, Denise Manahan-Vaughan

T8-3C Mechanisms regulating CtBP1 nucleo-cytoplasmic functions
Anika Dirks, Daniela Ivanova, Eckart D. Gundelfinger, Anna Fejtová

T8-4C Methylphenidate amplifies long-term potentiation in rat hippocampus involving ß-adrenergic and D1/D5 receptors and insertion of AMPA receptors
Bernardo Enrique Morales, Claudia Carvallo, Carlos Rozas, Darwin Contreras, Mario Carreño, Gonzalo Ugarte, Marc Leander Zeise

T8-5C Methylphenidate induces long-lasting metaplastic changes in the rat prefrontal cortex
Marc Leander Zeise, Héctor Burgos, Rocio Agurto, Christian Cofré, Patricio Saez, Alejandro Hernández, Bernardo Morales

T8-6C Molecular mechanisms mediating the specific effects of BDNF-TrkB signaling in hippocampal neurons
Nina Gödecke, Anita Remus, Marta Zagrebelsky, Martin Korte

T8-7C Phenotypic synaptic plasticity in the brain of the nectar-feeding ant *Camponotus rufipes*
Annekathrin Lindenberg, Wolfgang Rössler, Claudia Groh

T8-8C Role of Histone-Methyltransferases in Learning and Memory
Cemil Kerimoglu, Susanne Burkhardt, Eva Benito-Garagorri, Ana Martinez-Hernandez, Jerzy Dyczkowski, Andre Fischer

T8-9C Role of the synaptic vesicle protein Mover at bushy cell synapses
Friederike Wetzel, Thomas Dresbach

T8-1D Spike timing-dependent plasticity: contribution of the number of spike pairings in synaptic modification at Schaffer collateral-CA1 synapses
Efrain Cepeda-Prado, Leßmann Volkmar , Elke Edelmann

T8-2D Synaptic plasticity and the spine apparatus
Alexander Drakew, Anja Tippmann, Urban Maier, Michael Frotscher

T8-3D Synaptotagmin3 controls endocytosis of post-synaptic receptors to affect synaptic plasticity.
Ankit Awasthi, Saheeb Ahmed, Binu Ramachandran, Yo Shinoda, Henrik Martens, Carolin Wichmann, Camin Dean

T8-4D The APP interacting protein family Fe65 reveals a crucial role for synaptic function and plasticity
Susann Ludewig, Ulrike Herrmann, Meike Hick, Paul Strecker, Ulrike Müller, Suzanne Guenette, Stefan Kins, Martin Korte

T8-5D The Effect on the field potentials from the Hippocampus of Bad-Badý extract (Solanacea Species): is there a contribution on the memory?
Yalcin Yetkin, Mehmet A. Pak

T8-6D The Effects of Albumin on Plasticity Changes in the Hippocampus and on Response to Antiepileptic Drugs in the Entorhinal Cortex
Seda Salar, Ezequiel Lapilover, Julia Müller, Alon Friedman, Uwe Heinemann

T8-7D The neural cell adhesion molecule-associated polysialic acid regulates synaptic plasticity in the mouse prefrontal cortex
Hristo Varbanov, Gaga Kochlamazashvili , Herbert Hildebrandt,
Alexander Dityatev

T8-8D The role of metabotropic glutamate receptors in different forms of synaptic plasticity in vitro.
Amira Latif-Hernandez, Enrico Faldini, Tariq Ahmed, Detlef Balschun

T8-9D Time-restricted impact of matrix metalloprotease 3 activity on long-term plasticity of NMDARs-mediated synaptic transmission and LTP in apical and basal dendrites in mouse CA1 hippocampal region.
Patrycja Brzdak, Jerzy W. Mozrzymas, Tomasz Wojtowicz

T8-10D Activation of the metabotropic glutamate receptor mGlu5 determines the direction of synaptic plasticity at mossy fiber – CA3 and associational/commissural – CA3 synapses
Hardy Hagena, Denise Manahan-Vaughan

A high content *in vitro* screen for measuring regulation of synaptic structure/function by small molecules

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Synaptic dysfunction is a common early event in neuro-degenerative diseases and age-associated cognitive decline. Furthermore, a number of neuro-developmental disorders have also been linked to inappropriate synaptic connectivity. Importantly, loss of synaptic connectivity may prove to be a reversible phenomenon suitable for drug intervention. In contrast, neuronal loss occurring at disease end-stage is unlikely to be readily reversible. Indeed current therapeutics for Alzheimer's disease focus on enhancing the signaling properties of the remaining neuronal population rather than addressing the disease progression itself.

Synaptic plasticity describes the dynamic process by which neuronal connections are formed and maintained over time. For the majority of excitatory synapses this occurs via specialised structures known as spines. In the long term, changes in neuronal connectivity are underpinned by alterations in the number, localisation and structure of synaptic spines. However, despite a clear link between spines, synaptic connectivity, neuro-degenerative and neuro-developmental diseases there remains a distinct lack of tools to assay such processes within modern drug discovery work-flows.

Target *SynapticPlasticity* is an Evotec initiative which builds on >15 years CNS and drug discovery experience. We use the Opera high content imaging platform combined with sophisticated image analysis tools to detect colocalization of pre- and postsynaptic sites and to measure changes in synaptic structure and function. Additionally we developed a reproducible technique for labelling of single neurons in primary cultures to visualize dendritic spine size and morphology using staining with lipophilic dyes. Further, we created different image parameters for network analysis of the cultured neurons.

To use these high content imaging platforms on primary neuronal cultures we first aligned the culture conditions to achieve a highly connected neuronal culture which still is capable to be analyzed by script based automated image analysis tools. To assess the assay window we induced neuroplastic changes via drug treatment using "stressor compounds" that robustly decrease the density of synapses on cultured rat hippocampal neurons and found picrotoxin producing a consistent response. Additionally work focuses to identify tool compounds which increase the density of synapses and/or reverse the effect of the stressor stimuli. The aim is to establish a medium-throughput screening assay to identify neuroprotective specimen driving formation and/or stabilization of excitatory synapses.

Disruption in synaptic structure and function is thought to be a major determinant of neurodegenerative and psychiatric disorders. One example is Autism spectrum disorder (ASD), primarily characterized by behavioral and social impairments. The aim in this part of the project is to investigate developmental psychiatric phenotypes in neuronal cultures by modulation of risk gene expression via viral transduction to derive translational models for ASD. Thus several ASD related genes, linking to key regulatory checkpoints and a common molecular pathway in the transcriptional networks underlying autism [1)], were selected for AAV-shRNA mediated knock-down. Preliminary data indicates gene-dependent up- and down-regulation of synapse density.

[1)] Lanz et al.: Transcriptomic analysis of genetically defined autism candidate genes reveals common mechanism of action. Molecular Autism 2013, 4:45

A transgenic mouse model (BLEV) for visualization of the differential usage of BDNF exon IV and VI in the living organ

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Brain-derived neurotrophic factor (BDNF) is a key neurotrophin which plays an important role in the alteration of synaptic efficacy, the balance of inhibition / excitation and activity-dependent plasticity (Manadas et al., 2009; Reichardt, 2006). BDNF has a characteristic gene structure: a common protein-encoding exon (IX), which can be alternatively spliced to any of the eight non-coding upstream exons (I-VIII). This results in different BDNF transcripts but only in one protein (Aid et al., 2007; Timmusk et al., 1993). The role of the differential usage of the untranslated BDNF exons during plasticity changes is still unknown (Chiaruttini et al.; Pattabiraman et al., 2005; Rich and Wenner, 2007). In this study we focused on BDNF exon IV and VI, which have been previously shown to be differentially targeted in an activity dependent manner into a neuron´s soma and its dendrites (Baj et al., 2011; Chiaruttini et al., 2008; Tongiorgi). A transgenic mouse line was generated in which the transcription of BDNF exon IV and VI can be visualized in parallel via either a cyan (BDNF exon IV) or a yellow (BDNF exon VI) fluorescent protein without affecting the basic BDNF expression.
In a first approach, the construct for the generation of the mouse line was tested in cell culture models. The basic phenotype of the newly generated mouse line, BLEV (BDNF Live Exon Viewing), including BDNF expression and the activity-dependent usage of the BDNF promoters IV and VI was then analyzed in different tissues.

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Activity dependent modulation of ECM by extracellular proteolysis

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The chondroitin sulfate proteoglycan Brevican is one of the main components of the mature extracellular matrix (ECM). It is the smallest member of the family of lecticans. Together with other proteoglycans and glycoproteins such as tenascin-R, the lecticans form a tight meshwork around neurons. It has been shown that brevican and the other lecticans can undergo proteolytic cleavage, mainly by ADAMTS4 (a disintegrin and metalloprotease with thrombospondin motifs). It has been suggested that the ECM blocks synaptic plasticity by stabilizing synaptic contacts and preventing synaptogenesis in the adult. On the other hand the ECM provides important instructive information necessary for LTP, a measure for synaptic plasticity. Therefore, endogenous mechanisms that alter locally the structure of the ECM may be a mechanism to allow for synaptic rearrangements in the adult in a restricted area and time. Interestingly, it has been reported that during the first minutes to hours after LTP induction neurons show enhanced structural plasticity. Therefore we wondered whether brevican as a representative of the ECM is particularly proteolytically cleaved during LTP. To this end we induced chemical LTP in acute slices and performed quantitative western blotting to measure brevican cleavage. We found a marked increase in brevican cleavage compared to control slices 15-60 min after LTP induction. Cleavage was reduced to basal level when a broad-spectrum protease inhibitor was used during the experiment. This indeed indicates that proteolytic cleavage and therefore remodeling of the ECM may be involved in learning and memory processes that require structural plasticity.

Activity-dependence and target specificity of synapse formation during long-term potentiation

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Long-term potentiation (LTP) of synaptic connections results in the formation and stabilization of new dendritic spines in vitro [1]. Similarly, experience-dependent plasticity in vivo is associated with changes in the number and stability of spines [2]. However, to date, the contribution of excitatory synaptogenesis to the strengthening of synaptic connections remains elusive. Do new spines form functional synapses with the inputs stimulated during LTP induction and thereby follow Hebbian co-activation rules, or do they connect with random partners? Furthermore, at which time-point are de novo spines functionally integrated into the network?

We developed an all-optical approach to stably and exclusively stimulate a defined channelrhodopsin-2 (ChR2)-transduced subset of CA3 cell axons in mature hippocampal slice culture over extended periods of time (up to 24h). We continuously monitor synaptic activation and synaptic structure of CA1 dendrites using two-photon imaging. To control the dendritic location where LTP and associated spinogenesis are allowed to take place, we globally block Na+-dependent action potential firing and directly evoke neurotransmitter release by local light-evoked depolarization of ChR2-expressing presynaptic boutons (in TTX, 4-AP). We induce robust optical LTP specifically at this location by combining optogenetic activation with chemical pairing (in low [Mg2+]o, high [Ca2+]o, forskolin, and rolipram). Taking advantage of the NMDA-receptor mediated Ca2+ influx during synaptic activation we assess the formation of functional synapses using the genetically encoded Ca2+ indicator GCaMP6s (see figure).

We find that spines formed during the induction of optical LTP are more stable than spines formed under control conditions. A fraction of new spines responded to optical presynaptic stimulation within minutes after formation. However, the occurrence of the first synaptic Ca2+ response in de novo spines varied considerably, ranging from 8 min to 25 h. Most new spines became responsive within 5 h (1.5 ± 1.15 h, mean ± S.D., n = 22 out of 31), whereas the remainder showed their first response only on the second experimental day (19.7 ± 3.30 h). Ongoing experiments will show whether or not new spines preferentially contact the fraction of axons active during LTP and thus could be considered to be “Hebbian”.

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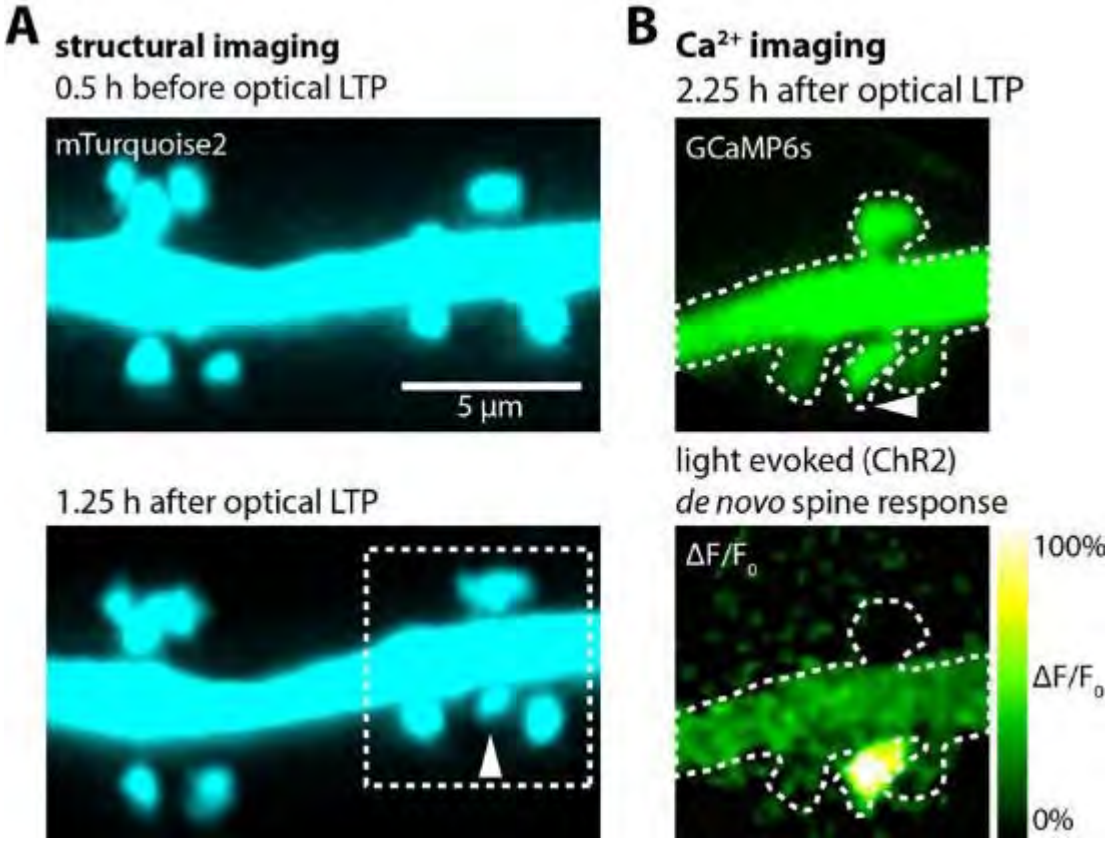
A
structural imaging
0.5 h before optical LTP
mTurquoise2
5 µm
1.25 h after optical LTP
B
Ca2+ imaging
2.25 h after optical LTP
GCaMP6s
light evoked (ChR2)
de novo spine response
ΔF/F0
100%
ΔF/F0
0%

Circuit analysis of functionally characterized neurons in the visual cortex of mice

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The function of neural circuits is determined largely by the specific connectivity between their neurons. Likewise, the specific response and tuning properties of single neurons arise mostly from the information carried by their synaptic inputs. However, little is known about the detailed relationship between the organization of synaptic connections and neural response properties at the level of single cells. Our first aim is to correlate the functional properties of single neurons with their circuit organization in the visual cortex of mice. The second aim is to study how changes in the response properties of single neurons during experience-dependent plasticity are reflected in modifications of their synaptic connectivity.

To address these questions, we first characterize the principal connections in cortical circuits of mouse primary visual cortex (V1) using laser scanning photo stimulation (LSPS). In acute coronal slices, we record electrophysiologically from a target neuron and map its cortical inputs by stimulation of its potential presynaptic neurons using UV glutamate uncaging (e.g. Callaway and Katz, 1993, Yoshimura, Dantzker and Callaway, 2005). We obtained preliminary connectivity data for the principal neurons in cortical layers 2/3, 4, 5 and 6.

In order to correlate the response properties of neurons with their circuit organization, we follow an *in vivo*/*in vitro* approach. This enables us to first characterize neurons with *in vivo* 2-photon calcium imaging, and to subsequently find back the same neurons *in vitro* in acute coronal slices for circuit analysis with LSPS. We express genetically encoded calcium indicators (GECIs) in neurons of visual cortex to characterize their visually evoked response properties with *in vivo* 2-photon calcium imaging. To study the changes in circuit organization after experience-dependent plasticity we will employ established paradigms such as monocular deprivation. Chronic calcium imaging with GECIs before and after plasticity induction followed by *in vitro* LSPS will then allow us to directly compare the differential changes in circuit organization that distinguish plastic from non-plastic neurons. This approach promises new and deeper insights into the relationship between plastic neural response properties and neural circuit structure and reorganization.

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Contribution of calcium-induced calcium release to synaptic plasticity at the mossy fiber synapse

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It is still debated to what extent calcium-induced calcium release (CICR) from internal stores contributes to calcium kinetics in dendritic spines. Cisterns of endoplasmic reticulum represent such stores in spines and dendrites. In some spines a structurally refined derivative of the endoplasmic reticulum, the spine apparatus, is found. It consists of several flat cisterns of smooth endoplasmic reticulum intervened by electron dense plates. The functional significance of this structural specialization is not known. The complex spines postsynaptic to hippocampal mossy fiber boutons regularly contain a spine apparatus. Synaptopodin-deficient mice do not form a spine apparatus in otherwise unaltered spines (Deller et al., 2003).

We recorded calcium transients in complex spines using two-photon calcium imaging of patch-clamped hilar mossy cells in organotypic entorhino-hippocampal slice cultures from wild type mice and Synaptopodin knock-out mice. In response to trains of back-propagating action potentials we found significantly smaller calcium transients in spines of Synaptopodin knock-out slice cultures compared to wild type spines. In contrast, there was no difference in calcium transients observed in dendrites of both genotypes. In order to clarify whether the additional calcium in spines containing a spine apparatus is attributable to CICR, we blocked putative calcium release channels using blocking concentrations of ryanodine. After wash-in of ryanodine, peak amplitudes of the recorded calcium transients were strongly reduced in wild type spines and reached the level of Synaptopodin-deficient spines without ryanodine treatment. However, calcium transients were also affected by ryanodine in spines of Synaptopodin knock-out mice, suggesting the presence of ryanodine receptors in these spines. We conclude that CICR contributes to calcium kinetics in spines of mossy fiber synapses and that CICR is enhanced in spines containing a spine apparatus when compared to spines harboring simple cisterns of endoplasmic reticulum.

Reportedly, the mossy fiber synapse (MFS) can undergo a postsynaptic form of LTP characterized by an NMDA receptor (NMDAR)-dependent enhancement of NMDAR-mediated transmission after tetanic stimulation (Kwon and Castillo, 2008). This type of potentiation requires postsynaptic calcium elevations. In addition, LTP of NMDARs was suggested to serve as a metaplastic switch, making the MFS competent for classical NMDAR- mediated LTP of AMPAR-mediated currents (Rebola et al., 2011). Therefore we suggest the spine apparatus to play a key role in synaptic plasticity at the MFS, shaping the postsynaptic calcium response by modifying CICR. We are currently testing this hypothesis in a paired-recording configuration: Two-photon calcium imaging of an individual mossy cell excrescence and parallel stimulation of the innervating mossy fiber bouton. We want to know to what extent the potentiation of NMDAR-mediated transmission is linked to CICR at mossy fiber synapses and hence altered in complex spines from Synaptopodin knock-out mice.

M.F. is Senior Research Professor for Neuroscience of the Hertie Foundation.

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Development and spike-time dependent plasticity of inhibition in a binaural E/I nucleus

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In the auditory brainstem, the Lateral Superior Olive (LSO) is the first nucleus that integrates convergent inputs from the left and right ears to determine the origin of sound in azimuth (Pollak, 2012). LSO principal cells receive indirect inhibitory inputs from the contralateral ear via the medial nucleus of the trapezoid body (MNTB), and direct excitatory input from the ipsilateral ear via the spherical bushy cells in the VCN. We found recently that unitary IPSCs arising from MNTB stimulation are surprisingly large, indicating a role for powerful inhibition in excitation/inhibition (E/I) integration in LSO (see abstract by Gjoni et al., this meeting). Here, we investigate the development, and possible plasticity mechanisms which could lead to a strengthening of unitary IPSCs in the MNTB to LSO pathway.

We made recordings of LSO neurons in brain slices of P6 - P18 old mice. At all these ages, we found that fiber stimulation on the medial side of the LSO, presumably activating inhibitory input from the MNTB, caused IPSCs which increased in "jumps" upon increasing the stimulation voltage in small increments (Kim & Kandler, 2003). This allowed us to estimate the number of functionally innervating fibers, as well as the unitary IPSCs amplitudes. We found that at P6 - P7, uIPSC amplitudes were small under conditions of high intracellular Cl- (~ 50 - 200 pA), and excitatory glutamatergic synaptic components were often apparent (Gillespie et al., 2005). From about P9 onwards, uIPSCs were typically much larger (~ 500 pA - 3 nA), indicating a quite abrupt increase in uIPSC size at ~ P8, clearly before the onset of hearing. Interestingly, the number of functionally observed inhibitory inputs did not change significantly between P6 - P20, which argues against a strong component of elimination of inhibitory synapses at these times.

We next asked whether activity-dependent plasticity might contribute to the developmental increase in uIPSC amplitude. Since it can be expected that in these auditory sensory synapses, pre- and postsynaptic spikes can occur in an ordered sequence, we tested classical spike-time dependent plasticity (STDP) protocols. We paired presynaptic afferent fiber stimulation of inhibitory inputs, with a postsynaptic spike induced by short current injections in current clamp mode (120 times, at 1 Hz). In P11 - P15 mice, pre- post pairings at short intervals (3 - 8 ms) induced a moderate, but long-lasting potentiation of inhibitory inputs. With the opposite spike timing (post - pre), a subtle long-lasting depression of IPSCs was observed, after a stronger, but transient depression. We applied the same protocols before hearing onset (P8 to P10). At this age, we did not observe potentiation but a small transient depression in pre - post pairings. At both ages, we never observed long-lasting plasticity with unpaired stimulation, whereas the more transient effects remained. This data suggests that use-dependent forms of plasticity could contribute to a developmental increase and refinement of inhibition after hearing onset (Sanes &Takacs, 1993). However, the large increase in inhibitory synaptic strength before hearing onset (see above) occurs at a time when at least in-vitro, STDP plasticity is not apparent. Thus, different plasticity mechanisms likely contribute to the initial growth of inhibitory synapses in LSO, versus their later use-dependent refinement after the onset of hearing.

Different forms of timing-dependent LTP can coexist at CA3-CA1 hippocampal synapses

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The commonly used models to study learning and memory storage processes in vitro are long-term potentiation (LTP) as well as long-term depression (LTD). These types of long-lasting strengthening or weakening of synaptic transmission can be induced with various stimulation patterns. A reliable tool to mimic experience-dependent synaptic plasticity is the spike timing-dependent plasticity (STDP) paradigm, which consists of repetitive single presynaptic stimulation paired with 1-4 action potentials (AP) fired in the postsynaptic neuron. Applying different STDP paradigms at CA3-CA1 synapses in acute slices, we can induce robust timing-dependent (t-) LTP. Interestingly, subtle changes in the number of postsynaptic APs used for pairing and in the number of overall repetition of pairings, respectively, led to t-LTP forms which markedly differ in the recruited intracellular signaling cascades mediating t-LTP expression.

Using whole cell patch clamp techniques in acute hippocampal slices, we show that the 1EPSP/1AP pairing (1 presynaptically induced excitatory postsynaptic potential paired with one postsynaptic AP) leads to presynaptically expressed t-LTP, which is induced in a dopamine dependent manner, while a 1EPSP/4AP STDP paradigm leads to postsynaptically expressed and BDNF dependent t-LTP. We further show that this 1:4 pairing induced t-LTP is mediated via BDNF action at postsynaptic TrkB receptors. Postsynaptic elevation of cAMP combined with repeated 4AP burst firing leads to a BDNF dependent increase of evoked EPSPs, mimicking and occluding the 1:4 pairing induced t-LTP. On the one hand this proves the specificity of the postsynaptic stimulation pattern for BDNF signaling; on the other hand this suggests postsynaptic BDNF secretion as underlying mechanism for the 1:4 t-LTP.
Independence of signaling and expression mechanisms between the 1:1 and the 1:4 paradigm induced t-LTP is shown by the absence of t-LTP occlusion between these 2 protocols. However, occlusion can be found by a slight modification of the protocols, indicating that not only the postsynaptic stimulation number, but also repeat numbers determine specificity of signaling and expression mechanisms (see also Cepeda-Prado et al., this meeting). Additionally to these differences regarding t-LTP at the stimulated synapse, hetero-synaptic effects as well as adaptive intrinsic changes are evident from our study. In experiments in which a non-potentiated second synaptic input is analyzed as control pathway, our BDNF-dependent postsynaptic STDP protocol leads to a hetero-synaptic spread of BDNF and a subsequent transient increase of evoked EPSPs in the unstimulated and independent control, while the 1EPSP/1AP paradigm does not show any such changes in the control pathway. Parallel to synaptic plasticity, we observe an additional type of plasticity, which is indicated by AP frequency changes in potentiated neurons. While presynaptically expressed plasticity leads to a postsynaptic increase in AP firing, postsynaptically expressed t-LTP is ineffective in this respect.

Taken together our data show, that similarly effective STDP can be induced with various patterns of STDP protocols at CA3-CA1 synapses. However, these t-LTP forms are mediated by activation of distinct signaling cascades and induced by different patterns of stimulation. Hence, our data indicate a multitude of potential forms of memory formation, which are expressed at a single type of synapse in the hippocampus.

Differential contribution of matrix metalloproteinases 3 and 2/9 to long-term potentiation of NMDAR-mediated field potentials and LTP in basal vs apical dendrites in mouse CA1 hippocampal region.

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Learning and memory formation require modification of the number and strength of existing synaptic connections. Both structural and functional synaptic plasticity are supported by proteolysis of extracellular matrix proteins, adhesion and signaling molecules (Sonderegger, 2014). We have recently shown that activity of matrix metalloproteases (MMP) plays a crucial role in expression of long-term potentiation (LTP) of field EPSPs (fEPSPs) and population spike amplitudes within CA3 region of the hippocampus (Wójtowicz & Mozrzymas, 2014). Interestingly, while MMP-3 inhibition resulted in impaired LTP induction and affected its early phase time course, MMP-2/9 inhibition affected only late-phase of LTP in CA3-CA3 synapses (Wójtowicz & Mozrzymas, 2014). Here we further addressed the question how MMP-3 proteolytic activity could support LTP induction and its early time-course following enhanced synaptic activity in the CA1 hippocampal region. To this end, we studied the impact of bath applied MMP-3 (NNGH, 10µM) and MMP-2/9 (SB3CT, 10µM) inhibitors on fEPSPs in CA3-CA1 hippocampal projection in mouse C57BL6 (P30-P50) acute brain slices. We found that NNGH reduced LTP of fEPSPs following high frequency stimulation (HFS, 4x100Hz) of Schaeffer collaterals in apical dendrites (131±10% and 98±6% of baseline EPSP in control and NNGH treated slices, respectively, n=8, $p<0.01$), similarly to previously described effect within CA3 region (Wójtowicz & Mozrzymas, 2014). Considering the crucial role of NMDARs in LTP, we next recorded pharmacologically isolated NMDAR-mediated field potentials in the presence of AMPA/kainate receptor antagonist DNQX (20µM) and 0mM Mg_{2+}. We found that HFS resulted in LTP_{NMDA} (140±33% of baseline $EPSP_{NMDA}$ area 1.5 hour post LTP induction, n=8) that was insensitive to SB3CT (153±22%, n=7) but was completely abolished by NNGH (75.8±2.9%, n=6, $p<0.05$).

MMP-3 cleavage of fibronectin and other matrix proteins may provide ligands for integrin activation (Zhang et al., 2012) which were reported to underlie differences in LTP consolidation in basal vs apical dendrites in CA1 region (Kramar and Lynch, 2003). Thus, we repeated above experiments in stratum oriens CA3-CA1 projection and we found that in contrast to stratum radiatum, MMP-3 inhibitor had no effect on HFS-induced LTP (189±15% and 227±19% of baseline EPSP in control and NNGH treated slices, respectively, n=8, $p>0.05$). In conclusion, a picture emerges that proteolytic activity of MMP-3 may differentially support induction and early phase of LTP in apical vs basal dendrites of CA1 pyramidal cells. Integrin-dependent NMDARs modulation is likely to be involved in this differential role of MMP-3.
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Dynein light chain - An essential factor for gephyrin clustering

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Inhibitory transmission in the central nervous system depends on the strength and plasticity of synaptic contacts, balancing the activity of excitatory synapses and vice versa. As major postsynaptic proteins, scaffolding molecules present crucial factors for the specific localization of neurotransmitter receptors at the postsynaptic membrane. At inhibitory synapses, the scaffolding protein gephyrin anchors glycine and $GABA_A$ receptors at the postsynaptic site. Synaptic clustering and translocation of gephyrin are highly dynamic processes, which are regulated by protein-protein interactions and post-translational modifications. However, the molecular mechanisms regulating gephyrin-mediated receptor clustering are not fully understood. Gephyrin was among others identified to interact with cytoplasmic dynein light chain (DLC1 and DLC2), which is a central component of the large dynein motor complex mediating long distance intraneuronal transport but is also involved in other cellular processes.
We characterized the interaction between gephyrin and DLC1 and determined a K_d of 2.14 ± 0.1 µM. Recent mass spectrometric analysis of gephyrin identified numerous phosphorylation sites in the central C domain of gephyrin, one of which is located within the DLC binding site. Mimicking a constitutive phosphorylation within the gephyrin-DLC binding motif (T205D) completely prevented the binding of DLC1 to gephyrin in vitro as well as in primary hippocampal neurons, revealing a phosphorylation-dependent regulation of this interaction. Furthermore, we provide evidence that gephyrin and DLC interact independently of the neuronal retrograde transport machinery, as we could not identify a ternary complex between gephyrin, DLC and the dynein motor complex. Moreover, inhibition of the dynein motor complex caused a significant increase in the number as well as size of gephyrin clusters in primary hippocampal neurons, indicating a novel, yet not explored DLC function in gephyrin cluster formation.

Endoplasmic reticulum dynamics and spine structural plasticity are correlated

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The precise role of intracellular organelles in spines is poorly understood, but there is evidence for effects on synaptic plasticity. The presence of endoplasmic reticulum (ER) allows mGluR-dependent depression and local calcium signalling in voluminous dendritic spines which bear strong synapses (Holbro et al., 2009 PNAS). Knocking out synaptopodin, a protein that is essential for ER organization into a 'spine apparatus', has modest effects on synaptic plasticity (Deller et al., 2003 PNAS; Vlachos et al., 2008 Hippocampus). We were interested in the temporal dynamics of ER and potential effects on synaptic structure. Therefore, we monitored the volume of dendritic spines and movements of GFP-labelled ER with multiphoton microscopy. ER movements in and out of spines were much more dynamic than previously thought, occurring on a time scale of minutes rather than days (Toresson and Grant, 2005 EJN). We could distinguish 2 classes of ER dynamics: In some spines (~10%), the ER remained present for hours. The majority of ER intrusions, however, were short-lasting (< 20 min). About 20% of CA1 hippocampal spines possess ER at any given time point but more than 50% were visited within 2 hours, and progressively more in longer time periods. Interestingly, the volume of dendritic spines was at its maximum at the time of ER insertion, pointing to a tight correlation between ER and structural plasticity. In addition, we have found that the fast ER dynamics depend on myosin Va activity and this turnover is positively modulated by AMPA/NMDA receptors while mGluRs exert the opposite effect. Our time-lapse analysis agrees with the concept that spine ER acts as a 'brake' on spine growth and synaptic potentiation (Holbro et al., 2009 PNAS).

Gad65-specific antibody effects on synaptic plasticity in hippocampal organotypic slices

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It is well known that Arc, for Activity-Regulated Cytoskeleton-associated protein, is implicated in synaptic plasticity in the hippocampus. This protein is translated only in activated dendritic spines where it stabilizes F actin polymers. However, the mechanisms underlying the regulation of its expression are still poorly understood. Moreover, very little is known about the impact of neuroinflammation on its expression.

In this study, hippocampal organotypic slices were used to investigate the induction of Arc expression and the synaptic plasticity in the presence of GAD-65 specific monoclonal antibodies. This experimental model offers the advantages of an *in vitro* model together with the preservation of the structure of a complex neuronal network. Arc expression was induced in this model by forskolin and IBMX, two drugs increasing the level of cAMP, confirming the results obtained by other groups *in vivo* or in primary cultured cells.

Two Gad65-specific human monoclonal antibodies (B96.11 and B78) arising from a patient with autoimmune polyendocrine syndrome type 2 were tested to determine their potential effects in hippocampal organotypic slices. Gad65 is the smaller isoform of glutamate decarboxylase which transforms glutamate to GABA at the level of synapses. It is the target of autoantibodies in various neurological disorders such as Stiff Person syndrome, cerebellar ataxia or limbic encephalitis but also in type 1 diabetes. It has been demonstrated that B96.11 recognizes an epitope located at amino acid residues 308-365 while B78 recognizes an epitope located at the C-terminus (512-540). It is also known that only B78 inhibits Gad65 enzymatic activity in the cerebellum and causes an increase of extracellular glutamate (Manto *et al*. 2011).

In hippocampal slices, after having demonstrated that Gad65 antibodies were able to enter living neurons, we observed that B78 led to a significant microglial proliferation when added at 0.5µg/ml during 3 days. This effect was specific as it was not induced by B96.11 antibody. Moreover, B78 prevented long-term potentiation and inhibited the induction of Arc expression in culture. This results could be explained by an alteration in dendritic spine morphology where Arc's function is critical. An increase in cell death rate was finally detected with both antibodies but at stronger dose applied during 10 days suggesting that these damages were not specific to Gad65 antibodies.

Based upon these results, it would be interesting to investigate the interaction between GABAergic neurons and microglia in the context of the synaptic plasticity.

Growth factor receptor bound-protein 2 is essential for social long-term recognition memory formation

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Learned experiences produce plastic changes in synaptic strength and their persistence has been postulated to be part of the cellular mechanisms underlying learning and memory. One of the molecular cascades implicated in the process of long-term memory formation is the Ras-controlled mitogen-activated protein kinase (MAPK) – extracellular regulated kinase (ERK) pathway. This cascade is activated by neurotrophins such as brain-derived neurotrophic factor which induce the autophosphorylation of receptor tyrosine kinases (RTK, tropomyosin releated kinase B). Phosphorylated RTKs are able to interact with several molecules and activate different downstream signalling pathways. Our interest is focused on the direct interaction with the growth factor receptor bound-protein 2 (Grb2) and Son of Sevenless. Once this complex is formed, a series of phosphorylation reactions leads to the activation of several nuclear events, which initiate cell-specific gene expression necessary for synaptic remodelling and long-term changes in synaptic efficacy. It is well known that MAPK activation plays an important role in the induction of neuronal plasticity and memory in adulthood, although the exact mechanisms remain to be investigated. In order to get first insights into distinct elements of this intracellular signalling cascade we focused on Grb2. Grb2 is suggested to be involved in memory formation, since Grb2 levels were increased in mouse hippocampus after training. Moreover, downregulation of Grb2 in the same area was linked with cognitive deficits. First, we produced conditional knock-out mice, which contain a floxed (loxP) Grb2 gene and in which the Cre-recombinase expression is controlled by the mouse calcium/calmodulin dependent protein kinase II alpha promoter. Second, we checked the protein levels of Grb2 in different parts of the brain. Mutant mice showed decreased levels of Grb2 protein in the hippocampus, frontal cortex, striatum and olfactory bulb, but not in the cerebellum. Third, we performed a general behavioural screening. Both, open field and fear conditioning tests revealed that cKO Grb2 mice are slightly more active than wild type animals. Preliminary results suggest that the genotype had no impact on the ability to successfully associate the tone and context with the electric foot shock, in the cued and contextual fear conditioning test, respectively. Despite Grb2 was found to be downregulated in the olfactory bulb of cKO Grb2 mice, mutant animals showed a similar performance in the olfactory habituation and dishabituation test as wild type animals. Finally, we investigated the recognition memory abilities of cKO Grb2 mice using the social discrimination test. cKO Grb2 mice show intact short- and intermediate-term social memory but an impaired long-term social memory. These results highlight the relevance of the MAPK-ERK pathway through Grb2 to successfully consolidate long-term social recognition memory. Further studies are in progress to investigate the consequences of decreased Grb2 levels on distinct molecular and behavioural parameters to get deeper insight into the cellular mechanisms underlying long-term social recognition memory function in mice.

Hippocampal synaptic plasticity can be induced via patterned afferent stimulation of the primary olfactory cortex

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Both the hippocampus and the cortex are essential for learning and memory mechanisms. All areas of the cortex are able to undergo experience-dependent modification, whereby most experience-dependent learning emerges from sensory inputs. But, the precise mechanisms for encoding of incoming sensory information into persistent representations in the CNS are still unclear.
This project focusses specifically on the olfactory system, as the oldest sensory system. The bypassing of thalamic modulation before the primary sensory cortex is involved, results in an earlier involvement of the limbic system in olfactory information processing, in contrast to all other sensory systems. So far only few studies investigated the interaction of the primary olfactory cortex, in rodents the piriform cortex, and the hippocampus in learning and memory mechanisms of olfactory sensory inputs. It has been examined that presentation of spatial olfactory cues results in a facilitation of short-term depression into long-term depression in hippocampal CA1 region (André and Manahan-Vaughan, 2013). Another study demonstrated that in the absence of sensory inputs a specific odor constellation is sufficient to generate stable place field in CA1 place cells, whereby a rotation of the odor constellation results in place field rotation (Zhang and Manahan-Vaughan, 2013).
The ability of patterned afferent stimulation applied in the anterior piriform cortex on the induction of synaptic plasticity in the dentate gyrus was investigated in freely behaving rats. Therefore, rats were chronically implanted with a recording electrode in the dentate gyrus and bipolar stimulating electrodes in the anterior piriform cortex, the olfactory bulb and the perforant path. Then, specific high-frequency and low-frequency stimulation protocols were applied in the anterior piriform cortex to examine their ability to induce synaptic plasticity in the dentate gyrus. High-frequency stimulation at 100 Hz resulted in a long-term potentiation, whereas low-frequency stimulation at 1 Hz induced a depression of synaptic responses in the dentate gyrus.
Altogether, these results indicate that the primary olfactory cortex has a direct long-lasting influence on hippocampal synaptic plasticity.

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Impaired short-term plasticity in the hippocampus of sphingosine-1-phosphate lyase deficient mice

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Sphingosine-1-phosphate, a bioactive lipid, plays an essential role in brain development. Its cellular concentration is under control of the sphingosine-1-phosphate lyase (S1PL). Mice with knockout of the S1PL gene show retarded growth, reduced body weight and a limited life expectancy. To overcome these limitations, a conditional knockout with a specific deletion of the S1PL in neuronal tissue was constructed. These mice exhibit a normal phenotype and allow the investigation of S1PL's role in various brain functions.

Neuroanatomical studies revealed morphological alterations in the structure of the entorhinal cortex and the CA1 region of the hippocampus in KO mice but not in their age-matched wild type littermates.

In the hippocampus the levels of the presynaptic marker proteins synaptobrevin, synapsin and bassoon were reduced, whereas the levels of synaptotagmin, SNAP25 and piccolo as well as the level of the postsynaptic density protein 95 (PSD-95) were unchanged. To test whether the reduction in presynaptic marker proteins affect synaptic plasticity, we performed electrophysiological measurements in the CA1 region. S1PL KO mice showed a significant decrease in paired pulse facilitation (PPF), which was age dependent and reached its maximum between 8 and 10 weeks p.p.. Post-tetanic potentiation (PTP) showed a slight enhancement, whereas long-term potentiation (LTP) was unchanged. These results indicate that elimination of the S1PL from neuronal tissue affects short-term synaptic plasticity but has no effect on long-term synaptic processes. To investigate whether inappropriate protein degradation might be involved in these effects we used the proteasome inhibitor epoxomicin. Following control recordings, slices where incubated in epoxomicin (15 µM) for approximately 5 h. This pharmacological treatment restored PPF almost to control values, suggesting that inappropriate protein degradation might be responsible for the impairment of synaptic plasticity in these sphingosine-1-phosphate lyase deficient mice.

Interplay between the dopaminergic system and the extracellular matrix in terms of learning

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The extracellular matrix (ECM) of the central nervous system which surrounds the pre- and the postsynapse consists of hyaluronan, chondroitin sulfate proteoglycans such as aggrecan and brevican, link proteins and tenascins. It was shown that the ECM functions as a cell-migration and diffusion barrier but also allows for example growth factors to be trapped and presented to their receptors at the cell surface.

Dopamine synthesized from tyrosine via L-DOPA is one neurotransmitter that plays a crucial role in learning. In the hippocampus dopamine acts on either D1- or D2-like dopamine receptors. It has been shown that activation of proteinkinase A caused by activated D1 dopamine receptors leads to a release of the growth factor BDNF. We now hypothesize that after activating dopamine receptors and activation of PKA not only BDNF but also ECM modifying proteases like Matrixmetalloprotease-9 and ADAMTS4 are released.

Here we want to investigate the impact of dopamine in remodeling of the extracellular matrix by proteolytic cleavage and its relevance for synaptic plasticity and learning. Therefore, D1-like or D2-like receptors were activated by dopamine receptor agonists SKF81297 or Quinpirole. By using immunocytochemical and western blotting analysis we found that brevican cleavage is increased after both D1 and D2 activation. This effect could be blocked by the protease inhibitors GM6001 and TIMP3. Taken together, these findings underline the possibility of an interplay between the dopaminergic system and the extracellular matrix, though the exact molecular and cellular mechansims remain to be clarified.

Lack of type 1 IFN receptor affects synaptic plasticity in mouse hippocampus

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Type 1 interferons (IFNs) are cytokines that can be released by glial and neuronal cells and have not only antiviral and immunomodulatory but also neuromodulatory actions. In this respect, it could be shown that rat interferon has a dose-dependent inhibitory effect on basal synaptic transmission, short- and long-term potentiation and glutamate-mediated excitatory postsynaptic potentials in acute hippocampal slices. The alterations of NMDA-induced and voltage-activated Ca^{2+} currents in cultured neurons have been shown as well. Type 1 IFNs bind to the type 1 IFN receptor (IFNAR). Previous studies showed that early IFNAR triggering is essential for prevention of viral spread over the CNS. Thus, mice that were devoid of IFNAR were not able to cope with viral infections.

In the current study, we investigated the functional role of IFNAR under homeostatic conditions, i.e., in non-immune-challenged mice. Six-week-old wild-type (WT) and IFNAR-deficient ($IFNAR^{-/-}$) mice of both genders were used to study the role of IFNAR in the brain. Following anesthesia and decapitation, brains were removed from the skull and divided into two hemispheres. The transverse slices (400 µm) were cut from the hippocampus of one hemisphere and were used for recordings of field excitatory postsynaptic potentials (fEPSPs) evoked by stimulation of Schaffer collaterals in stratum radiatum of the CA1 region of hippocampus. The second hemisphere was used for Golgi silver impregnation to study the morphology of hippocampal neurons.

Dependence of EPSP slope on stimulation intensity was assessed from input/output curves. The slices obtained from WT and $IFNAR^{-/-}$ mice exhibited similar input/output relations, which suggests that basal synaptic transmission is not affected by the absence of type 1 IFN receptor. Short-term synaptic plasticity (STP) was not altered in hippocampal slices taken from mice of both groups as revealed by examining paired-pulse facilitation (PPF). To study possible differences in long-term synaptic plasticity (LTP), theta-burst stimulation (TBS, four bursts at 100 Hz repeated 10 times in a 200 ms interval, repeated three times in a 10 seconds interval) was applied. Slices taken from $IFNAR^{-/-}$ mice showed significantly lower magnitude of LTP compared to WT (1.11 ± 0.001 (n=28) and 1.39 ± 0.01 (n=21), respectively, $p<0.001$, F value 61.35, one-way ANOVA).

Morphological analysis of CA1 pyramidal neurons revealed a significantly decreased spine density of both apical and basal dendrites in $IFNAR^{-/-}$ mice compared to WT (1.84 ± 0.04, $p<0.001$, t test, and 1.55 ± 0.03, $p<0.001$, t test, compared with 2.29 ± 0.04 and 1.98 ± 0.04, respectively).

Our results indicate the importance of the IFNAR for processes of synaptic plasticity in the adult, healthy brain. Further experiments are needed to identify cell types, signaling pathways and mechanism that are involved.

Light-inducible transcriptomic and epigenomic changes underlying brain plasticity in honeybees

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Honeybee workers perform different tasks in the colony throughout their adult life span. This is accomplished via division of labor, whereby young bees progress through a series of tasks inside the hive (cleaning, nursing), and older bees start foraging at ~3 weeks of age. However, division of labor is not exclusively age-regulated, but also context-dependent. Nurses may start precocious foraging when the colony is in need of foragers, and foragers can revert back to nurses in case of shortage of nurses. The honeybee, therefore, is an ideal model to investigate neuronal mechanisms of environmentally induced behavioral plasticity.

The shift from in-hive to outside tasks involves adaptations to different environments. One major difference in the outside world is exposure to light as bees leave the dark pheromone-filled hive. Light plays an essential role for foragers, particularly for visual navigation and spotting food sources. Therefore, foragers need to be optimally prepared by adaptive changes in the neuronal circuitry. Transition to foraging is associated with remarkable changes in brain structure and associated synaptic plasticity (Groh et al. 2012, J Comp Neurol). Exposure of adult workers to light is sufficient to induce structural synaptic plasticity in visual subcompartments of the mushroom bodies (Scholl et al. 2014, Dev. Neurobiol.).

Our aim is to study how light-inducible neuronal plasticity in visual centers of the brain is controlled at both transcriptional and epigenomic levels. In a broader context we wish to understand how light contributes to the genome-environment interplay that generates strikingly different phenotypes and behaviors with no conventional genetic changes. We will describe our experimental set-up designed to integrate environmental exposure with molecular changes in the brain and present some preliminary data on gene expression in the honey bee brain.

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Long-term plasticity and fear learning in adult heterozygous BDNF knockout mice

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The neurotrophin BDNF (brain-derived neurotrophic factor) has been shown to be an important mediator of synaptic strength and to be crucially involved in learning and memory processes. Several recent studies have demonstrated an important role of BDNF also in amygdala-dependent cued fear learning. Recently, we could demonstrate that heterozygous BDNF knockout ($BDNF^{+/-}$) mice exhibit a learning deficit in response to a weak fear conditioning protocol when animals became older than 3 months of age (Endres & Lessmann, 2012, Learn. Mem.). In order to analyze this learning deficit at the cellular level, we analyzed synaptic plasticity in amygdala slice preparations.

Since we previously showed impaired long-term potentiation (LTP) at the thalamic input to the lateral amygdala (LA) already in one month old $BDNF^{+/-}$ mice (Meis et al., 2012, J.Physiol.), we hypothesized that age-dependent changes of plasticity at cortico-LA afferents might cause the observed learning deficit. Therefore, we tested LTP at this input structure of the amygdala in 3-4 months old $BDNF^{+/-}$ mice and their wild type (WT) littermates. However, we observed unimpaired LTP in $BDNF^{+/-}$ mice at this synapse as well as at other intra-amygdala synapses, i.e. lateral-basal and basal-central amygdala synapses.

In order to better understand how fear learning alters synaptic plasticity in the LA, we performed *ex vivo* occlusion experiments. In fear conditioned WT mice, in contrast to pseudo-conditioned animals, LTP at cortico-LA synapses was occluded 24 h after fear conditioning training, stressing the fear learning relevance of our applied LTP paradigm. Interestingly, LTP at the same synapses was not occluded in fear conditioned $BDNF^{+/-}$ mice. This lack of occlusion 24 h after fear conditioning parallels the fear learning deficit in adult $BDNF^{+/-}$ mice. Currently we are testing occlusion of LTP at cortico-LA synapses 4 h after fear conditioning. First results show that LTP at this time point is also not occluded in adult $BDNF^{+/-}$ mice. This observation is paralleled by impaired fear memory in $BDNF^{+/-}$ mice at this time point. Interestingly, fear memory was still unimpaired 30 min after fear conditioning, indicating that the acquisition of fear memory is still intact in adult $BDNF^{+/-}$ mice.

In conclusion, our results suggest that in adult $BDNF^{+/-}$ mice the acquisition of fear is still intact, as indicated by intact short-term fear memory 30 min after fear conditioning. As we observed neither occlusion of LTP nor intact fear memory 4 or 24 h after fear conditioning, the observed learning deficit seems to result from an unsuccessful early consolidation process.

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Long-term potentiation in perforant-path dentate gyrus synapses of freely behaving rats requires activation of ß-adrenergic receptors

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Noradrenaline, released from the locus coeruleus, modulates hippocampus-dependent learning and synaptic plasticity. These effects are primarily mediated via ß-adrenergic receptors (ß-AR). The different hippocampal subfields differ in their responsiveness to ß-AR-regulation in vivo. In the CA1 region, long-term potentiation (LTP), but not long-term depression (LTD), requires ß-AR-activation, whereas in mossy fiber-CA3 synapses, neither form critically requires ß-AR-activation. However, synaptic plasticity that is facilitated by spatial learning depends on ß-AR in both hippocampal subfields.
Here, we investigated whether LTP in the dentate gyrus in vivo, that is induced by perforant path-stimulation, or LTP, that is facilitated by spatial learning, requires the activation of ß-AR. Interestingly, we detected that both forms of LTP are sensitive to ß-AR-modulation: receptor-antagonism in both conditions abolished the persistence of LTP and impaired spatial learning.
These findings suggest that LTP in the dentate gyrus critically requires activation of ß-AR. This dependency is likely to relate to the crucial role of the dentate gyrus as a gateway for the processing and synaptic encoding of novel experience by the hippocampus.

Mechanisms regulating CtBP1 nucleo-cytoplasmic functions

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C-terminal-binding protein 1 (CtBP1) is a dual-function protein with well-established functions in the nucleus as a transcriptional co-repressor and in the cytoplasm as a fission mediator. In neurons, CtBP1 is enriched in presynapses, but can also be observed in the nucleus and in the cytoplasm analogously to non-neuronal cells.
Using quantitative immunocytochemical stainings and live imaging of fluorescently tagged CtBP1 (FRAP and photoactivation), we demonstrated that the distribution of CtBP1 between the three pools is activity-regulated. We revealed that nuclear presence of activity-modulated CtBP1 plays a crucial role in the transcriptional regulation of a set of plasticity-relevant genes. Synaptic targeting and retention of CtBP1 is mediated by its direct interaction with the presynaptic scaffolds Bassoon and Piccolo, which is regulated by neuronal activity via modulation of cellular NAD/NADH balance. This interaction indirectly controls the nuclear pool size of CtBP1 by sequestering it at presynapses and thereby making it unavailable for nuclear import and transcriptional repression. In this way, CtBP1 might function as molecular messenger translating information about activity levels at presynapses into regulation of gene expression.
In the present study, we disected the signaling pathways contributing to control of synapto-nuclear shuttling of CtBP1. We identified the effects of calcium influx on CtBP1 nucleo-cytoplasmic translocation using the NMDAR agonist NMDA to stimulate mature rat cortical cultures. Quantitative immunostainings of CtBP1 showed the dependence of its nuclear export on NDMAR activation and presynaptic calcium influx. In addition, we could show that the activity-dependent CtBP1 nucleo-cytoplasmic shuttling only appears in excitatory neurons. Furthermore, inhibition of the two partially calcium dependent kinases PKA and PAK1 impairs the nucleo-cytoplasmic translocation of CtBP1 giving rise to the assumption that CtBP1 dynamics depend on the phosphorylation state of the protein.

Methylphenidate amplifies long-term potentiation in rat hippocampus involving ß-adrenergic and D1/D5 receptors and insertion of AMPA receptors

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Methylphenidate (MPH, Ritalin©) is widely used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and as a drug of abuse. MPH increases extracellular levels of dopamine and noradrenaline by blocking monoaminergic transporters. Recently, our group and others have demonstrated that MPH modifies synaptic plasticity in hippocampus. However, the cellular and molecular mechanisms involved in this process are still unknown. Using an electrophysiological approach and Western blot analysis we investigated the effect of MPH on Long-Term Potentiation (LTP) in rat hippocampus slices. 3-4 weeks old Sprague-Dawley rats were decapitated under halothane anesthesia, and hippocampus slices (400 µm thick) were prepared. LTPs were induced by applying theta burst stimulation (TBS, 5 trains, 100 Hz) at the Schaeffer collaterals and recorded in the striatum radiatum of the CA1 area. Superfusion of hippocampus slices during 20 min with MPH, significantly and dose-dependently increased the magnitude of LTP induced by TBS from 143.3 ± 3.1 % (controls; n=5,6) to 146.2 ± 2.8 % (3nM; n=3,3; p>0.05), 164.7 ± 10.4 % (50nM; n=5,7; **p<0.01), 194.3 ± 5.8 (5µM; n=6,8; ***p<0.001), and 196.4 ± 4.2% (50µM; n=4,4; ***p<0.001). The experimental data fitted a Hill curve with an EC50 of 73.44 ± 6.32 nM and a maximum response of 52.93 ± 0.63 %. Paired-pulse facilitation (PPF) curves remained unchanged after perfusion with MPH, suggesting that the effect of MPH does not involve modifications of presynaptic components. The increase induced by MPH was inhibited by the ß-adrenergic blocker timolol (5 µM), from 194.3±5.8% (TBS+MPH, n=5,7) to 152.7±1.7% (TBS+MPH+TIM; n=4,4; **p<0.01). A significant difference was observed in the ratio for 20 ms interstimulus intervals of the curves obtained before and after the MPH-induced LTP increase in the presence of TIM (1.40 ± 0.03 vs 1.54 ± 0.03, respectively; n=5,7; *p< 0.05), suggesting that the ß-adrenergic receptors may participate at a presynaptic level. Considering that MPH acts on the dopamine transporter DAT, we evaluated the participation of dopaminergic transmission in the effect induced by MPH. Interestingly, LTP increase was also inhibited by 5 µM of SCH23390, a D1/D5 receptor blocker, from 193.1 ± 8.0 % (TBS + MPH; n = 5, 7) to 144.4 ± 3.2% (TBS + MPH+ SCH: n = 5,7; *** p < 0.001). PPF experiments revealed no significant presynaptic contribution of the SCH23390 effect. To determine whether the facilitation of TBS-dependent LTP induced by MPH involves the insertion of new AMPA receptors in the post-synaptic membrane, we collected CA1 areas from hippocampal slices used in LTP experiments and performed Western blot analysis of the phosphorylation state of Ser845 and Ser831 residues in GluR1. CA1 areas from slices showing an enhanced TBS-dependent LTP after perfusion with 5 µM MPH exhibited a significant increase of 28 ± 6 % in phosphorylation of Ser845 residues (n=9,9; * p<0.05) compared to slices with TBS-dependent LTP without MPH. This modification was detected specifically on Ser845 residue and no significant change in the phosphorylation state of Ser831 was observed (n=9,9; *p > 0.05). The SCH23390 inhibited the phosphorylation of Ser845 induced by MPH (n=4,4; *p > 0.05).

These results suggest that the increase of TBS-dependent LTP caused by MPH in CA3-CA1 synapses is based on a polysynaptic mechanism involving ß-adrenergic and D1/D5 dopaminergic receptors promoting the insertion of AMPA receptors in the postsynaptic membrane through phosphorylation of

Ser845 residues in GluR1 subunits.

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Methylphenidate induces long-lasting metaplastic changes in the rat prefrontal cortex

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Methylphenidate (MPH) is extensively used therapeutically in children and adolescents suffering from Attention Déficit/Hyperactivity Disorder. Its influence on learning and academic performance, however, is disputed. Further, there is an ongoing discussion, but relatively few knowledge about its long-term effects. Recently, it has been shown that MPH acutely applied has metaplastic effects, i.e. it augments long-term potentiation in the hippocampus and prefrontal cortex in vitro.
We investigated whether metaplasticity induced by MPH is long-lasting and correlated with its influence on learning in a visuo-spatial behavioral test.
We combined a radial maze test and induction of Long-Term Potentiation (LTP) in the prefrontal cortex in vivo. Rats (PND 42) were tested for 15 consecutive days after having received injections ip of 0, 0.2, 1 and 5 mg/Kg each day of testing. Without further administration of MPH, LTP was induced 15-18 days after the last injection of MPH by electrical stimulation of the callosal commissures and recorded in the prefrontal cortex (L: 2,5; A: 5,0 mm).
Times to complete the task in the maze were reduced in animals that had received 1 mg/Kg MPH, but increased at 5 mg/Kg confirming earlier studies. The latter dose elicited behaviors that are likely to interfere with learning, such as excessive rearing and stereotyped movements.
LTP in the prefrontal cortex two weeks after the last MPH injection was strongly increased in the groups that had received 1 or 5 mg/Kg (from 20 to 80%, approx.), but was not significantly changed in the 0.2 mg/Kg group. In another group of rats that had received exactly the same treatment except for starting of the testing/injections one week later (PND 49), LTP after two weeks was undistinguishable from controls. Further, when rats from the former group (start of tests/injections at PND 42) were allowed to survive for 5 months longer the LTP enhancement had completely vanished. What is more, LTP was virtually absent.
We conclude that:
• At a dose of 1 mg/Kg learning is improved and LTP enhanced – compatible with a role of metaplasticity in improving test performance.
• MPH-induced enhancement of LTP (metaplasticity) lasts for at least two weeks.
• This LTP increase is abolished or even reverted 5 months later.
• The metaplastic effect must be induced not much later than in the sixth week indicating the existence of a sensitive period.

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Molecular mechanisms mediating the specific effects of BDNF-TrkB signaling in hippocampal neurons

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Brain-derived neurotrophin factor (BDNF) has been proposed to be a crucial factor in promoting neuronal survival, differentiation and activity-dependent synaptic plasticity in the central nervous system. Furthermore, BDNF and its receptor TrkB are supposed to play a relevant role in regulating neuronal architecture and spine morphology during postnatal development. Interestingly, BDNF has been shown to exert specific effects within different areas of the brain. Indeed, medium spiny neurons (MSN; interneurons) of the striatum show a strong impairment in their morphology while hippocampal or cortical pyramidal neurons (excitatory) are only slightly altered. However, the cellular and molecular mechanism mediating these differences in BDNF function has remained unresolved. To address this question we used a loss-of-function approach where BDNF was depleted in primary hippocampal neurons by using the Cre-LoxP recombination system. Here, we compared the neuronal architecture and spine morphology of excitatory and inhibitory neurons form primary hippocampal cultures. In parallel, we used an immunohistochemical approach to analyze the BDNF-TrkB signaling cascade concentrating especially on the activation of the TrkB receptor. The results suggest diverse levels of responsiveness to BDNF for different hippocampal neuronal populations. Indeed, measurements of the fluorescence intensity level for phosphorylated TrkB reveal a reduced TrkB phosphorylation within hippocampal interneurons. This reduction is accompanied by a significant decrease in dendritic complexity. In contrast, in excitatory hippocampal pyramidal neurons both the TrkB phosphorylation and the neuronal architecture show no differences when compared to control neurons. The analysis of the loss-of-function approach for BDNF indicates that while inhibitory neurons of the hippocampus are strongly impaired in their architecture, excitatory neurons within the same brain region only show a mild phenotype in their morphology. To confirm this hypothesis current experiments are performed by using a Synaptotagmin IV knock-out mouse as a BDNF gain-of-function approach. In this mouse model, specifically the activity-dependent release of BDNF is significantly increased. Taken together, our results reveal a crucial role for BDNF-TrkB signaling in regulating the neuronal architecture of postnatal inhibitory neurons. The mild effect of BDNF observed for the dendrites of excitatory pyramidal neurons in the hippocampus suggests the existence of a different molecular mechanism activating the TrkB receptor and by this means compensating the lack of BDNF signaling.

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Phenotypic synaptic plasticity in the brain of the nectar-feeding ant *Camponotus rufipes*

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In the polymorphic nectar-feeding ant *Camponotus rufipes*, media sized workers undergo an age-related division of labor: after an indoor period of 4-6 weeks (W. Gronenberg, *personal communication*), workers leave the nest as foragers to orientate and navigate in the environment. Foragers of *C. rufipes* are mainly night active, but are able to shift their foraging activity to daylight conditions (Jaffe and Sanchez, 1986; Acta Client Venez; Gronenberg et al., 1996; J Exp Biol). This remarkable seasonal plasticity in sensory foraging strategies is likely triggered by environmental cues (e.g. temperature, humidity and day length). We hypothesize that neuronal plasticity in the mushroom bodies (MBs), higher sensory integration centers in the insect brain, are involved to accommodate changes in sensory tasks associated with the transition from nursing to foraging and the plasticity in sensory foraging strategies. In the present study, we started to investigate how the factors age (as a baseline for further manipulations) and the exposure to light influence neuronal plasticity in the MB input regions, the calyces.

Using synapsin-immunolabeling for whole-mount brains, we combined volume measurements with the quantification of synapsin-positive presynaptic boutons in the calyx (Groh et al. 2012, J Comp Neurol). In particular, we investigated how the effect of age and the exposure to light affect microglomeruli, synaptic complexes in the olfactory MB calyx lip and visual calyx collar. Our results show an age-related volume increase of both calyx subregions (in constant darkness), while the density of synaptic boutons of both calyx subregions varies with ongoing age. In addition, we found that the density of MG significantly decreased in the collar, but not in the lip after exposing 28 day old dark reared ants to light (12 h LD/ 12 h DD) for one and four days. This light exposure effect is similar to the results obtained in *Cataglyphis fortis* and the honey bee *Apis mellifera* (Stieb et al. 2010, 2012; Dev Neurobiol; Scholl et al. 2014; Dev Neurobiol). Our results indicate that the high degree of structural plasticity is primarily driven by visual experience and may play an important role in the timing of behavioral transitions from nursing to foraging. In the future, we will analyze how changes in the ambient temperature influences the workers nest as well as foraging activity affect neuronal plasticity in the MBs. Supported by DFG SFB 1047 “Insect Timing” (B5, B6).

Role of Histone-Methyltransferases in Learning and Memory

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Epigenetic mechanisms have long been known to be crucially involved in learning and memory. Especially the role of histone acetylation in general, and individual histone acetylases and deacetylases in particular, has been thoroughly studied. However, in spite of extensive knowledge regarding the role of histone acetylation, that of histone methylation – another important epigenetic modification has remained elusive. Mice lacking histone methyltransferase myeloid/lymphoid or mixed-lineage leukemia 2 (Mll2/Kmt2b) in their forebrain have recently been shown to have memory impairment and downregulation of genes important for synaptic signaling and plasticity in hippocampal dentate gyrus (Kerimoglu et al., 2013). Here we report memory deficits in conditional knock-out mice lacking Mll1 (Kmt2a), a close homolog of Mll2, in the forebrain. In addition, absence of Mll1 also leads to de-regulation of synaptic plasticity genes in dentate gyrus and CA region of hippocampus. Interestingly, in spite of close homology of these two histone methyltransferases, gene expression repertoires dependent on their function are quite distinct from each other.

Role of the synaptic vesicle protein Mover at bushy cell synapses

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The cochlear nucleus (CN) contains conventional excitatory and inhibitory synapses, as well as highly specialized giant glutamaterigic nerve terminals called endbulbs of Held. There is considerable structural and functional heterogeneity among these axosomatic synapses, in particular with respect to short-term plasticity, but the molecules accounting for the features of these synapses are unknown. We found that a previously uncharacterized synaptic vesicle protein and binding partner of the presynaptic scaffolding protein Bassoon, called Mover, is localized to CN synapses. Using immunohistochemistry we studied the expression and localization of Mover at CN synapses.

In the ventral CN, we detected Mover at excitatory endbulbs of Held as well as at conventional inhibitory synapses contacting bushy cell somata. Quantitative analysis of fluorescence intensities revealed higher Mover levels at Vgat-positive terminals compared to Vglut1-positive terminals. Moreover, we found that Mover is heterogeneously distributed among endbulbs of Held, such that some endbulbs have significantly more Mover than others. In contrast, Mover levels were similar among inhibitory synapses. This suggests that Mover might confer synapse-specific presynaptic properties to endbulbs and thus account for their functional heterogeneity. We are currently testing this hypothesis using electrophysiological analysis of bushy cell synapses in acute slices from Mover knockout mice.

Spike timing-dependent plasticity: contribution of the number of spike pairings in synaptic modification at Schaffer collateral-CA1 synapses

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The canonical form of spike timing-dependent plasticity (STDP) is commonly induced by high repeat numbers of single pre- and postsynaptic spike pairings synchronized within a precise temporal window (~10ms). Positive time intervals (pre-post) induce long-term potentiation (LTP) and negative time intervals (post-pre) long-term depression (LTD). Most of the protocols use between 60 to 100 spikes pairings for STDP induction. However the physiological impact of the number of spike pairings has not been evaluated systematically so far. Taken into account that the spike itself might represent the computational unit for processing of information into the brain, the number of repeats has been underestimated in most studies about STDP.
The current study aimed at elucidating the influence of the number of spike pairing on the mechanisms for STDP induction at Schaffer collateral (Sc)-CA1 synapses. Briefly, STDP was induced with different numbers of spike pairings (i.e., 3, 6, 12, 25, 50 and 70 repeats) of one presynaptic action potential (AP), leading to an excitatory postsynaptic potential (EPSP) in combination with one postsynaptic AP (1EPSP/1AP) with a Δt= +10ms at low frequency (0.5Hz) in acute hippocampal slices. Here we show a novel 1EPSP/1AP protocol, which induced robust t-LTP with only six spike pairings (1EPSP/1AP_6X). We also observed, that this STDP paradigm provokes significant changes in short term plasticity at Sc-CA1 synapses, suggesting that this kind of plasticity is not dominated by presynaptic changes. Furthermore, our data show that different numbers of spike pairings may have a strong influence on the influence of different neuromodulators on successful STDP. It is known, that dopamine D1-like receptors play a role in dopaminergic signaling in CA1 region of the hippocampus. We previously demonstrated that STDP induced with 1EPSP/1AP_70X at Sc-CA1 synapses could be completely blocked using a specific antagonist for this D1 receptor (SCH23390, 10µM). However, when using only 6 repetitions of the 1EPSP/1AP_6X STDP protocol D1 receptor block was only partial. Moreover, recently in our laboratory, we have found that different numbers and patterns of spikes pairs cause changes in the locus (i.e. pre- vs. postsynaptic) and the molecular mechanisms leading to t-LTP expression at Sc-CA1 synapses (see Edelmann et al, poster presentation).
Taken together, these findings lead us to conclude that STDP can be induced with a large range of spike pairings that differentially activate molecular pathways leading synaptic modification, which means that the number of spike pairings might orchestrate the activation of essential neuromodulatory pathways for integration and processing of memories in the hippocampus.

Synaptic plasticity and the spine apparatus

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Calcium plays a critical role in synaptic plasticity by linking synaptic processes at the plasma membrane to the cytosolic compartment of the postsynaptic neuron. The spine apparatus is a structurally refined derivative of the endoplasmic reticulum that is found in large, presumably potentiated dendritic spines. The function of this organelle is still enigmatic. The complex spines of hippocampal hilar mossy cells and CA3-pyramidal cells, which are postsynaptic to the large mossy fiber boutons in the hippocampus, regularly contain a spine apparatus. Pursuing the hypothesis that the spine apparatus as an internal calcium store contributes to cytoplasmic calcium kinetics, we compared calcium transients in spines from wild type mice and spines from Synaptopodin-deficient mice, which do not form a spine apparatus. We used two-photon calcium imaging and two-photon glutamate uncaging to record calcium responses to various stimulus protocols in individual spine heads of patch-clamped hilar mossy cells in organotypic entorhino-hippocampal slice cultures. In wild-type spines as well as in spines from Synaptopodin knock-out mice we found a remarkable heterogeneity of the amplitude ratios of electrical postsynaptic events and the accompanying calcium transients, pointing to different states of mossy fiber plasticity at individual spine synapses that evolved spontaneously in the slice cultures. Even though there was a tendency to obtain larger maximal EPSP and EPSC amplitudes upon single pulses of optically released glutamate in wild type spines, the respective calcium transients were not different between genotypes. However, calcium transients that were elicited by strong stimulation using trains of glutamate uncaging pulses paired with back-propagating action potentials had smaller amplitudes and slower time constants in spines devoid of a spine apparatus when compared to wild-type spines. We hypothesize that the spine apparatus is not required for basal synaptic transmission but contributes to calcium transients during strong synaptic stimulation. We now aim to analyze synaptic plasticity at individual spines using glutamate uncaging as well as direct stimulation of the presynaptic mossy fiber bouton. We expect that these experiments will elucidate the contribution of the spine apparatus to synaptic plasticity at individual synapses.

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Synaptotagmin3 controls endocytosis of post-synaptic receptors to affect synaptic plasticity.

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Activity-induced regulation of surface post-synaptic receptor number is a well-known mechanism underlying synaptic plasticity, but the molecular machinery mediating this calcium (Ca2+)-dependent process is poorly understood.

In a synaptotagmin-pHluorin screen, we found that synaptotagmin 3 (syt3) undergoes Ca2+-dependent endocytosis in hippocampal neurons upon stimulation with high KCl, 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl) propanoic acid (AMPA), N-Methyl-D-aspartic acid (NMDA) or field stimulation, with kinetics similar to those of pHluorin-tagged glutamate receptors. Using biochemical approaches and a specific antibody against syt3, we found that syt3 is present at synapses, and is enriched on post-synaptic membranes.

Upon stimulation with AMPA or NMDA, syt3-overexpressing neurons internalize more GluA1 receptors than control neurons. No difference in GluA1 surface expression was observed in the basal conditions. Consistent with this observation, over-expression or knockdown of syt3 post-synaptically did not change the amplitude or frequency of miniature excitatory post-synaptic currents (mEPSCs). Acute hippocampal slice experiments are underway to test for changes in basal NMDA, AMPA and γ-Aminobutyric acid (GABA) receptor surface composition in syt3 knockouts. Surface cross-linking assays in stimulated brain slices will also reveal the role of syt3 in the activity-mediated internalization of these receptors.

Interestingly, syt3 undergoes endocytosis in 2 mM Ca2+ and exocytosis in 0.5 mM Ca2+, in response to stimulation. Its enrichment in the post-synaptic membrane suggests that it is spatially coupled to activity-mediated Ca2+ currents in the post-synaptic membrane. Blockade of NMDA receptors or L-type calcium channels partially blocked syt3 internalization, and blockade of both completely abolished internalization. Ca2+ sensing by the C2 domains of syt3 may effectively transduce post-synaptic calcium signals. In addition, recombinant syt3 C2AB bound to the endocytic adaptor protein AP-2, and to SNAP-47 and VAMP-3 SNARE isoforms, which may specifically mediate membrane fusion post-synaptically.

The internalization of AMPARs is necessary for the decay of early-long term potentiation (LTP) at hippocampal CA3-CA1 synapses. Consistent with syt3 internalizing receptors, early-LTP in syt3 knockout hippocampal slices failed to decay and persisted for hours. Additionally, the maintenance of late-LTD is reduced, while late-LTP is unaffected in syt3 knockouts. Behavioral tests are underway to test for defects in short- and long-term memory in the syt3 knockout mice.

In summary, we found that syt3 is involved in a post-synaptic mechanism of plasticity by which receptors are internalized following stimulation to promote decay of synaptic potentiation.

The APP interacting protein family Fe65 reveals a crucial role for synaptic function and plasticity

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The key role of the Amyloid Precursor Protein (APP) in Alzheimer´s Disease pathogenesis forming Aß plaques is well established, whereas its physiological function is poorly understood. The Fe65 protein family was shown to interact with APP. The family of adaptor proteins consisting of Fe65 and two homologous proteins (Fe65L1, Fe65L2) bind the highly conserved YENPTY-motif of APP thereby regulating its subcellular trafficking and proteolytic processing. The APP derived intracellular fragment (AICD) serves as an effector molecule. Several studies implicated Fe65 in AICD nuclear translocation and as mediator of gene transcription. Interestingly, loss of the full-length Fe65 isoform in aged mice is associated with impaired performance in learning and memory tasks similar to aged APP-knock-out (KO) mice. APP-KOs also have a defect in long-term potentiation (LTP). As the Fe65 and APP protein family show similar developmental expression patterns, are enriched in neurons and present in growth cones and synapses, we performed LTP recordings on different transgenic mice. The applied theta-burst stimulation (TBS) induces a LTP of synaptic strength at hippocampal Schaffer collateral synapses modelling their functional and structural changes occurring during learning and memory processes.
First we examined the role of the Fe65 proteins, as suggested downstream factors for APP, in synaptic transmission and plasticity in the rodent hippocampus, as well as possible compensatory mechanisms of its homologous Fe65L1. The recordings revealed that only the Fe65/Fe65L1 DKO resulted in strongly impaired LTP, while Fe65 or Fe65L1 single KOs showed only minor defects in induction and maintenance of LTP compared to littermate control mice. Analyzing basal synaptic transmission yielded no differences between genotypes with exception of altered presynapse functionality in Fe65L1 single KO mice. In a second approach we used mice in which the interaction site for Fe65 at APP is disrupted. Mice expressing an APP construct lacking the YENPTY motif on an APLP2 deficient background (double mutant, DM) were analyzed to circumvent a potential masking effect of the homologous APLP2 protein. The recordings revealed a significant LTP defect in the DM animals. These observations suggest that the unaltered LTP of APP-KI mice published in Ring et al., 2007, was due to the compensatory role of the homologue APLP2. These data suggest that as far as one of these APP family proteins have an intact YENPTY motif LTP remains unaltered. Furthermore the analysis of synaptic transmission points towards altered postsynapse function in the DM, while the presynapse seems to be unaffected. Moreover we analyzed Late-LTP (L-LTP) in the DMs which depends on protein expression and could be triggered through the interaction of AICD with Fe65. The L-LTP recordings revealed lower LTP levels compared to littermate controls.
Taken together our results suggest an important role for the APP/Fe65 protein family interactions in activity-dependent synaptic plasticity. We additionally showed that the homologues of both protein families are able to mask the LTP deficit seen in their corresponding double mutants. These findings strongly support the already detected similarities between the APP and Fe65 protein family and underscore their crucial relevance for synaptic plasticity. (supported by the DFG, FOR1332)

The Effect on the field potentials from the Hippocampus of Bad-Badý extract (Solanacea Species): is there a contribution on the memory?

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The aim of the present study was to investigate the effect on the field potentials recorded from hippocampal slices of the extract of Hyoscyamus reticulatus L of Hyoscyamus species of the Solanaceae family, which called Bad-Badý in eastern Turkey. As it well known, hippocampus is an important organ on the memory: the question is that is there a positive or negative contribution on the memory of the Solanaceae extract? The extract includes especially hyoscyamine and scopolamine which are alkaloid derivatives. Transverse slices of 500 micrometer thickness prepared from the hippocampus of young adult Wistar rats were incubated for at least 60 min in an artificial cerebrospinal fluid of the following composition (mM): NaCI 124, KCl 3, MgS04 1.25, CaCl2 2.5, KH2P04 1.25, NaHCO) 22, Glucose 10. The solution was continuously gassed at 33°C with a mixture of 95% O2 and 5% CO2 to attain a steady-state oxygenation level and maintain a pH of 7.4, The slices were super fused at I ml/min in a narrow chamber. Half-maximal population spikes were recorded in stratum pyramidal of area CA1 as averages of 8 sweeps. Recording electrodes were glass micropipettes filled with I M NaCI (resistance 8-10 M). Orthodromic stimulation of the Schaffer collateral pathway was performed at a frequency of 0.1 Hz (100 sec pulses) using a twisted pair of Teflon coated wires. All drugs i.e. the extract of plant Hyoscyamus reticulates L. From the family Solanaceae was purified in our chemistry and pharmacology department. The rate of yield was 15.63 % (15,63g extract from 100g Plant). The extraction was solved in DMSO (dimethyl sulfoxide, Sigma-Aldrich Chemie Gmbh, German) and diluted in artificial cerebrospinal fluid at various concentrations. By a special computer program the amplitudes were recorded as a relative, changing graphs and the size of the hippocampus field potentials. Measurements and recordings were performed every 5 minutes.

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The Effects of Albumin on Plasticity Changes in the Hippocampus and on Response to Antiepileptic Drugs in the Entorhinal Cortex

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Dysfunction of the blood-brain barrier has been shown to play a role in epileptogenesis following brain insult via leakage of serum albumin and transforming growth factor beta (TGF B) signalling. Expressional changes after activation of that signalling pathway suggested to be related with alterations of the network excitability in the brain. Pharmacoresistance is affecting 30 % of epileptic patients and the ratio increases in focal and partial epilepsies. Recent studies indicate that blood-brain barrer dysfunction is common among patients with pharmacoresistant epilepsy. We therefore evaluated: (1) the effect of brain exposure to serum albumin on homo- and heterosynaptic plasticity in the hippocampal network, (2) the effect of interstitial albumin on pharmacoresistance.

Extracellular recordings were performed in acute rodent entorhinal cortex-hippocampus slices, from CA1 stratum radiatum and stratum pyramidale and from medial entorhinal cortex. Albumin was applied intraventriculary 24 hrs before slicing in order to assess the effect of roboust transcriptional changes following the application. Schaffer collaterals (SC) were activated for homosynaptic plasticity experiments. Combined SC&Temporoammonic pathway or SC&alveus stimulation were used evaluate the heterosynaptic plasticity changes. Seizure-like events (SLEs) were induced by 4-Aminopyridine. Standard antiepileptic drugs (phenytoin, valproic acid, carbamazepine and Phenobarbital) were used in the presence of acute albumin or 24 hrs after its application. Unbound drug concentrations were quantified by ultrafiltration and high-pressure liquid chromatograpy.

SC-induced popspikes were more potentiated following 20 Hz stimulation and less depressed following LTD protocol. This effect was reversible by TGF ß receptor II blocker. Following repetitive high frequency temporoammonic pathway stimulation, SC-induced potentials were no longer reduced but enhanced in amplitude. There was no difference in the heterosynaptic interactions following combined SC&alveus stimulation. Antiepileptic drugs failed to suppress SLEs in the presence of albumin. This effect was not seen in rodents that were pretreated with albumin 24 hrs before the experiments suggesting the acute buffering effect of albumin on antiepileptic drugs.

These findings showed that following exsudation of albumin there are significant alterations in synaptic plasticity that might represent the transformation of the healthy tissue following an initial insult into a hyperexcitable state. A dysfunctional blood-brain barrier with acute extravasation of albumin could contribute to pharmacoresistance. The choice of an antiepileptic drug with low albumin-binding affinity may help in seizure control.

The neural cell adhesion molecule-associated polysialic acid regulates synaptic plasticity in the mouse prefrontal cortex

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The neural cell adhesion molecule (NCAM) belongs to the immunoglobulin superfamily of CAMs and mediates cell-to-cell and cell-to-matrix adhesion. The unusual glycan polysialic acid (PolySia or PSA) is added to NCAM by the polysialyltransferases ST8SiaII/STX and ST8SiaIV/PST. Alterations in the expression of NCAM and PolySia are associated with schizophrenia in humans and impaired hippocampal synaptic plasticity in mice. However, the role of PolySia-NCAM in synaptic plasticity in the medial prefrontal cortex (mPFC) remains unclear. Mice overexpressing the NCAM extracellular domain (NCAM-EC) show reduced perisomatic GABAergic innervation and long-term potentiation (LTP) in the mPFC (Brennaman et al., 2011). Using field EPSP recordings and whole-cell patch-clamp recordings in acute brain slices, we show that LTP in the mPFC depends on N-methyl-D-aspartate (NMDA) receptors and that mPFC LTP in NCAM-EC mice could be fully restored by the NMDA receptor agonist D-cycloserine and the glycine transporter type 1 inhibitor sarcosine. We further demonstrate that NCAM-deficient mice and ST8SiaIV-deficient mice, but not mice lacking ST8SiaII, exhibited similar deficits in mPFC LTP. In both NCAM- and ST8SiaIV-deficient mice, sarcosine rescued abnormal mPFC LTP. Notably, the enzymatic removal of PolySia in brain slices led to impaired mPFC LTP, unchanged NMDA/AMPA amplitude ratio, but increased signaling through GluN2B-containing NMDA receptors. Thus, these results suggest a key role of PolySia carried by NCAM in regulation of mPFC LTP and demonstrate the reversibility of synaptic deficits following genetic manipulations of these schizophrenia-associated molecules.

Brennaman LH, Kochlamazashvili G, Stoenica L, Nonneman RJ, Moy SS, Schachner M, Dityatev A, Maness PF. Transgenic mice overexpressing the extracellular domain of NCAM are impaired in working memory and cortical plasticity. Neurobiol Dis. 2011 Aug;43(2):372-8.

The role of metabotropic glutamate receptors in different forms of synaptic plasticity in vitro.

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The ability of neurons to modulate their structure and function is an underlying mechanism of learning and memory (Abraham and Bear, 1996). Long-term potentiation (LTP) and long-term depression (LTD) are both assumed to occur during the physiological processes of learning and memory formation and to sustain the latter (Abraham, 2008). It has been argued that metaplasticity refers to the plasticity of synaptic plasticity (Abraham and Bear, 1996; Abraham 2008), indicating a higher order level of plasticity (Abraham, 2008). A form of metaplasticity is depotentiation (DP), commonly seen as the mechanism by which “naïve” synapses that have recently undergone LTP can reset their synaptic strengthening to a more “efficient” state to store new information (Abraham, 2008). Bortolotto et al. (1994) first reported metabotropic glutamate receptors (mGluRs) as being involved in DP. Further experimental evidence indicated that both subtypes of group I mGluRs (mGluR1 and mGluR5) have distinct functions in synaptic plasticity in the hippocampal CA1 region (Gladding et al., 2008). However, their role in DP was not addressed yet in detail and appear to be distinct from those involved in NMDAR-dependent DP (Zho et al., 2002). Therefore, we investigated the mechanisms responsible for NMDAR and mGluR-dependent DP by combining electrophysiological recordings in vitro with a pharmacological approach.
Transverse hippocampal slices were prepared and maintained as described in Balschun et al. (1999). When we applied a standard DP protocol which consisted of LTP induced by a single theta burst stimulation (TBS, 100 Hz-2s) followed by DP (5Hz-2 min; Balschun et al., 1999) we found that the NMDAR competitive antagonist (D-AP5) and the group I mGluRs antagonists (YM 298198 and MPEP) failed to affect DP. To establish whether more robust DP protocols required a substantial NMDAR and mGluR activation, we tested 5Hz-LFS protocols of 3, 5 and 8 minute duration, applied 6 min after TBS-LTP induction. Ultimately, we established that the 8 min DP protocol yielded a pronounced DP. Therefore, this protocol was used as the standard for all subsequent measurements. Bath-application of the competitive NMDAR antagonist D-AP5 (50µM) immediately after the LTP-inducing TBS episode led to a significant impairment of DP. Application of the mGluR5 antagonist MPEP (40µM) did not alter the expression of DP, excluding a role of mGluR5 in DP. In contrast, the selective mGluR1 antagonist, YM 298198 (1µM) caused a marked enhancement of DP. These results point to a distinct stimulation-strength dependent involvement of NMDAR and mGluR1 in DP. While DP triggered by 2 min of 5 Hz is independent of the activation of NMDAR and group I mGluRs, DP induced by a strong 8 min protocol requires activation of NMDARs and is enhanced by concomitant inactivation of mGluR1. Interestingly, 1xTBS LTP was dependent of mGluR5 activation but did not require activation of mGluR1. Somewhat surprisingly MPEP failed to affect a strong LTD (3x LFS; Ahmed et al 2011), whereas YM did so. These contrasting data in conjunction with our DP studies suggest a reciprocal involvement of mGluR1 and mGluR5 in different forms of synaptic plasticity. Future work will disentangle the detailed interaction of NMDARs and group I mGluRs in these forms of synaptic plasticity.

Time-restricted impact of matrix metalloprotease 3 activity on long-term plasticity of NMDARs- mediated synaptic transmission and LTP in apical and basal dendrites in mouse CA1 hippocampal region.

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Matrix metalloproteinases (MMPs) activity plays an important role in learning and memory (Huntley, 2012, Wright and Harding, 2009). However, how different MMP subtypes contribute to neuronal plasticity remains poorly understood. We have recently shown that MMP inhibition interferes with long-term potentiation (LTP) of fEPSPs and population spike amplitudes within CA3 region of the hippocampus (Wójtowicz & Mozrzymas, 2014). Interestingly, while MMP-3 inhibition significantly impaired LTP induction and early phase time course, MMP-2/9 inhibition affected only late-phase of LTP in CA3-CA3 synapses (Wójtowicz & Mozrzymas, 2014). However, the mechanism underlying time-restricted and MMP subtype-specific actions with respect to LTP phases remains unknown. Since differences in LTP consolidation, early phase and magnitude were reported in basal vs apical dendrites in CA1 region (Kramar and Lynch, 2003) we initially compared the impact of MMP-3 inhibitor (NNGH, 10µM) on fEPSPs recorded in strata radiatum and oriens of CA3-CA1 hippocampal projection in mouse C57BL6 (P30-P50) acute brain slices. We found that in contrast to stratum radiatum, LTP evoked in stratum oriens was completely insensitive to MMP-3 inhibitor. In another set of experiments, we found that consolidation of fEPSP LTP evoked with high frequency stimulation (HFS, 4x100Hz) in apical dendrites was significantly attenuated when NMDAR antagonist APV (50µM) was applied not only during induction but also up to 15 minutes post LTP induction, confirming that NMDARs activity support LTP consolidation beyond the moment of induction. Taken into account that MMP-3 can cleave NR1 subunit of NMDARs (Pauly et al., 2008) and MMP-9 was implied in regulating lateral mobility of this receptor (Michaluk et al., 2009), we next studied the crosstalk between MMP activity and NMDARs function following LTP induction. To this end we studied the impact of MMP-3 and MMP-2/9 (SB3CT, 10µM) inhibitors on pharmacologically isolated NMDAR-mediated field potentials in the presence of AMPA/kainate receptor antagonist DNQX (20µM) and 0mM Mg^{2+}. We found that HFS resulted in robust LTP_{NMDA} that was completely abolished in the presence of NNGH inhibitor but not SB3CT. Moreover, MMP-3 inhibitor affected LTP_{NMDA} only when applied prior to or up to 15 minutes post LTP induction.

In conclusion, we suggest that MMP-3 driven modulation of NMDARs in the narrow time window of minutes following LTP induction may underlie the supportive role this MMP subtype has in LTP consolidation. The narrow time window required for MMP activity may be a general rule, since we previously found a similar effect of broad spectrum MMP inhibitor (FN439) on LTP in mossy-fiber-CA3 projection (Wójtowicz & Mozrzymas, 2010). This study also reveals different requirements for MMP-3 activity in LTP at Schaeffer collaterals to apical and basal dendrites in CA1 region of the hippocampus and further indicates the importance of MMP-3 activity in various forms of neural plasticity and likely information processing in the hippocampus. Supported by grants N N401541540 and 3/Pbmn.

Activation of the metabotropic glutamate receptor mGlu5 determines the direction of synaptic plasticity at mossy fiber – CA3 and associational/commissural – CA3 synapses

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Introduction: The direction of synaptic plasticity within the hippocampus is affected by spatial experience. The CA3 region plays a pivotal role in the processing of spatial information and hence spatial experience. CA3 receives two inputs formed by mossy fibers (MF) and associational/commissural fibers (AC) that are believed to in help in engaging working memory, pattern separation and pattern completion. However, it remains unclear, how information at these synapses is differentially encoded. We speculate that the metabotropic glutamate receptor, mGlu5, could be a key player, given its contribution in enabling synaptic plasticity in other hippocampal subfields.

Methods: Male Wistar rats (7-8 weeks, Charles River, Germany) were anaesthetized (Pentobarbital 52 mg/kg) and underwent chronic implantation of hippocampal electrodes, as described previously (Hagena and Manahan-Vaughan, 2010) to enable monitoring of evoked potentials at MF-CA3 and AC-CA3 synapses. Long-term depression (LTD) was induced by low-frequency stimulation (LFS) at 1 Hz and with 900 pulses. Long-term potentiation (LTP) was elicited using 4 trains of 100 pulses at 100 Hz. The mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP; Biozol) was dissolved in 5 µl of 0.9% NaCl and 1.8 µg was injected via the intracerebral ventricle. The specific mGlu5 receptor agonist (R,S)-2- chloro-5-hydroxyphenylglycine (CHPG, 0.5µg) was dissolved in 1 N NaOH and bought to the desired molarities with 0.9% NaCl.

Results: Antagonizing the metabotropic glutamate (mGlu) receptor, mGlu5, with MPEP inhibits LTP at MF-CA3 synapses, but not at AC-CA3 synapses. On the other hand mGlu5 antagonism prevents LTD at AC-CA3 synapses but not at MF-CA3 synapses. To further evaluate the finding that activation of mGlu5 preferentially leads to LTP at MF-synapses, whereas LTD is promoted at AC-synapses, we assessed the outcome of activation of mGlu5 receptors with a 50 Hz stimulation protocol. This frequency corresponds to Tm and depicts the activation threshold between LTP and LTD. 50 Hz stimulation in the presence of CHPG results in a synaptic potentiation of MF-CA3 but not of AC-CA3 responses.

Conclusion: The mGlu5 receptor may act as a switch that alters signal-to-noise ratios during information encoding and thereby supports a highly specific storage of information in CA3.

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Poster Topic

T9: Glia, Glia-Neuron Interactions

T9-1A A photoactivatble Cre system based on a caged tamoxifen analog for permanent genetic manipulation of cells in the brain
Sidney Cambridge, Betsi Flores, Ludovic Jullien, Alexandre Specht

T9-2A Activity-dependent regulation of sodium/bicarbonate co-transporter 1, NBCe1, in rodent hippocampus and cortex
Eleni Roussa, Magdalena Schrödl-Häußel

T9-3A Astrocytic Neuronal Lactate Shuttle: Fuel Substrate for Maintenance of Ionic Homeostasis in Rat Hippocampus
Eskedar Ayele Angamo, Uwe Heinemann

T9-4A Astrogliosis: changes in potassium buffering in Alzheimer's
Lana M Osborn, Lieneke Kooijman, Willem Kamphuis, Elly M Hol, Wytse J Wadman

T9-1B ATP sensing and dynamics in brain cells
Ulrike Winkler, Andrea Trevisiol, Pauline Seim, Yvonne Enzbrenner, Aiman S. Saab, Klaus-Armin Nave, Johannes Hirrlinger

T9-2B Characterization of panglial networks in barreloids of the murine juvenile thalamus
Lena Claus, Stephanie Griemsmann, Ronald Jabs, Christian Henneberger, Christian Steinhäuser

T9-3B Electrophysiological characterization of ion channels in astrocytes proliferating in response to acute brain injury
Stefanie Götz, Lars Kunz, Benedikt Grothe, Magdalena Götz

T9-4B Functional role of presynaptic NMDA receptors during the induction of long-term depression at neocortical L4-L2/3 synapses in juvenile rats
Florian B. Neubauer, Rogier Min, Thomas Nevian

T9-5B Heterogeneous gap junctional coupling of astrocytes in the auditory brainstem
Jonathan Stephan, Simon Wadle, Charlotte Bold, Philipp Naumann, Christian P. Moritz, Julia Langer, Christine R. Rose, Eckhard Friauf

T9-1C Impact of neuronal activity on glial energetic metabolites as determined with genetically encoded FRET-based nanosensors in organotypic mouse hippocampal slices
Iván Ruminot, Jana Schmaelzle, Alejandro San Martin, Sebastián Ceballo, L. Felipe Barros, Joachim W. Deitmer

T9-2C Lactate supports sodium homeostasis of neurons, but not of astrocytes, under metabolic stress in the mouse hippocampus
Daniel Ziemens, Claudia Karus , Christine R. Rose

T9-3C Microglia comprise functionally distinct cellular subsets with specialized phagocytotic capacity
Alexander Adalbert Götz, Angela Borisch, Martin Weber, Uwe-Karsten Hanisch

T9-4C Solulble Neuregulin-1 modulates disease pathogenesis in rodent models of Charcot-Marie-Tooth disease 1A
Robert Fledrich, Ruth M. Stassart, Klink A, Thomas Prukop, Tamer A. M. Abdelaal, Christine Stadelmann, Wolfgang Brück, Klaus-Armin Nave, Michael W. Sereda

T9-1D The role of myelin in cognitive processing: direction and strength of lateralization.
Livia de Hoz, Klaus-Armin Nave, Sharlen Moore

T9-2D The role of myelin in temporal and spectral processing in the auditory system
Sharlen Yared Moore Corona, Klaus-Armin Nave, Livia de Hoz

T9-3D The role of the monocarboxylate transporters (MCTs) in neurons and astrocytes in the uptake of ketone bodies
Linda S. Forero Quintero, L.F Barros, H.M. Becker, J.W. Deitmer

T9-4D Tight Junction Barriers in the Fiber Layer of the Fish Retina
Lidia Garcia Pradas, Corinna Gleiser, Andrea Wizenmann, Hartwig Wolburg, Andreas F Mack

T9-5D Water deprivation induced neuro-astrocytic interactions in both supraoptic and paraventricular nuclei of hypothalamus of brain Meriones shawi which improved the control of vasopressin on kidney water channel named aquaporines-2.
Abdeljalil ELGOT, Omar ELHIBA, Halima GAMRANI

A photoactivatble Cre system based on a caged tamoxifen analog for permanent genetic manipulation of cells in the brain

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With the advent of the optogenetic tools, light has proven invaluable for manipulation of cells in vivo. Several light-activated gene expression systems have been developed which unfortunately all suffer from very low efficiencies and thus lack the necessary robustness for in vivo applications. Based on the potent tamoxifen-inducible Cre-ERT2 system, a caged tamoxifen analog was synthesized to permanently mark and genetically modify cells in a variety of biological systems with high spatiotemporal resolution. In vitro experiments in cell culture demonstrated that brief irradiation with DAPI excitation light is sufficient for robust Cre-mediated induction of fluorescent reporter genes. We are now in the process to establish this tool for ex vivo and in vivo biological systems, including single cells in mouse brains using two-photon microscopy. Of course, light-dependent manipulation of cells in the living brain will be a very powerful method for a wide spectrum of applications and we plan to establish this tool for in depth characterization of microglia migration. Photoactivation of Cre will be used for constitutive expression of fluorescent proteins to permit long-term in vivo tracking and morphological analysis of individual microglia. Microglia exhibit a well-understood short-distance mobility as they locally direct and extend cellular processes, but their in vivo long-range migration has not yet been described thoroughly. Particularly in respect to Alzheimers disease, there is a high medical relevance to understanding microglial migration to and interaction with amyloid plaques.

Activity-dependent regulation of sodium/bicarbonate co-transporter 1, NBCe1, in rodent hippocampus and cortex

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Intracellular and extracellular pH in brain undergo transient changes during neuronal activity and large changes during seizure activity and during metabolic and respiratory disturbances. For neurons, regulation of intracellular pH is of particular importance because many ion channels are sensitive to both intracellular and extracellular pH. Subsequently, neural cells try to cope to maintain pH homeostasis by several mechanisms, among them via activation and regulation of acid-base transporters. The electrogenic sodium/ bicarbonate co-transporter 1, NBCe1, the product of the Slc4a4 gene, is a potent candidate to regulate intracellular pH and has been shown to lead to depolarization-induced alkalinization of both astrocytes and neurons. The aim of the study was to elucidate regulation mechanisms of NBCe1, identify putative underlying signalling pathways, and investigate the biological significance of NBCe1 under control conditions and after activity-dependent pH changes. Expression, regulation, and activity of NBCe1 was examined in acute hippocampal slices and primary cultured hippocampal and cortical astrocytes using the 4-AP (4-aminopyridine) model of epilepsy in vitro in the presence or absence of inhibitors for Src, ERK, and JNK signalling pathways. The results show activity-dependent upregulation of NBCe1 mRNA level and protein abundance in hippocampal slices, an effect that was abolished following inhibition of JNK signalling pathway. Seizure-like events increased membrane expression of NBCe1 as well, an effect that was not observed after inhibition of Src/ERK signalling. In cultured astrocytes, 4-AP treatment caused activation of astrocytes. Moreover, depolarization caused significant increase of NBCe1 protein that was prevented in the presence of either JNK or Src inhibitor. Inhibiting of Src signalling also affected depolarization-induced increased membrane expression of NBCe1. These data demonstrate regulation of NBCe1 transcript, functional expression, and activation after activity-dependent and depolarization-induced pH changes via Src/ERK and JNK signalling pathways.

Astrocytic Neuronal Lactate Shuttle: Fuel Substrate for Maintenance of Ionic Homeostasis in Rat Hippocampus

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The brain consumes about 20% of oxygen and 25% of glucose taken by the human body; and 50 % of this consumption is spent on recovery of transmembrane ionic gradient. The brain can utilize different energy substrates like pyruvate, lactate, glutamate etc. Even though the majority of ATP is produced through oxidative metabolism, glycolysis and glycogenolysis can serve as fast energy source in state of high neuronal activity. The astrocytic neuronal lactate shuttle hypothesis postulates that lactate produced in astrocytes gets shuttled to neurons through monocarboxylate transporter 2 (MCT2) during high energy demand. In this study, we evaluated the effect of lactate shuttle on ionic homeostasis and energy metabolism using the MCT2 inhibitor, 4-a-cyano- hydroxycinnamate (4-CIN) in rat hippocampus.

More precisely, stimulation induced Na^+, Ca^{2+} and K^+ transients were measured using Na^+, Ca^{2+} and K^+ ion sensitive microelectrodes respectively during 4-CIN application, while oxygen consumption was measured with Clark-style oxygen sensor microelectrodes. NAD(P)H and FAD signals, which are indicators of cytosolic and mitochondrial energetic metabolism, were monitored with live florescence imaging.

We found that application of MCT2 inhibitor resulted in prolongation of half decay time of stimulus induced potassium and sodium transients in CA3 stratum pyramidale region, indicating slower uptake of potassium and sodium efflux. Furthermore, it increased baseline extracellular potassium concentration with disturbance of ionic homeostasis. We did not observe any significant effect on calcium transients in both stratum pyramidale and radiatum region. Oxygen consumption also decreased during 4-CIN application; the drug quenched blue florescence rendering the NAD(P)H signal to be masked by an artifact. The sharp overshoot phase of FAD signal decreased but there is an overall baseline shift towards increased oxidation. 4-CIN has no effect on intracellular PH. High energy consumption by the Na^+/ K^+ ATPase pump could be the contributing factor for preferential disturbance of Sodium and potassium homeostasis; inhibiting the neuronal lactate transporter leads to decreased availability of energy substrate leading to decreased oxygen consumption. In conclusion, lactate shuttled to neurons partly meets the energy demand for ionic homeostasis.

Astrogliosis: changes in potassium buffering in Alzheimer's

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Alzheimer's disease (AD) is the main cause of dementia in the elderly and begins with a subtle decline in episodic memory followed by a more general decline in overall cognitive abilities. Though the exact trigger for this cascade of events remains unknown the presence of the misfolded amyloid-beta (Aß) protein triggers reactive gliosis, a prominent neuropathological feature in the brains of AD patients. It is likely that the physiological function of astrocytes is affected in AD, resulting in altered synaptic physiology and having consequences for the stability of microcircuits within key brain regions. Indeed, deficits in neuronal inhibition have been observed after viral induction of reactive astrocytosis in acute hippocampal slices of the mouse (Ortinski et al., 2010). We showed with microarray data from an aged mouse model of Alzheimer's that reactive astrocytes change from a neuro-supportive molecular phenotype to an immune-activated phenotype (Orre et al., 2014). Further analysis of our microarray data revealed crucial changes in astrocyte-specific genes associated with ion homeostasis including a global down-regulation of glutamate (GLT-1 and GLAST) and GABA (GAT-1) transporters, Na/K-ATPase (AMOG), and outward (TWIK-1 and TREK2) and inward rectifiers (Kir4.1 and Kir5.1). Further immunohistochemical analysis of KIR expression surprisingly revealed localized increases of channel expression in astrocytes directly surrounding amyloid plaque deposits. Currently, we are characterizing the electrophysiological changes in astrocytes occurring during different stages of astrogliosis in the APPswe/PS1dE9 mouse model focusing on potassium currents in the membrane using patch-clamp and field potential recordings. The functional implications of these changes on the surrounding neuronal network are studied in acute hippocampal slices made from mice at different ages. Our preliminary results suggest that functional changes in reactive astrocytes disrupt potassium homeostasis thereby perturbing normal neuronal and synaptic function. We hypothesize that astrogliosis is an important player in the development of dementia. If astrocytes have a modulatory role in normal neuronal communication, reactive astrocytes are likely to interfere with synaptic efficacy and plasticity.

ATP sensing and dynamics in brain cells

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To provide proper maintenance of brain function appropriate supply of energy is essential. Deficiency of energy delivery as e.g. during stroke or other injuries in the central nervous system will very quickly severely impair brain activity. However, while the consequences of energy depletion during pathophysiological events have well been documented, much less is known about physiological dynamics in energy load of brain cells. In general, ATP is the major energy carrier of cells and changes in the intracellular ATP concentration have been postulated to contribute to cellular signalling. Therefore, we first have addressed dynamics of intracellular ATP in primary cultured astrocytes by time-lapse microscopy using a genetically encoded fluorescent sensor for ATP expressed by transient transfection. The two major ATP producing pathways, glycolysis and mitochondrial respiration, contribute to maintenance of ATP content in astrocytes. The level of ATP in astrocytes decreased after application of the neurotransmitter glutamate which was also confirmed by biochemical determination of ATP content in these cells. The drop of ATP induced by glutamate was dependent on the activity of glutamate transporters, while other neurotransmitters like dopamine had only minor effects. Further we investigated ATP dynamics in acutely isolated tissue preparations from transgenic mice expressing the FRET-based ATP sensor driven by a neuron-specific promoter. The expression pattern of the sensor show labelled neurons in all brain regions including cortex, hippocampus and cerebellum as well as neurons in the retina. Preliminary results show an activity dependent modulation of intracellular ATP levels in cerebellar Purkinje cells and retinal ganglion cells. These results confirm the hypothesis that intracellular ATP levels are indeed subject to fast and reversible changes during physiological activity of at least two types of brain cells. The use of the genetically encoded fluorescent biosensor for ATP contributes to a detailed understanding of brain energy metabolism and is particularly suitable for further studies addressing factors which affect regulation of ATP.

Characterization of panglial networks in barreloids of the murine juvenile thalamus

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The thalamus plays an important role in relay and modulation of information to the cortex. The ventral posterior nucleus (VPM) is part of the somatosensory system and contains elongated cell clusters called barreloids. These barreloids receive sensory input from individual vibrissae and transmit their output to the corresponding cortical barrels. While the barrel cortex is well investigated, little is known about the properties of barreloids and their astrocytic networks. Astrocytes are connected via gap junction channels, which consist of connexins and allow small molecules to pass.

Recent studies have shown that astrocytes in the thalamus differ in various aspects from their counterparts in other brain regions. For instance, extensive panglial coupling between astrocytes and oligodendrocytes was more prominent in the adult thalamus compared to hippocampus (Griemsmann et al. 2014, Cereb Cortex doi:10.1093/cercor/bhu157). In the present study we developed a method to visualize barreloids in acute brain slices and investigated cell type distribution and glial coupling inside these functional compartments.

Investigation of glial coupling networks within the barreloids was achieved by modifying slice preparation. To improve visualization of barreloids in acute slices the cutting plane was optimized. We then combined electrophysiological, immunohistochemical approaches and electroporation to determine size, shape and cellular composition of barreloids and coupled networks. Experiments were performed in transgenic mice with astrocyte- or oligodendrocyte-specific fluorescence labeling (hGFAP-EGFP; PLP-GFP) and in wild type mice (C57/Bl6J) between postnatal days 14-17.

The optimal cutting plane to visualize barreloids in murine acute brain slices was roughly horizontal, tilted up anteriorly for 5 degrees and laterally for 30 degrees. Patch-clamp experiments with biocytin filling of astrocytes in hGFAP-EGFP and PLP-GFP mice demonstrated that the shape of glial networks in the VPM follows the barreloid structure. Barreloid borders restricted coupling and generated oval network shapes. Dye coupling was asymmetric when the initial tracer-filled cell was located close to a border. Oligodendrocytes and astrocytes equally contributed to the panglial coupling networks within the barreloids. Immunohistochemistry revealed a preferential location of oligodendrocytes close to barreloid borders. Neurons were homogenously distributed, although their processes were aligned in parallel to the barreloid axis. In contrast to cortical barrels where axons are located between barrels, axons of thalamic neurons mainly run within barreloids.

The finding that glial coupling networks in the VPM are orientated along barreloids raises the question of their dependency on neuronal activity. The functional impact of panglial coupling in the thalamus deserves further investigation.

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Electrophysiological characterization of ion channels in astrocytes proliferating in response to acute brain injury

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Glial cells are the most abundant cells in the brain and astrocytes represent one important subclass. They play a major role in brain injury and several neurological diseases by becoming reactive under these conditions. Juxtavascular astrocytes with their soma directly adjacent to blood vessels are prone to selectively proliferate after traumatic brain injury (TBI) in the somatosensory cortex of mice (Bardehle et al., 2013). We want to characterize the expression pattern of ion channels and their electrophysiological properties in juxtavascular astrocytes because it is known that ion channels and especially K^+ channels play important roles in cell-cycle progression and therefore division. Ion channel characteristics of juxtavascular astrocytes will be compared with that of non-dividing non-juxtavascular ones. We use the BAC Aldh1l1 eGFP transgenic mouse strain at the age of postnatal day 25-35, which expresses eGFP in astrocytes. In acute slice preparations of the somatosensory region of the cerebral cortex, we perform whole-cell patch-clamp recordings as well as immunohistochemical stainings to identify ion channel subtypes expressed by astrocytes. We have focused on inwardly rectifying K^+ (K_{ir}) channels, due to their contribution in maintaining the resting membrane potential and the redistribution of potassium across astrocytic membranes. We also concentrated on rapidly inactivating K^+(K_v) channels underlying the A-type current, because it is known that at least one subtype is present in hippocampal astrocytes. By means of immunohistochemistry we identified the channel subunits K_{ir} 4.1 and K_{ir} 6.2; as well as the K_v 4.3 channel giving rise to A-type currents. In accordance with immunohistochemistry, both juxtavascular and non-juxtavascular astrocytes show a reduction of K^+ currents and a depolarization after bath application of Ba^+, which is an effective blocker of K_{ir} 4.1 channels. Our study shows that ion channels known from well-studied hippocampal astrocytes are also expressed in those of the cerebral cortex. In addition, the same channel subtypes can be found in juxtavascular and in non-juxtavascular astrocytes in the same cortical region. This provides the opportunity for the future to study the proliferative role of these channel types in juxtavascular astrocytes when they become reactive in the cause of TBI. (The study was funded by SyNergy – the „Munich Cluster for Systems Neurology“).

Functional role of presynaptic NMDA receptors during the induction of long-term depression at neocortical L4-L2/3 synapses in juvenile rats

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Spike-timing dependent depression (t-LTD) at layer 4-to-layer 2/3 synapses in the developing primary somatosensory cortex is presynaptically expressed but depends on a chain of retrograde signaling events initiated at the postsynapse. We previously have shown that endocannabinoids which are postsynaptically released during the induction of t-LTD do not directly signal to the presynaptic membrane but rather act on cannabinoid receptors located on the third cellular synaptic element, the astrocyte. Activation of astrocytic cannabinoid receptors triggers an increase in astrocytic calcium activity leading to the release of glutamate from the astrocyte which, in turn, activates presynaptic NMDA receptors (pre-NMDAR). Hence, pre-NMDAR are at the interface between the postsynaptic/astrocytic events of LTD induction and the presynaptic signal transduction pathway which eventually leads to the presynaptic expression of LTD. Here we investigate whether calcium influx through pre-NMDAR is a necessary part of this presynaptic pathway. Making use of our finding that astrocytes are mediating the effect of endocannabinoids, we have established an experimental paradigm which allows us to image the putative calcium influx through presynaptic NMDA receptors. Bath application of 2-AG resulted in astrocyte-dependent induction of LTD, bypassing the requirement of timing-dependent activation of neurons. Using high-resolution two-photon calcium imaging and appropriate pharmacology we then monitored whether axonal boutons show pre-NMDAR-dependent calcium increases upon astrocyte activation.

Heterogeneous gap junctional coupling of astrocytes in the auditory brainstem

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Astrocytes form gap junctional networks, which participate in homeostasis by redistribution of ions and neurotransmitters within the syncytium. In the auditory brainstem, astrocytes are heterogeneously distributed and strongly aggregate in its nuclei. Quantitative mass spectrometry revealed connexin (Cx) 43 expression in various auditory brainstem regions. *Post hoc* quantitative western blotting and immunohistochemistry revealed different Cx43 levels in the superior olivary complex compared to the inferior colliculus (IC) pointing to a heterogeneity of astrocytic coupling between those regions. In order to analyze the degree of astrocyte coupling and the network shape in the lateral superior olive (LSO) and the IC, we utilized acute brainstem slices from C57Bl6 mice at postnatal days 6 to 20. Astrocytes were identified *a priori* by sulforhodamine 101-labeling before subjecting them to whole-cell patch-clamp recordings. The intracellular solution contained a gap junction permeable tracer and an impermeable fluorescent dye to label the network and the patched cell, respectively. After tissue fixation, the tracer was tagged with a fluorescent dye and the brainstem nucleus was marked immunohistochemically to define the network location. Additionally, we performed wide field sodium imaging in order to analyze the capability of ion redistribution within both regions of interest. The network size was age-dependent and differed between LSO and IC. Networks showed great variation regarding their size and were predominantly restricted to a single nucleus. Unexpectedly, networks originating from astrocytes located in the internuclear space between the LSO and the neighboring superior paraolivary nucleus extended into both nuclei. While astrocytic networks are almost circular in many CNS regions, those in central parts of LSO and IC were often oriented orthogonally to the tonotopic axis, while fewer networks were circular or oriented along the tonotopic axis. Electrical stimulation of individual astrocytes in both the LSO and the IC caused sodium elevations in their somata. Additionally, sodium elevations were consecutively present also in neighboring cells, indicative of a spread of sodium through gap junctions. Surprisingly, not only neighboring SR101-labeled astrocytes, but also SR101-negative cells responded to the stimulus. In turn, stimulation of such SR101-negative cells induced a sodium elevation in SR101-positive astrocytes as well as SR101-negative cells, confirming bidirectional coupling of both cell types. Taken together, our results demonstrate heterogeneous astrocyte networks in the auditory brainstem regarding their size and orientation. Moreover, these networks do not only include SR101-positive astrocytes, but apparently include additional cells, likely forming a panglial network. We assume that the morphological properties of the networks in the LSO and IC lead to a spatially restricted homeostasis and therefore to a limited “spill-over” of information towards neighboring isofrequency bands.

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Impact of neuronal activity on glial energetic metabolites as determined with genetically encoded FRET-based nanosensors in organotypic mouse hippocampal slices

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Little is known about the dynamics of energetic metabolites during neuronal activity, partly because the current isotopic techniques do not have enough spatio-temporal resolution to discriminate cell types. Attributable to the development of new genetically-encoded optical FRET nanosensors for glucose (Takanaga et al., Biochim. Biophys. Acta. 2008) and lactate (San Martin et al., PLoS One 2013), it is now possible to measure the dynamics of these energetic metabolites in single identified cells with a resolution of seconds. The aim of this work was to evaluate the impact of neuronal activity on the astrocytic glucose and lactate in mouse organotypic hippocampal slices. For this purpose, hippocampal slices were cultivated in a gas/medium interface (Stoppini et al., J. Neurosci. Meth., 1991). After 10 days, the slices were transfected with serotype 5 adenoviral vectors containing the sensors for glucose or lactate.

After 2 weeks of culture, the architecture and viability of the hippocampus was evaluated with Toluidine blue staining, and by Ca^{2+} and H^{+} imaging. The expression pattern of the nanosensors was cytosolic, and exclusively in astrocytes due the positive tropism of adenovirus to this cell type. The function and sensitivity of all sensors tested showed similar sensitivity for glucose and lactate to that observed with the nanosensors in vitro or expressed in cell cultures. The FRET-nanososensors were used to study the dynamics of glucose and lactate in astrocytes exposed to epileptogenic activity, high K^{+} and surrounding electrically-stimulated Schaffer collateral synapses. Our results reveal some complex changes of these metabolites attributable to their transport across the cell membrane and varying glycolytic activity.

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Lactate supports sodium homeostasis of neurons, but not of astrocytes, under metabolic stress in the mouse hippocampus

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Neurons and glial cells maintain a steep inwardly directed electrochemical gradient for sodium ions. This gradient is generated by the Na^+/K^+-ATPase and is essential for sodium-based electrical signaling of neurons, as e.g. required during action-potential generation. Activation of Na^+/K^+-ATPase following channel-mediated influx of sodium into neurons is in fact the major ATP-consuming process in the brain. There is strong evidence that astrocytes support neurons in this task by supplying them with lactate as a metabolite to generate ATP. This neuro-metabolic coupling between astrocytes and neurons might be especially relevant under conditions of hypo- or aglycemia because neurons, in contrast to astrocytes, do not possess own glycogen stores.

To test this hypothesis, we studied the consequences of glucose deprivation and/or inhibition of glycolysis on sodium homeostasis and regulation in neurons and astrocytes in the CA1 region of acute mouse hippocampal tissue slices, using fluorescence imaging with the sodium-sensitive dye SBFI-AM. Within 40 minutes after removal of glucose from the saline, baseline sodium concentration of neurons and astrocytes increased by about 1.2 mM and 4.0 mM as compared to time-matched control conditions (20 mM glucose). At the same time, the recovery from intracellular sodium transients, evoked by local application of D-aspartate, was slightly slowed in neurons, indicating a moderate reduction in Na^+/K^+-ATPase activity under these conditions. After about 100 minutes in glucose-free saline, recovery from D-aspartate-induced sodium signals was prolonged in both cell types, an effect, which was more pronounced in astrocytes.

Inhibition of glycolysis by NaF enhanced the effects observed after removal of glucose. Within 40 minutes, baseline $[Na^+]_i$ increased by about 21 mM in astrocytes and by about 4 mM in neurons as compared to control conditions. Decay time constants of D-aspartate-induced sodium signals in both neurons and glial cells nearly doubled. In neurons, the effects of inhibition of glycolysis were almost completely compensated by addition of the downstream metabolite lactate and baseline $[Na^+]_i$ and sodium transients were restored to the levels observed in 0 mM glucose. In contrast to this, lactate did not rescue sodium homeostasis in astrocytes during inhibition of glycolysis in glucose-free saline.

Taken together our data indicate that lactate can fully support efficient sodium extrusion under aglycemic conditions in neurons, but not in astrocytes in situ, strengthening the idea of a lactate shuttle between these two cell types.

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Microglia comprise functionally distinct cellular subsets with specialized phagocytotic capacity

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Microglia-mediated phagocytosis and clearance activities are essential for a proper CNS development and maturation of the neuronal circuitry as well as to guarantee tissue integrity and proper CNS functionality throughout life. Microglia can respond to virtually all homeostatic disturbances of the CNS, ranging from infection and injury to neurodegenerative and autoimmune processes. Microglia can thereby commit to a range of reactive phenotypes which are determined by the 'activating' stimuli as well as the situational context. Effective clearance of infectious agents, such as bacteria, is critical in fight of infection, while removal of tissue debris, such as damaged myelin, is required to facilitate repair attempts and remyelination. Yet it is still unclear whether the responding microglia consist of a uniform cell population with identical profiles of induced genes and effector functions or whether subsets contribute individual factors and actions to organize 'the reactive phenotype'. Here, we studied the capacity of mouse microglia to phagocytose DsRed-expressing *E. coli* DH5a and fluorescence-labeled myelin in vitro. We observed that clearance activities for both exogenous and endogenous materials are carried by subsets. Pre-stimulation of cells with bacterial lipopolysaccharide (LPS), a cell wall component of Gram-negative strains signaling through Toll-like receptor (TLR) 4, resulted in an increase in the population of *E.coli*-engulfing microglia, while concomitantly decreasing the subset engaging with myelin uptake. Deficiency in CD14 (in $cd14^{-/-}$ microglia), the prototypical TLR4 co-receptor, affected the *E.coli*-positive population, suggesting a further division of cells into those with CD14-dependent versus –independent mechanisms. Introducing conditions for a microglial MHCII expression by interferon-γ (IFNγ) treatment, an additional population split was observed. While IFNγ resulted in MHCII+ and MHCII- subsets, combination with a phagocytosis task resulted in cells which clear or do not clear the material in either combination with or without MHCII expression, as a molecular prerequisite for professional antigen presentation. Co-culture experiments revealed microglia double-positive for MHCII expression and myelin uptake as functional antigen-presenting cells (APC) which are able to activate myelin-oligodendrocyte glycoprotein (MOG)-specific T cells, as indicated by the release of pro-inflammatory cytokines (such as IL-2 and IL-12). Thus, at least a subset of microglia could serve as efficient APCs for myelin-specific T cells—a potentially dangerous activity with regard to autoimmunity. Characterization of typical microglial 'markers' and a deep sequencing approach, however, could not pre-identify actual functional orientations for myelin phagocytosis, suggesting that the organization of diversity relies on subtle mechanisms. Together the data demonstrate functional diversity among microglia by phagocytotic clearance, challenging a notion of an uniformly acting population.

Solulble Neuregulin-1 modulates disease pathogenesis in rodent models of Charcot-Marie-Tooth disease 1A

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A progressive loss of axons determines the clinical phenotype of chronic demyelinating peripheral neuropathies, such as the incurable Charcot Marie Tooth disease type 1A (CMT1A). Even though most affected patients seek medical advice in young adulthood, a moderate walking disability and electrophysiological abnormalities are usually already present in childhood. We found that Schwann cells in rodent models of CMT1A mount a cell autonomous dysdifferentiation program already during early postnatal myelination which is then sustained throughout life. Importantly, dysdifferentiation is associated with a disturbed balance of PI3K and ERK signaling. Here, we demonstrate that the soluble type I isoform of Neuregulin-1 (Nrg1) drives diseased Schwann cells towards differentiation via modulation of PI3K signaling. In a preclinical therapeutic trial, Nrg1 treatment ameliorates perturbed postnatal peripheral nerve development and restores nerve function in CMT1A rodent models. These findings support a model in which myelination independent Schwann cell differentiation is crucial for the maintenance of axonal support. Targeting Schwann cell differentiation may hence constitute a promising, common therapeutic approach in peripheral nerve disorders.

The role of myelin in cognitive processing: direction and strength of lateralization.

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The present project aims to understand better the role of fast and reliable conduction speed on cognitive processing. Myelin plays a crucial role in action potential propagation but the precise effect this has on cognitive processing is unknown. The corpus callosum is a highly myelinated path linking both hemispheres. It is necessary for the timed and precise co-ordination between the two sides of the brain. In the absence of a corpus callosum, the degree of handedness is strongly affected in rodents. We tested lateralization in mice without myelin using the paw preference task.

The paw preference test was first reported by Collins in 1974. Individual mice are put into a small cubicle that has a narrow open tube in the middle of one of the walls. The mouse is required to reach a pellet placed in the tube by introducing one paw into the tube. The paw used (right or left) is recorded and the direction and strength of the lateralization calculated over trials.

We first tested shiverer mice in this task. This mouse has a natural mutation of the myelin basic protein (MBP) gene that results in abnormal myelination in the absence of axonal degeneration. We found that while wt mice were strongly lateralized and had an equal distribution of paw preference in the population, shiverer mice were significantly less lateralized and had a tendency to be either ambidextrous or left handed.

The data suggest that stable lateralization is dependent on the presence of myelin and opens questions about the mechanisms underlying efficient lateralization and interhemispheric interactions.

The role of myelin in temporal and spectral processing in the auditory system

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This project aims to understand better the role of fast and reliable conduction speed on sensory processing. Myelin plays a crucial role in speeding up action potential propagation and axonal maintenance. Since the auditory system is highly optimized for the processing of stimuli that are encoded in time, we tested auditory processing in a mouse model (shiverer) with abnormal myelination in the absence of axonal degeneration. We recorded acutely from the primary auditory cortex of shiverer and wild type (wt) mice, while presenting them with different stimuli patterns to test frequency coding, frequency discrimination and temporal processing.

Frequency coding was tested presenting pure tones at different intensities. Frequency discrimination was assessed using stimulus-specific adaptation (SSA). SSA occurs when two frequencies, one highly repeated and one rarely presented, are different enough that adaptation to the constant one can occur in the absence of adaptation to the other. SSA is reflected in a larger response to the rare frequency and decreases with dF, the rate of stimulus presentation, and higher repetition of the rare frequency. Temporal processing was assessed using standard protocols of gap detection (for temporal acuity) and different click rates (for temporal reliability).

Shiverer mice had longer stimulus response latencies in all protocols as expected. Temporal processing was impaired: shiverer had higher gap detection thresholds and could not follow click rates as well as wt. This was presumably because of the relevance of the timing and refractory period within a path. Also, there were abnormally larger responses in shiverer with no obvious difference in jitter. However, frequency coding and discrimination was normal, probably because information is carried in parallel frequency lines.

The role of the monocarboxylate transporters (MCTs) in neurons and astrocytes in the uptake of ketone bodies

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The SLC16 gene family of monocarboxylate transporters (MCTs) comprises 14 isoforms, the first four of which (MCT1-4) mediate electroneutral co-transport of one high energy metabolite like lactate and ketone bodies with one proton. In the brain, MCT1 is expressed ubiquitously, MCT2 predominantly in neurons and MCT4 exclusively in astrocytes (Pierre, K. *et al.*, J. Neurochem., 2005). It has been suggested that MCTs play an important role in the treatment of refractory epilepsy in patients that follow a ketogenic diet (Lauritzen, F. *et al.*, Brain Struct Funct., 2013); some studies on animal models and humans have shown that ketone bodies are able to diminish the occurrence of seizures (Greene, A. *et al.*, J. Neurochem., 2003; Nehlig, A., PLEFA., 2003; McNally M. *et al.*, J.Neurochem., 2012). However, the molecular mechanisms by which ketone bodies reduce the incidence of seizures under ketogenic diet still remain poorly understood. In this study, we have characterized the transport of ketone bodies in primary neuron-astrocyte co-culture from mouse cerebral cortex using H^+ imaging. The intracellular proton concentration ($[H^+]_i$) was measured using live fluorescence imaging with confocal laser microscopy and SNARF-5F as proton sensitive probe. Determination of the Km value for lactate and ketone bodies as measured in both cell types indicated activity of MCT1 and MCT2 in neurons and MCT1 and MCT4 in astrocytes. On the other hand, we mimicked ketogenic conditions in the co-culture through the incubation of the cultures with 2 mM ß-Hydroxybutyrate (BHB) over three days. Astrocytes incubated with BHB showed an increase in the uptake of lactate and acetoacetate (ACA) in comparison with untreated astrocytes. However, no significant changes in the uptake of lactate, BHB and ACA in neurons were observed. From these results we hypothesize that the MCTs expressed in cortical astrocytes are up-regulated in the presence of high concentration of BHB in the extracellular environment, and that a similar scenario might be expected under ketogenic conditions.

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Tight Junction Barriers in the Fiber Layer of the Fish Retina

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The endfeet of Müller cells and the underlying basal lamina represent the interface between the retina and the vitreous humor in the vertebrate eye. However, these structures do not form a tight barrier. Investigating the vitreous-retinal interface in the eye of the cichlid fish *Astatotilapia burtoni* we discovered conspicuous tight junctions in the retinal nerve fiber layer using freeze fracture electron microscopy. These tight junctions formed branching strands between myelin-like wrappings of ganglion cell axons, and differed morphologically from any known myelin tight junction strands. In addition, we found elaborate meshwork of tight junction strands on large membrane faces belonging to glial cells in the nerve fiber layer. Immunohistochemical stainings against the adaptor protein ZO-1 labelled structures in the nerve fiber layer as did antibodies against the mammalian claudin-1. Claudins are integral membrane proteins found in every tight junction studied so far.
To further characterize the involved claudins of these tight junctions, we performed a PCR analysis to uncover expression of candidate claudins in the retina. Specific primers to *A. burtoni* predicted claudin sequences let us identify claudin-1, 3, 5a, 5b, 9 and 11 in the retina. Currently, we are testing whether these junctions form a barrier in the nerve fiber layer separating the vitreous from the neural retina.
The existence of these tight junctions in the fiber layer of teleosts in contrast to mammals is interesting because neurons and glial cells are continuously added from a circumferential peripheral proliferation zone in the fish retina. This implies that neuronal signaling occurs in close proximity to locations where processes of cell growth and proliferation have to be regulated. In the retinal periphery, the nerve fiber layer is missing or extremely thin. We speculate that the peripheral growth zone might therefore have access to growth promoting substances derived from ciliary blood vessels whereas in the central retina this might be prevented by the described tight junctions.

Water deprivation induced neuro-astrocytic interactions in both supraoptic and paraventricular nuclei of hypothalamus of brain Meriones shawi which improved the control of vasopressin on kidney water channel named aquaporines-2.

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Supraoptic (SON) and paraventricular (PVN) nuclei are part of the hypothalamic system, they constitute the main source for vasopressin (AVP) and they represent obvious examples of activity-dependent neuroglial plasticity. Under sever conditions of dehydration, AVP neurons, release AVP which stimulates the expression of the kidney water channel named aquaporines type 2 (AQP-2), necessary for the reabsorption of water and reduces significantly the dieresis. The aim of the present investigation is to clarify the underlying central and peripheral mechanisms allowing the desert rodent Meriones shawi to regulate its body water content and resist to dehydration. Thus, GFAP, AVP and AQP-2 immunoreactivities were used successively as activation indicators of astrocytes, AVP neurons and medulla kidney AQP-2. Hence, we studied the immunoreactivity in various hydration states: water ad libitum, one and three months of water deprivation. Our results showed that dehydration of Meriones induced a significant decrease of GFAP accompanied by a significant increase of AVP immunoreactivities, the latter concerns both cell bodies and fibers in the same hypothalamic nuclei SON and PVN. Peripherally, a significant increase of AQP-2 immunoreactivity in the medullar part of Meriones kidneys was simultaneously seen. These results show that both astrocytes and AVP neurons display a remarkable structural and physiological plasticity on both SON and PVN with an excessive release of AVP, which acts probably on AQP-2 allowing probably to Meriones a great ability to water retention. These various changes at both central and peripheral levels might be the basis of control of body water homeostasis, providing to M. shawi a strong resistance against dehydration.

Poster Topic

T10: Aging and Developmental Disorders

T10-1A A novel form of infant-onset mitochondriopathy
Bianca Hartmann, Hao Hu, Nadine Kraemer, Luciana Musante, Björn Fischer, Hans-Hilger Ropers, Thomas Wienker, Christoph Huebner, Angela M. Kaindl

T10-2A Advanced Paternal Age as a Risk Factor for Autism: Effects on Behavior and Brain Morphology in Rats and Humans
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T10-3A Autistic-like Behavior and Altered Neurotransmitter Levels in Mice Lacking the Post-synaptic Scaffolding Protein *SHANK1*
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T10-6A Ultrasonic vocalizations of neonatal rats prenatally exposed to valproic acid.
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T10-1B Early treatment of an M-channel epilepsy phenotype prevents disordered behavior, brain structure and activity
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T10-4B Modeling morphological and functional brain diseases by ablation of I(h) during development
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T10-5B MYELIN ABNORMALITIES IN MENTAL DISEASES: FOCUS ON MYELIN BASIC PROTEIN (MBP)
Giulia Poggi, Susann Boretius, Wiebke Möbius, Klaus-Armin Nave, Hannelore Ehrenreich

T10-6B Novel alternative splice variants of mouse *Cdk5rap2* cause a lack of microcephaly phenotype in conditional *Cdk5rap2 LoxP/hCMV Cre* mutant mouse
Nadine Krämer, Lina Issa-Jahns, Gerda Neubert, Ethiraj Ravindran, Olaf Ninnemann, Angela M. Kaindl

T10-1C Novel mid-hindbrain malformation, microcephaly, and intellectual disability
ETHIRAJ RAVINDRAN, Hao Hu, Nadine Kraemer, Olaf Ninnemann, Luciana Musante, Eugen Boltshauser, Detlev Schindler, Hans-Hilger Ropers, Thomas Wienker, Christoph Hubner, Angela M Kaindl

T10-2C Retracted

T10-3C Probiotic bacteria protects dopaminergic neurons in a rotenone model of PD in rats
Mohamed Moheb Elgamal, Mohamed Salama, Mohamed Alaa, Mahmoud Elkotb, Hussein Sheashaa , Mohamed Sobh

T10-4C Recombinant human Erythropoietin modulates neurogenesis and vasculogenesis in the hypoxic mouse brain during early development
Mandy Richter-Kraus, Susan Jung, Florian Brackmann, Regina Trollmann

T10-5C Reelin function in the adult dentate gyrus
Jasmine Pahle, Jo Kristin Welzel, Michael Frotscher, Bianka Brunne

T10-6C Synaptic Proteins And Their Relationship To Brain Aging In Male and Female Zebrafish (*Danio rerio*)
Michelle Marie Adams, Ayca Arslan-Ergul, Dilara Halim, Elif Karoglu, Bahriye Erkaya, Ferda Altaytas, Ozlen Konu

T10-7C The multiple roles of reelin in neuronal migration and layer formation: beyond the simplistic view
Nieves Mingo-Moreno, Rebecca Wallrafen, Jochen F. Staiger, Robin J. Wagener

T10-1D The expression of myelin-associated genes is reduced in mild focal cortical dysplasia
Catharina Donkels, Dietmar Pfeifer, Susanne Huber, Julia Nakagawa, Vera van Velthoven, Astrid Weyerbrock, Josef Zentner, Carola A. Haas

T10-2D Switched to T10-7C

T10-3D The NF2 tumor suppressor protein merlin in peripheral nerve regeneration
Alexander Schulz, Stephan L. Baader, Andrey Irintchev, Otto .W Witte, Helen Morrison

T10-4D Theoretical modeling of cerebral organoids: Microcephaly and the Griffiths singularity
Karen G. Petrosyan, Chin-Kun Hu

T10-5D Touchscreen-based visual pairwise discrimination and reversal learning in the primate brain aging model *Microcebus murinus*
Sandra Ammersdörfer, Daniel Schmidtke, Marine Joly, Elke Zimmermann

T10-6D Visuo-Spatial Paired Associate Learning (PAL) in a Strepsirrhine Primate (*Microcebus murinus*): New Insights into Early Primate Cognition from a Computer-Based Learning Task
Daniel Schmidtke, Sandra Ammersdörfer, Elke Zimmermann

A novel form of infant-onset mitochondriopathy

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Introduction:

Mitochondripathies are diseases caused by a defect in energy metabolism and therefore manifest predominantly as multi-systemic diseases. High-energy consuming organs such as muscle, heart, and the central nervous system are therefore primarily affected leading to symptoms such as muscle weakness, cardiomyopathy and intellectual disability. The prevalence of mitochondriopathies is estimated to be about 1:10.000. The majority of mitochondriopathies is caused by mutations of nuclear- rather than mitochondrial-encoded genes. Here we studied a consanguineous family with a new form of a mitochondriopathy.

Methods:

The causative homozygous gene mutation was identified by whole-exome sequencing and confirmed by Sanger sequencing. Histochemical and biochemical methods as well as electron microscopy and cell culture studies were performed using patient material (primary fibroblasts, muscle biopsy specimen).

Results and discussion:

Using whole-exome sequencing, we detected homozygous point mutations in a so-far not disease-associated gene in four children of a consanguineous family of Saudi Arabian descent with a novel mitochondrial disease. Main features include intellectual disability, motor developmental delay with muscle weakness, epilepsy, deafness, optic nerve atrophy, ataxia, and a myelination disorder. The mutated gene product is a nuclear-encoded, mitochondrially located protein. It is required for the maintenance of mitochondrial integrity, selective proteolysis, cell proliferation, and resistance against apoptosis. The function of this protein in brain and muscle still remains unknown. In the human fibroblasts of a patient, we detected a significant fragmentation of the mitochondrial network suggesting impaired mitochondrial dynamics as well as decreased proliferation rates. We are currently studying further pathomechanisms underlying the disease, also in light of putative interaction with other mitochondrial proteins. We are seaking for patients with a similar phenotype.

Advanced Paternal Age as a Risk Factor for Autism: Effects on Behavior and Brain Morphology in Rats and Humans

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It is widely recognized that advanced maternal age is a risk factor for bearing a child with mental retardation, such as Down syndrome. In contrast, however, few people are aware of the fact that advanced paternal age (APA) constitutes a risk factor for mental illness in offspring as well. Children born to older fathers have an increased risk of developing severe neurodevelopmental disorders, such as autism (ASD) and schizophrenia, as shown in a number of well-conducted epidemiological studies, with some of them even reporting accumulating risk across generations. This is particular relevant as ASD diagnoses have climbed steadily since the 1970s – along with a marked increase in the number of fathers older than 40 years in the past couple of decades (e.g. Germany: 10.5% in 2000, but 18.8% in 2012). It is estimated that approximately 10% of the increase in ASD diagnoses is due to APA. To study APA effects on brain and behavior, a large cohort of 670 healthy subjects was investigated with the schizotypal personality questionnaire (SPQ-B) and the NEO-FFI. It was found that APA had linear effects on SPQ-B sum scores and all of its subscales as well as neuroticism after controlling for maternal age, subjects' age, sex and level of education. In addition, APA was linearly correlated with increased grey matter volume in the right parahippocampal cortex and the right inferior frontal cortex in a subsample of 342 subjects, which is in line with reports of increased grey matter volumes in these brain areas in ASD. However, despite the fact that epidemiological studies demonstrated an association between APA and neuropsychiatric disorders, the underlying causality is not yet understood since experimental evidence in humans is not feasible. The development of animal models that allow environmental and genetic confounds to be controlled is needed in order to establish causality between risk factors and brain pathophysiology. Therefore, we recently developed a rat model, comparing offspring from young (2 months) and old (12 months) fathers, while maternal age was the same in both conditions (2 months). By means of this comparison, we found that rats from old fathers display behavioral alterations with relevance to all ASD core symptoms, including social communication deficits and impaired reversal learning. This finding indicates that at least part of the APA effects obtained in humans are not due to differences in personality traits or socio-economic status that have been repeatedly reported when comparing young and old fathers, but may be linked to reduced quality of spermatocytes due to epigenetic modifications or accumulating genetic deficits, i.e. mutations, as a consequence of "copy errors" during cell division, as recently suggested. Furthermore, rats' brain morphology was investigated at different developmental stages; since there is evidence for altered hippocampal size in ASD diagnosed patients.

Autistic-like Behavior and Altered Neurotransmitter Levels in Mice Lacking the Post-synaptic Scaffolding Protein *SHANK1*

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Autism spectrum disorders (ASD) are a class of neurodevelopmental disorders characterized by persistent social communication deficits across multiple contexts, together with repetitive patterns of behavior. Among the most promising ASD candidate genes is the *SHANK* gene family, including *SHANK1*. To study the contribution of *SHANK1* mutations to ASD symptoms throughout development, *Shank1+/+, Shank1+/-, and Shank1-/-* mice were compared in behavioral assays developed to detect social communication deficits and aberrant cognitive phenotypes as pups, juveniles, and adults. When assessing isolation-induced ultrasonic vocalizations as a measure for communication during early development, call rate exhibited the typical inverted U-shaped developmental pattern in all genotypes. However, *Shank1-/-* pups were found to be developmentally delayed and characterized by a less prominent inverted U-shaped call emission pattern, reflecting an overall reduction in ultrasonic calling. Those deficits in *Shank1-/-* mice were even more prominent when pups were tested for isolation-induced USV across various social contexts, including the exposure to maternal odors and odors from a stranger adult male. As juveniles, social approach and recognition were evident irrespective of genotype. In contrast, object recognition was affected by the *Shank1* deletion, with *Shank1-/-* mice being severely impaired, not showing a preference for the novel object. In addition, alterations in major biogenic amine levels, precursors and metabolite concentrations were found in various brain regions of juvenile *Shank1-/-* mice. In adulthood, *Shank1-/-* males and controls displayed normal social approach, but impaired social recognition. Object recognition was additionally impaired in adult *Shank1-/-* males. Conversely, adult *Shank1-/-* females exhibited deficits in social recognition only. In summary, the present findings indicate that *Shank1* deletions lead to communication deficits and an aberrant cognitive phenotype, together with age- and sex-dependent effects on social behavior.

BDNF Deletion in the cochlea/lower brainstem leads to central plasticity changes over age

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Tissue-specific deletion of brain-derived neurotrophic factor (BDNF) in the whole cochlea, dorsal cochlear nucleus, and inferior colliculus was found to be preventive against loss of auditory brainstem response (ABR) thresholds, ABR wave I amplitudes, and loss of inner hair cell (IHC) synaptic ribbons after exposure to traumatizing sound (Zuccotti et al., 2012). The present study aimed to assess if the deletion of BDNF in the cochlea/lower brainstem alters the vulnerability of hearing, sound processing, and cortical plasticity over age.

We compared the auditory function by using auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) measurements on young and aged conditional BDNF Pax2 KO mice. We also analyzed the influence of acoustic noise exposure on hearing loss in young and aged animals. We furthermore investigated plasticity dependent genes and patterns of perisomatic disinhibiton in the inferior colliculus and the auditory cortex of the KO mice and the respective aged-matched controls over age.

Analyses of tissue-specific BDNF KO mice over age showed profound differences in auditory function and noise vulnerability between KO mice and the controls. We discuss the results in the context of a differential role of BDNF for bottom-up / top-down circuits that may prevent vulnerability during aging.

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Marie Curie Research Training Network CavNET MRTN-CT-2006-035367, Deutsche Forschungsgemeinschaft, grant DFG-En294/2-4, DFG Kni316/4-1

CB1 receptor signaling influences astroglial morphology and distribution in the ageing hippocampus

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Astrocytes interact with neighboring glial cells, neurons and the vasculature through complex processes. They become activated in response to neuroinflammation, which may be indicative for the progression of neuroinflammatory process. Astrocytes are prone to become activated in the aging brain, characterized by enhanced processes and production of pro-inflammatory cytokines. This age-dependent hyperactivity of astrocytes may contribute to the dynamics of neurodegenerative processes in the aging brain. It is not fully known, however, which factors regulate astroglial activity during ageing. Previously we found that disturbed cannabinoid signaling between GABAergic neurons and microglia leads to enhanced microglia activity, which is associated with early onset of age-related changes in the brain. Here, we asked whether deletion of CB1 receptors from GABAergic neurons alters age-related changes in the distribution and morphology of astrocyte. In wild-type animals brain ageing is associated with a layer-specific increase in the GFAP-positive areas. GABA-Cnr1-/- mice show an exacerbated increase in the size of astrocyte processes and abnormal astrocyte distribution in the ageing hippocampus. Our results suggest that cannabinoid signaling on GABAergic neurons influences astrocyte activities in ageing.

Ultrasonic vocalizations of neonatal rats prenatally exposed to valproic acid.

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder of which symptoms are often evident from early infancy. In recent years, animal models have contributed significantly to our understanding of ASD and to the search for new therapeutic targets. In one particular animal model with a high translational validity, pregnant rats are injected at post-natal day 12.5 with a high dose of valproic acid (VPA; 500mg/kg), which yields an autistic-like phenotype in the offspring. Communicative deficits are among the main symptoms of ASD. In rodent models, early communicative behaviour can be assessed using a maternal separation paradigm. Pups that are taken from the nest vocalize in response to their isolation to notify their mother and facilitate retrieval to the nest. In this study, we evaluated maternal separation-induced ultrasonic vocalizations of six-day old pups after prenatal VPA treatment. The control pups produced a high level of ultrasonic calls independently on the testing environment. In contrast, in VPA-treated pups, we observed a reduction of USV`s when the pups were tested on the home cage bedding, while a novel environment provoked a high number of USV calls. These data demonstrate that male VPA-exposed pups show an atypical reaction to maternal stimuli, which can be interpreted as dysfunctional attachment behaviour. This context-dependent dissociation in communicative behaviour might be considered as a novel marker for early assessment of autism-like behaviour in rodents.

Early treatment of an M-channel epilepsy phenotype prevents disordered behavior, brain structure and activity

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The developing nervous system is especially vulnerable during critical developmental time windows. Insults in periods can produce long-term consequences, including neurological diseases such as epilepsy. We report a pharmacological intervention during a critical neonatal period, which prevents pathology in a genetic epilepsy model.

Mice with dominant-negative Kv7.2 subunits, which are in humans linked to neonatal epileptic encephalopathy, are particularly vulnerable to altered M-currents during early development. Normal Kv7 expression during the first two postnatal weeks produced no phenotype, even if M-currents were suppressed from week three onwards. Animals with dominant-negative Kv7.2 subunits expressed in early development, on the other hand, were hyperactive and prone to seizures. These mice exhibited disturbed hippocampal morphology, including astrogliosis and CA1 pyramidal cell dispersion.

We assessed early M-current's importance for network activity by performing in-vivo silicon probe recordings in awake neonatal (P6 to 11) control and mutant mice. Cortical activity included spontaneous events in the beta frequency (10 to 25-Hz) range in upper layers. Such "spindle burst" events occurred more frequently in mutants. Prominent neonatal hippocampal events included sharp waves and "stratum radiatum oscillations" of 2 to 5-s in the 15 to 30-Hz range; both were similar in controls and mutants.

We then tested whether we could prevent the disease phenotype by targeting treatment to the identified Kv7/M critical susceptibility period. Barbiturates and other GABA mimetics are used to treat neonatal seizures, but efficacy and prophylactic safety is debated. Instead, we administered the loop diuretic and sodium-potassium-chloride cotransporter (NKCC1) antagonist bumetanide, a proposed therapeutic option in neonatal epilepsy. Bumetanide reduces intracellular chloride concentrations of immature cortical neurons in vitro, attenuating GABA-mediated depolarization.

In Kv7/M-current-deficient mice, bumetanide treatment from P0 to P14 normalized neonatal in-vivo cortical spindle burst rates, prevented hippocampal structural damage, and restored wild-type behavior. Although in-vivo hippocampal firing rates at P10 to P11 were similar across groups, neonatal bumetanide treatment prevented mutant-specific alterations in spike train auto-correlation structure, including bursting.

We suggest prophylactically-safe interventions targeted to key developmental windows may be an effective strategy for protecting at-risk patients against disease pathology.

Functional analysis of novel collybistin missense mutations associated with intellectual disability

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Selected GABAA receptor subtypes are clustered at inhibitory synapses via interactions with the scaffolding protein gephyrin, which in turn is targeted to inhibitory synapses by collybistin, a GDP-GTP exchange factor with neuroligin-dependent activity. Collybistin harbours three key functional domains: a N-terminal regulatory src homology 3 (SH3 domain), a RhoGEF domain (that binds gephyrin and activates the small GTPase Cdc42) and a phosphoinositide-binding pleckstrin homology (PH) domain that is vital for correct membrane apposition. Collybistin-mediated clustering of gephyrin does not appear to be dependent on Cdc42, since collybistin mutants lacking any detectable GEF activity are able to induce submembrane gephyrin clustering. Rather, collybistin binding to phosphatidylinositol 3-phosphate (PI3P), a phospholipid found in cell membranes, is pivotal since a PH domain mutant (R303N/R304N) interfering with phosphoinositide binding abolishes gephyrin recruitment to synaptic sites. We have previously shown that genetic defects in the human collybistin gene (ARHGEF9) give rise to a range of symptoms consistent with loss of several GABAAR subtypes, including anxiety, seizures and intellectual disability. Here, we report the structure-function analysis of new collybistin missense mutations in patients with intellectual disability, affecting amino acids in the RhoGEF and PH domains. All mutations resulted in defective collybistin-mediated submembrane clustering of EGFP-gephyrin in recombinant systems. Mutant collybistin proteins co-localised with gephyrin in cytoplasmic aggregates. Although only one of the mutations identified (R356Q) is predicted to affect a PH domain residue required for binding to PI3P, pull-down assays from HEK293 cells transfected with pRK5-myc-collybistin revealed that all missense mutations investigated impaired binding to PI3P. Taken together, these results suggest that mutations in the either the RhoGEF or PH domains can impair binding of collybistin to PI3P, and that this is the most common pathomechanism for collybistin dysfunction in intellectual disability.

Gene Therapy in Fragile X Syndrome

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Fragile X Syndrome (FXS) is a genetic disorder causing cognitive impairment and is the leading known genetic cause of autism. The genetic defect is caused by an expanded CGG repeat in the 5' untranslated region of the FMR1 gene on the X chromosome. The FMR1 gene codes for Fragile X Mental Retardation Protein (FMRP). FMRP is an mRNA binding protein that controls translation of its bound mRNA substrates. The expanded CGG repeat causes an elimination or drastic reduction in the level of FMRP.
To determine if FMRP transgene expression in the central nervous system could reverse phenotypic deficits in the Fmr1-knockout mouse model of fragile X, we used a single-stranded adeno-associated viral (AAV) vector that contained a major isoform of FMRP. Transgene expression was driven by a neuron-selective promoter. The vector was delivered to the brain via a single bilateral intra-cerebroventricular injection into neonatal Fmr1 knockout mice. Transgene expression and behavioral assessments were conducted 3-4 and 7-8 weeks post-injection. Western blotting and immunocytochemical analyses of AAV-FMRP injected knockout mice revealed FMRP protein in the striatum, hippocampus, retrosplenial cortex and cingulate cortex. Cellular expression was selective for neurons and reached approximately 50% of wild-type levels in the hippocampus and cerebral cortex at 7 months post-injection. The pathologically elevated repetitive behavior and the deficit in social dominance behavior observed in PBS-injected Fmr1 knockout mice, were reversed in AAV-FMRP injected mice. These results provide proof-of-principle that gene therapy using a viral vector can correct specific behavioral abnormalities in a mouse model of fragile X syndrome.

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Modeling morphological and functional brain diseases by ablation of I(h) during development

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The hyperpolarization-activated cyclic nucleotide-gated nonselective cation (HCN) channels mediate I(h) and are important determinants of the biophysical properties of neurons. The channels consist of homo- or heterotetramers of HCN1-4 subunits. Changes in expression patterns, subcellular localization, or biophysical characteristics of HCN channels have been associated with neurological dysfunctions ranging from motor learning deficits to diseases such as epilepsy. Recently, *de novo* mutations in the *HCN1* gene in human patients have been linked to epileptic encephalopathy with concomitant neurological abnormalities, including autistic features, ADHD, absence of language, behavioral disturbances, ataxia, and delay of motor development (1).

By expression of a dominant-negative (non-conducting) HCN subunit (HCN-DN), we generated transgenic mice with a conditional and subunit-unspecific functional knock-out of I(h) at different developmental stages. HCN-DN expression under the control of either the EMX1 or CaMKII alpha promoters enabled prenatal or peri-/postnatal ablation of I(h) in forebrain projection neurons.

EMX1 promoter-mediated early prenatal ablation of the HCN/h current resulted in severe morphological abnormalities in the developing brain. Brain volume and cortex thickness were strongly reduced in HCN-DN-expressing mice. In contrast, CaMKII alpha promoter-driven early postnatal suppression of HCN channel-activity did not affect brain morphology, but resulted in behavioral abnormalities. HCN-DN mice displayed delayed somatosensory development with respect to sensorimotor reflexes, cognitive deficits in working memory and spatial learning and memory, as well as hyperactivity. Post-weaning onset of HCN-DN expression resulted in slight learning and memory deficits, but no hyperactivity.

Our results demonstrate distinct roles of HCN/h-channel activity during pre- and postnatal development of the central nervous system of the mouse. The different age-dependent developmental phenotypes observed in our I(h)-deficient mice may provide a model to investigate the range and variability of neurological dysfunctions associated with *de novo HCN1* mutations in human patients.

(1) C. Nava et al. (2014), *De novo* mutations in *HCN1* cause early infantile epileptic encephalopathy, Nature Genetics; doi:10.1038/ng.2952

MYELIN ABNORMALITIES IN MENTAL DISEASES: FOCUS ON MYELIN BASIC PROTEIN (MBP)

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During the last two decades, gene-expression studies, proteomic studies, association studies, analyses via electron microscopy and imaging have consistently reported myelin abnormalities in mentally ill patients. Among several myelin-related molecules, Myelin Basic Protein (MBP) has become more and more appealing for the attempt of understanding the role of myelin abnormalities in neuropsychiatric diseases. A reduction of about 30-40% of MBP expression has been detected in the left enthorinal cortex of schizophrenic patients and a recent study, based on convergent functional genomic, has reported MBP as one of the top candidate genes for schizophrenia. Moreover, a diminished level of MBP has been discovered in post-mortem brain of bipolar and depressive patients compared with controls. MBP is one of the major myelin structural proteins. It accounts for almost 30% of the total protein content of myelin in the CNS and it is crucial for the opportune apposition of the cytoplasmic surface of compact myelin layers. Due to its essential role in the correct formation of the myelin sheath, it is plausible that a reduction in MBP expression could be detrimental for the brain connectivity and functionality, hence leading to the appearance of specific symptomatology reported in mental diseases. In order to clarify the role of the reported anomalies, we focused our attention on the characterization of a mouse line expressing only 50% of MBP, i.e. the heterozygous strain of shiverer mice. Through the combination of several methods (electron microscopy, imaging, spectroscopy and behavioral and molecular analysis), we managed to take a step further in unraveling the role of the reduction of MBP in mental diseases.

Novel alternative splice variants of mouse *Cdk5rap2* cause a lack of microcephaly phenotype in conditional *Cdk5rap2 LoxP/hCMV Cre* mutant mouse

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Autosomal recessive primary microcephaly (MCPH) is a rare neurodevelopmental disorder characterized by a pronounced reduction of brain volume and intellectual disability. A current model for the microcephaly phenotype invokes a stem cell proliferation and differentiation defect, which has moved the disease into the spotlight of stem cell biology and neurodevelopmental science. Homozygous mutations of the Cyclin-dependent kinase-5 regulatory subunit-associated protein 2 gene *CDK5RAP2* cause MCPH3. To further characterize the pathomechanism underlying MCPH, we generated a conditional *Cdk5rap2 LoxP/hCMV Cre* mutant mouse. Further analysis, initiated on account of a lack of a microcephaly phenotype in these mutant mice, revealed the presence of previously unknown splice variants of the *Cdk5rap2* gene that are at least in part accountable for the lack of microcephaly in these mutant mice.

Novel mid-hindbrain malformation, microcephaly, and intellectual disability

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During development, the mid-hindbrain, comprised of brainstem (midbrain, pons, medulla oblongata) and cerebellum, forms at the anterior part of the neural tube. The latter is divided into transient segments, or rhombomeres, through localized expression of genes. This segmentation is highly important for the proper development of various brain regions, and several proteins are known to contribute to localized gene expression. Therefore, developmentally related structures are often co-affected in diseases that are associated with early midbrain-hindbrain developmental disorders.
In this study, we report on a novel mid-hindbrain malformation phenotype in two affected children of a consanguineous family of Kurdish-Turkish descent. The phenotype of our patients includes facial dysmorphism with long eyelashes, horizontal pendular nystagmus, strabismus, hyperopia, retinal dystrophy, muscular hypotonia, and disturbance in fine motor movements. Intellectual deficit (IQ <50) and speech delay was also observed. Cranial magnetic resonance imaging (MRI) revealed microencephaly, hypoplasia of the pons and grooves, and caudal vermis or cerebellar hypoplasia in patients. We identified a homozygous nonsense mutation in a gene not linked to any disease so far and found both mRNA and protein levels to be reduced in patient samples when compared to controls. This indicates a nonsense-mediated RNA decay. We further identified abnormal spindle morphology, reduced cell size, and reduction of neurogenesis with an increase in the precursor cell pool. These processes likely contribute to the microcephaly phenotype in our patients. We are currently further studying the pathomechanism underlying the novel disease.

Probiotic bacteria protects dopaminergic neurons in a rotenone model of PD in rats

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OBJECTIVE:Our proposed hypothesis was that the decline in sex hormones in ageing process may represent the major player in Parkinson's disease (PD)pathogenesis based on a wealth of researches showing some protective effects for sex hormones administration to PD patients/animals.
BACKGROUND:Great advances in understanding the pathogenesis of PD have been achieved in the last 3 decades . Since most of PD cases are encountered in elderly, a positive relation between ageing and PD has been suggested.
DESIGN/METHODS:The study design included 120 C57BL/6 mice half of them are old age with mean 1.5 years ,other half are young age with mean 3 months and each half subdivided according sex difference to males and females . They were divided into 6 equal groups. The 1st received daily intraperitoneal injection of 0.5% carboxymethyl cellulose (CMC)3mL/kg for 35 days. The 2nd group undergone surgical operations , castration for males and oophorectomy for females .The 3rd group received Rotenone suspended in 0.5% CMC intraperitoneally at a dose of 3 mg/kg, daily for 35 days . The 4th group received the same rotenone regimen plus daily intraperitoneal hormone replacement therapy (HRT), for male Testosterone Enanthate at a dose of 500 µg and for female Estradiol Benzoate 50 µg/kg daily for 35 days . After same surgical operations regimen by 2 weeks, the 5th group received same rotenone regimen for 35 days & the 6th group received same rotenone and HRT regimens for 35 days . All animals were evaluated regarding locomotor disturbance through a video tracking system(Anymaze@ StoeltingTM) . After 35 days the animals were sacrificed and their brains were evaluated biochemically and by immunostaining against ant-TH antibodies.
RESULTS:Preliminary results showed significant decline in behavioral performance of sex hormone deprived groups (both young and old)
CONCLUSIONS:Primarily, behavioral assessment showed positive results suggesting putative role for sex hormone deprivation regardless age, this data, however, will be confirmed by postmortem tissue analyses.(on going).

Recombinant human Erythropoietin modulates neurogenesis and vasculogenesis in the hypoxic mouse brain during early development

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Hypoxia-inducible transcription factors are characterized as the most important adaptive mechanisms modifying the degree of hypoxic-ischemic lesions of the developing brain during early stages of cerebral maturation. Considering future neuroprotective therapeutics, most studies focus on effects on hypoxia-induced pro-apoptotic mechanisms of the immature brain. In addition, crucial hypoxia-induced disturbances of early neuronal and vascular development are important to consider in the context of pharmacologic activation of endogenous neurotrophic systems. Thus, we aimed to investigate in vivo effects of recombinant human erythropoietin (rhEPO) on vasoactive and vasoproliferative factors modulating cerebral vasculogenesis, neuronal proliferation and migration in an established mouse model of neonatal acute systemic hypoxia.

To this end, neonatal C57BL/6NCrl mice (P7) were exposed to systemic hypoxia (8% O2) for 6h followed by rhEPO treatment (2500/5000 IU/kg, ip) at the end of the incubation period, as well as upon 24h and 48h. After a reoxygenation period of 72h mRNA expression (TaqMan RT-PCR) of neurotrophic and vasoactive factors (VEGF-A, VEGFR-1, VEGFR-2, Nrp-1, Nrp-2, ANG-1, ANG-2, TIE-2) as well as cell-type and region-specific expression patterns (parietal cortex, hippocampus; RNA In-situ-Hybridization) were investigated in comparison to vehicle-treated normoxic and hypoxic controls. Vascular development was assessed by PECAM-1/CD31 immunofluorescence staining focusing on vessel length, branching and area (AxioVision, Imaris). Additionally, neuronal proliferation was assessed by Bromdesoxyuridin (BrdU), neuronal maturation by Doublecortin (DCX) and NeuN immunofluorescence staining.

Under normoxia, there were no significant changes of cerebral mRNA expression of neurotrophic and vasoactive factors due to rhEPO in comparison to controls. However, rhEPO led to a significant reduction of ANG-2 mRNA levels, and consequently, to an increase of ANG-1/ANG-2 mRNA ratio in favour of vessel stabilizing ANG-1. Furthermore, rhEPO induced a significant increase of cortical vessel length, branching and area associated with significant activation of VEGF-A mRNA expression (up to 300%). In contrast, hypoxia-inducible VEGF-A mRNA expression was reduced due to rhEPO treatment under hypoxia compared to normoxia ($p<0.001$). In comparison to controls, rhEPO induced a significant increase of ANG-1/ANG-2 mRNA ratio (3.5-fold; $p<0.001$) under hypoxia. Moreover, rhEPO led to a dose-dependent significant increase of DCX-positive signals in the subventricular zone ($p<0.001$) and the dentate gyrus ($p<0.01$) under hypoxia. We observed also a rhEPO dose-dependent increase of BrdU-NeuN-positive cells in the cortex as well as in the hippocampus under hypoxia (up to 8.2-fold, $p<0.001$) and normoxia (up to 4.7-fold, $p<0.001$).

Present results implicate protective effects of rhEPO on neuronal proliferation and cerebral vascular development under acute systemic hypoxia in the developing mouse brain. Alternative signaling pathways independent from the HIF system seem to be involved in rhEPO-induced regulation of VEGF-A-, ANG-1 and ANG-2, as well as neuronal proliferation. Based on present data, we conclude that, especially during regeneration processes of the immature brain, comprehensive analysis of specific mechanisms modulating vasculogenesis and neuronal maturation are an important prerequisite to assess pharmacological neuroprotective treatments.

Reelin function in the adult dentate gyrus

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Reelin is a large secreted glycoprotein which is mainly known for its important role in neuronal migration during brain development. In reeler mice lacking this signaling protein, neurons fail to reach their final positions, leading to severe malpositioning and morphological alterations of neurons throughout many brain regions including the dentate gyrus. In addition, more recent publications have shown the importance of Reelin in the adult brain and its involvement in various diseases like schizophrenia, depression and epilepsy. Until now the function of Reelin in the adult brain is not well understood. It has been associated with the stabilization of neuronal architecture, synapse modification, long-term potentiation and adult neurogenesis. However, due to the severe developmental defects in the reeler mutant, it is difficult to distinguish between direct effects of Reelin in the adult brain and indirect effects caused by the overall disturbed anatomy of the brain. To address this problem we used a conditional Reelin knockout mouse, which was kindly provided by Hans H. Bock (Düsseldorf) and Joachim Herz (Dallas), in this study. To switch of Reelin expression selectively in the adult brain but not during development, we took advantage of the different expression patterns of Reelin at these two stages. While Cajal-Retzius cells in the outermost layers of various brain areas are the main Reelin source during embryonic development, at early postnatal stages interneurons take over and become the main Reelin source in adulthood. Based on this, we combined this mouse line with the floxed Reelin allel with a transgenic mouse line expressing cre recombinase under the control of an interneuron specific promotor (Dlx5/6 cre), originally published by Monory et al (Neuron 51, 2006, pp 455-466). Here we show, that this conditional mouse model indeed undergoes normal brain development and lacks Reelin selectively in the late postnatal and adult brain. Using this new model we were able to study the role of Reelin in the stabilization of neuronal architecture and in adult neurogenesis independently of developmental defects. (Supported by the DFG: FR 620/12-1)

Synaptic Proteins And Their Relationship To Brain Aging In Male and Female Zebrafish (*Danio rerio*)

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Evidence suggests that the brain is sexually dimorphic in zebrafish (*Danio rerio*). However, whether the zebrafish brain ages in a sexually dimorphic manner is not well-established and just beginning to be examined. The aim of the current study was to determine the pattern of age-related changes of three key synaptic proteins associated with excitatory and inhibitory synapses, pre-synaptic vesicle protein, synaptophysin, post-synaptic density-95 protein, and gephyrin protein. We hypothesized that these levels would likely decline but the pattern of aging would be different in male and female zebrafish. Whole brain tissues were isolated from young (6-8 months), middle-aged (12-14 months) and old (27-30 months) male and female zebrafish (AB strain). Animals were maintained and raised in standard conditions in the zebrafish facility at Bilkent University, BilGen Genetics and Biotechnology Center, Ankara, Turkey. The extracted brain tissue was homogenized in RIPA buffer and subjected to Western Blot analysis to determine differences in the relative protein expression levels. Our results demonstrated that there was a significant decline in synaptophysin protein levels with age, as well as a significant age by gender interaction. We found that females tend to preserve synaptophysin levels in old age, whereas in males synaptophysin levels are reduced in old age. For gephyrin, we observed a significant main effect of gender with females having more gephyrin than males. Finally, for post-synaptic density-95, we found no significant main effect of age or gender but there was a significant age by gender interaction. Our data revealed that in females post-synaptic density-95 levels were preserved in older ages as compared to males. Thus, our data suggest that selected synaptic protein levels decline with age and might be differentially expressed according to gender. In addition, the state of these protein levels during aging depends on the sex of the animal. Therefore, both genders should be examined to determine whether these alterations in synaptic proteins affect synaptic function, especially across lifespan.

The multiple roles of reelin in neuronal migration and layer formation: beyond the simplistic view

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The adult mammalian neocortex is a six-layered structure divided into specialized areas, well-defined by a distinct cytoarchitecture and specific wiring patterns. During development, at approximately embryonic day 13, a wave of newborn neurons migrate from the ventricular zone towards the outer brain surface, splitting a preexisting neuronal layer named preplate. Subsequent cohorts of postmitotic neurons migrate and bypass preformed layers within the forming cortical plate, shaping the neocortex in an “inside-out” fashion. The secreted extracellular glycoprotein reelin, which binds to ApoER2 (apolipoprotein E receptor type 2) and VLDLR (very low density lipoprotein receptor) receptors, plays a key role during corticogenesis, orchestrating neuronal migration and neuronal maturation. However, the detailed mechanisms of reelin function remain controversial. Theories aiming for the precise role of this protein, to a big extend, are based on the phenotype of a mouse mutant with a homozygous loss-of-function mutation of the *reelin* gene (i.e. *reeler* mouse). It is assumed that in the *reeler* brain, neurons are not able to split the preplate, which remains as a single superficial layer called superplate. Moreover, laminated *reeler* structures do not show normal laminar morphology, displaying a neocortical phenotype described as “inverted”. On the basis of layer specific mRNA expression and triple cell birth dating approaches, we could demonstrate that the *reeler* cortex is disrupted in a more complex and area-specific fashion. For example, cells in the somatosensory cortex show massive intermingling of layer II/III and IV fated cells, that are sandwiched by layer V and VI fated cells. The visual cortex on the contrary, despite of its substantial cell scattering, showed at least a tendency of an inversion of the main laminar compartments. Reelin loss also leads to a non-homogeneous expression pattern of ApoER2 and VLDLR through the anterior-posterior axis of the neocortex. Furthermore, in early cortical development, we found individual postmitotic neurons entering the *reeler* preplate, indicating that preplate splitting is not fully inhibited. These results suggest diverse cell-type and/or area specific roles of reelin on corticogenesis. In summary, we see the necessity to reconsider the role of reelin and its different effects on neuronal migration during cortex formation.

The expression of myelin-associated genes is reduced in mild focal cortical dysplasia

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Focal cortical dysplasia (FCD) are local malformations of the human neocortex and are frequent causes of medically intractable focal epilepsy, associated with a high seizure frequency. FCD can occur as isolated forms or associated with other principal lesion (e.g. hippocampal sclerosis).The histological abnormalities of FCD are characterized by radial and/or tangential disturbances of lamination. In addition, dysmorphic neurons and/or Balloon cells occur in the severe forms of FCD (Blümcke et al. 2011). To date little is known about the pathomechanisms leading to the architectural and functional abnormalities associated with FCD. Since FCD type IIB displays the most severe pathohistological pattern and can reliably be detected in MRI, so far most investigations concentrated on this FCD type. Therefore, we focused in this study on mild FCD types, and performed a whole transcriptome screening to understand the molecular mechanisms leading to this cortical malformation.

In the present study, we performed a screening of the whole human transcriptome on dysplastic (FCD type Ia, IIa, IIIa) and non-dysplastic temporal lobe tissue, with the aim to identify transcriptional mechanism leading to this cortical malformation. To this end, RNA from human dysplastic temporal neocortex from children (n=7, mean age 2.5 years, range 0.8 - 5.1 years) and from adolescent or adult patients (n=9, mean age 20.2 years, range 6.9 - 36.5 years), who all had undergone surgical treatment due to intractable epilepsy, was compared to RNA from non-dysplastic (n=7; mean age 17.6 years, range 1.8-27.4 years) specimens by hybridization of sense strand DNA to Human Gene 1.0 ST Affymetrix arrays covering 28869 genes. Principle component analysis showed that the global gene expression pattern of dysplastic and non-dysplastic cortex was strongly overlapping. However, when expression levels of all genes were individually compared by analysis of variance, we found that in all dysplastic cases approximately 231 transcripts were differentially expressed when compared to controls. Interestingly, the majority of those genes were down-regulated. Accordingly, real-time polymerase chain reaction analysis confirmed that the expression of MOG, MAG, MYRF, CNP and MBP was significantly reduced. In addition, we could demonstrate by in situ hybridization and immunohistochemical staining of neocortical tissue that the density of myelin basic protein mRNA-expressing cells and of myelin fibers were drastically reduced in the dysplastic cortex of adolescent and adults patients.

In summary our study revealed a reduced expression of oligodendrocyte-specific genes encoding myelin-associated proteins indicating a disturbance of oligodendrocyte differentiation and myelin sheet formation/maintenance in mild FCD.

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The NF2 tumor suppressor protein merlin in peripheral nerve regeneration

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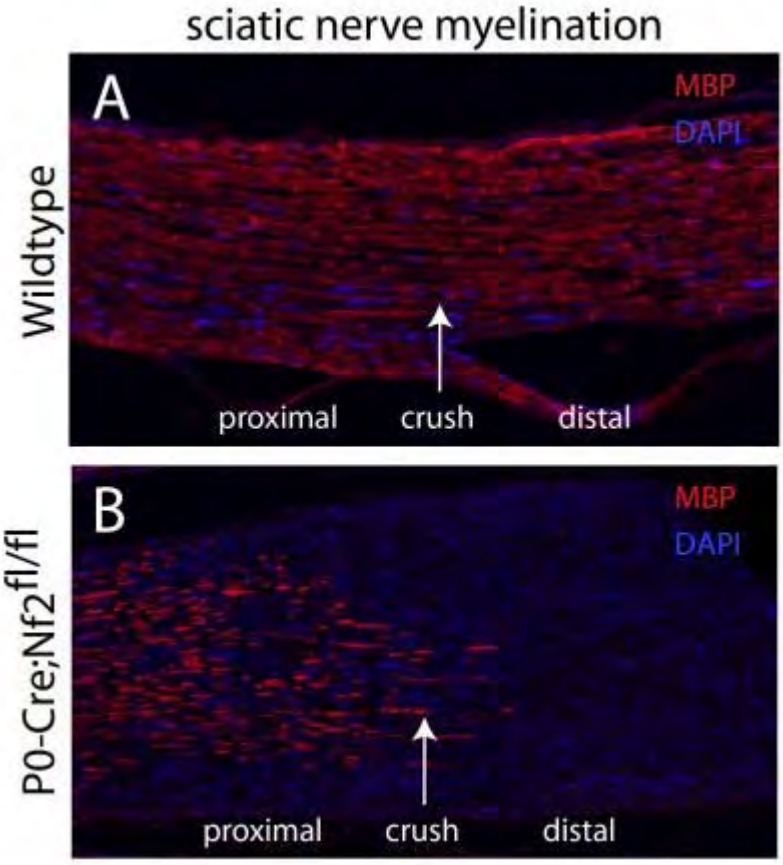
In contrast to neurons of the central nervous system (CNS), nerve cells of the peripheral nervous system (PNS) show high regenerative capacity. However, regeneration following peripheral nerve injuries is often incomplete and slow, indicating the need for improvement through biomedical research. Furthermore, the mechanistic understanding of cellular and molecular repair programs is still incomplete.
The tumor suppressor protein merlin is mutated in the hereditary tumor syndrome Neurofibromatosis type 2 (NF2), which is clinically characterized by Schwann cell-derived tumors. Previously, we were able to show the first mechanistic insight how and why NF2 patients additionally suffer from axonal polyneuropathy with a prevalence up to 70% (Schulz et al., 2013).
We have now investigated the role of the tumor suppressor protein merlin in peripheral nerve regeneration in vivo. Both the cell type-specific loss of merlin in neurons and Schwann cells of the PNS impair sciatic nerve regeneration following experimental crush injury.
Mice with conditional knockout of merlin in Schwann cells or neurons show impaired functional recovery of motor performance as measured by a novel video-based gait assessment (single-frame motion analysis) and by electrophysiological techniques. Immunohistochemical and ultrastructural analysis furthermore revealed that animals bearing Schwann cell-specific knockout of merlin (P0-Cre;$Nf2^{fl/fl}$) suffer from a complete remyelination defect of axons distal to the crush lesion even 5 weeks after crush injury (see Figure). To our knowledge, this represents the most devastating phenotype regarding peripheral regeneration studies in vivo.
Conclusively, it shows the significance of the NF2 tumor suppressor merlin for both peripheral nerve regeneration and maintenance. Our results thus have important implications for the pathogenesis of the NF2 disease as well as for the general understanding of nerve regeneration following traumatic lesions.


sciatic nerve myelination
A
MBP
DAPI
Wildtype
proximal
crush
distal
B
MBP
DAPI
P0-Cre;Nf2fl/fl
proximal
crush
distal

Theoretical modeling of cerebral organoids: Microcephaly and the Griffiths singularity

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Recently, researchers in the group of Juergen Knoblich have developed a human pluripotent stem cell-derived three-dimensional organoid culture system, termed cerebral organoids [1]. The cerebral organoids were shown to reiterate features of human cortical development. Besides, the experimental data showed that not only can the organoids mimic the developmental process but also demonstrate a premature neuronal differentiation, the phenomenon that could explain the microcephaly, a neurodevelopmental disorder.

We have developed a theoretical statistical physics model that could help to describe the phenomena observed in these experiments. Our model consists of a stochastic partial differential equation for the density of neuronal cells. In the model we included the processes of growth, competition, cell-type transformation, and three-dimensional diffusion. In order to proceed with the nonlinear non-equilibrium system we have applied methods recently presented in [2,3]. Using the model we were able to describe the time evolution of the neuronal matter as well as the phenomenon of microcephaly. Among main results is also the existence of the Griffith's singularity in the free energy of the system that makes yet another link between neuronal systems and condensed matter objects [4].

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Touchscreen-based visual pairwise discrimination and reversal learning in the primate brain aging model *Microcebus murinus*

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The grey mouse lemur (*Microcebus murinus*), a promising novel non-human primate model of brain aging, is increasingly used for studying various aspects of neurodegenerative pathologies, such as Alzheimer's disease. Our goal was to translate neuropsychological tasks (e.g. CANTAB tests) that are routinely used in humans to differentiate healthy ageing from ageing-related diseases to the model mouse lemur. In this study, we used for the first time a touchscreen-based behavioural test method, in which subjects can directly respond to visual stimuli on a touch-sensitive screen, to examine cognitive function and age-related impairments in the grey mouse lemur. This touchscreen-based procedure has several advantages, e.g. a high degree of standardization, minimized operator-subject interaction, and, above all, a high translational potential due to its similarity to the human CANTAB tests.

Our study revealed that comparable to humans, monkeys, and rodents, young (mean age = 2.6 years) as well as aged mouse lemurs (mean age = 7.9 years) could be successfully trained to interact with the touch-sensitive screen and to respond for food reinforcement. By applying two paradigms, a visual pairwise discrimination (PD) task and reversal learning (PDR), we examined different facets of cognition in the two age groups: perceptual learning / non-hippocampal, associative stimulus-reward learning and cognitive flexibility.

We found age-associated cognitive decline in the acquisition of the visual discrimination and in the reversal learning, i.e. the aged mouse lemurs needed significantly more trials to reach the task criteria as compared to their young conspecifics. Furthermore, a much higher inter-individual variability in the task performances was revealed in the aged adults. The individual performances in PD and PDR task correlate significantly, suggesting that individual learning performance is unrelated to the respective task. In the PDR task, we further found a significantly higher perseverance in aged compared to young adults, indicating an age-related deficit in cognitive flexibility. However, the animals' attention and motivation did not differ between age groups in the respective tasks. Whether the impaired learning performances of our aged sample reflect healthy age-related decline or pathological cognitive deficits is currently under investigation (e.g. using structural MRI).

Thus, our study provides the first touchscreen-based data on cognitive skills and age-related dysfunction in the novel primate aging model mouse lemur. Findings open exciting perspectives for comparative approaches in aging, personality, and for evolutionary research.

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Visuo-Spatial Paired Associate Learning (PAL) in a Strepsirrhine Primate (*Microcebus murinus*): New Insights into Early Primate Cognition from a Computer-Based Learning Task

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The Grey Mouse Lemur, *Microcebus murinus*, is currently discussed as a promising new model in ageing research, especially for Alzheimer's disease, as its brain undergoes changes during ageing that closely resemble the patterns of pathological ageing that can be found in the human brain (e.g. pathological tau-protein metabolism, beta amyloid plaques, and cerebral atrophies).

Using a touchscreen-based pairwise discrimination/pairwise discrimination reversal paradigm (PD/PDR), we could recently show that both visual, appetitive conditioning learning and cognitive flexibility in *M. murinus* are affected by ageing. Here, we present first results from a comparable study on visuo-spatial paired associate learning (PAL). Due to its spatial component, the PAL test is highly sensitive to alterations of the hippocampal formation and it has proven to have a high predictive value for the early diagnosis of Alzheimer's disease in humans. Despite the fact that the cognitive abilities of prosimian primates are traditionally considered as being poor as compared to simians and that mouse lemurs have been deemed untrainable in laboratory behavioural tests by some primatologists, our preliminary results show that young as well as old adults of *M. murinus* can learn to successfully solve the rather complex PAL task.

The relevance of this new insight is twofold: (1) The adjustment of the PAL protocol to *M. murinus* is the next important step towards establishing a Cambridge Neuropsychological Test Automated Battery (CANTAB) for mouse lemurs comparable to those used in Alzheimer's research in humans, monkeys, and rodents. (2) The finding that *M. murinus* can be trained to solve the PAL test in a touchscreen-based operant conditioning environment demonstrates that the cognitive abilities and the trainability of this prosimian primate are more advanced than often suggested. Standardized, touchscreen-based cognitive testing in mouse lemurs, thus, has proven to be a valuable tool for research into the nature of early primate cognition and will allow for improved comparative studies on this matter in the future.

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Poster Topic

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T11-4C miRNAs in neurite outgrowth and regeneration of midbrain neurons
Anna-Elisa Roser, Rashi Halder, Jerzy Dyczkowski, Mathias Bähr, André Fischer, Paul Lingor

T11-5C Molecular imaging of CaV3.2-channel promoter regulation in hippocampi of living mice during epileptogenesis
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T11-6C Neuronal Differentiation of Niemann-Pick Type C1 Patient-Specific Induced Pluripotent Stem Cells
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T11-7C Posttranslational modification and mutation of histidine 50 trigger alpha-synuclein aggregation and toxicity
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T11-3D The effect of cognitive and motor impairments on the P300 sources in Parkinson's disease patients
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T11-5D The functional link between autophagy and the trans-cellular spread of alpha-synuclein
Georgia Minakaki, Anna Bergmann, Wei Xiang, Holger Meixner, Jürgen Winkler, Jochen Klucken

T11-6D The hippocampal CA2 region in temporal lobe epilepsy
Ute Häussler, Katrin Rinas, Susanne Huber, Carola A. Haas

T11-7D The Role of *Drosophila* APPL (Amyloid Precursor Protein Like) Protein in Brain Function and Behaviour.
Franziska Rieche, Burkhard Poeck, Roland Strauss

T11-8D The role of intracellular Ca^{2+} stores for neuronal dysfunction in a mouse model of Alzheimer's disease
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T11-9D *a*Abnormal modification of ALS-associated mutant E102Q Sigma receptor-1leads to ER stress-mediated defects in protein degradation and endosomal trafficking.
Alice Dreser, Jan Tillman Vollrath , Antonio Sechi, Andreas Roos, Istvan Katona, S Bohlega , A Al-Saif , Dominik Wiemuth , Hannelore Heidtmann, Jörg Vervoorts , Marc Dohmen , Tania Rizo , Akila Chandrasekar , Hülya Daimaguler , Joachim Weis, Anand Goswami

T11-10D Deletion of the amyloid precursor protein family members APP and APLP2 results in aberrant changes of the mouse hippocampal presynaptic active zone proteome
Jens Weingarten, Melanie Laßek, Benjamin Müller, Marion Bäumlisberger, Tabiwang N.

Arrey, Amparo Acker-Palmer, Ulrike Müller, Michael Karas, Walter Volknandt

T11-11D Deletion of the amyloid precursor protein results in aberrant changes of the hippocampal presynaptic active zone proteome in mouse brain
Melanie Laßek, Jens Weingarten, Benjamin Müller, Marion Bäumlisberger, Tabiwang N. Arrey, Amparo Acker-Palmer, Ulrike Müller, Michael Karas, Walter Volknandt

^{201}TlDDC-SPECT imaging of alterations in CNS K^+-metabolism in mouse models of dementia

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Neuronal activity and membrane potential are coupled to transmembrane potassium (K^+) -turnover rates and intra- to extracellular K^+-gradients. We have recently shown, using histochemical methods in rodents, that the K^+-probe thallium (Tl^+) or the chelate complex thallium diethyldithiocarbamate (TlDDC), respectively, can be used for mapping acute (stimulus-dependent) and chronic (disease related) changes in neuronal activity as well as a breakdown of neuronal K^+-gradients. We here show, in mouse models of dementia, that pathological alterations in CNS K^+-metabolism can be imaged in vivo using ^{201}TlDDC single-photon emission computed tomography (SPECT).

TlDDC is an electroneutral lipophilic compound. After crossing the blood brain-barrier (BBB) Tl^+ is released from TlDDC. When animals are intravenously injected with TlDDC, neurons in the CNS take up Tl^+ in an activity-dependent manner from the extracellular space. With increasing Tl^+-uptake, Tl^+-efflux increases in return and Tl^+ redistributes over time. When Tl^+-influx and –efflux equilibrate, the intra- to extracellular Tl^+-gradients are related to the intra- to extracellular K^+-gradients.

Tl^+-redistributions have been studied over several decades in clinical routine using ^{201}Tl-SPECT. These studies have proven particularly useful in the diagnosis of myocardial ischemia and malignant tumors, diseases that are accompanied by acute or chronic up- or down-regulations of the Na,K-ATPase.

^{201}TlDDC-SPECT now makes it possible to study ^{201}Tl-uptake and redistribution with a similar rationale in the CNS. The close coupling of changes in K^+-metabolism and chronic up- or down-regulation of neuronal activity as well as cell death suggests the use of ^{201}TlDDC as a tracer for diagnosing and monitoring neurodegenerative diseases in preclinical and clinical studies.

Altered localization and abnormal modifications of Sigma receptor-1 in amyotrophic lateral sclerosis

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SigR1 alterations have been found in various neurodegenerative diseases including AD and PD and pathogenic mutations in SigR1 were recently discovered in ALS-FTLD/FTLD and in juvenile ALS cases. Moreover, there is abundant evidence for neuroprotective effects of SigR1 in a wide range of neurological disorders. Conversely, shRNA knockdown of sigma receptors in human cells has been shown to induce apoptosis. We therefore hypothesized that SigR1 is crucial for neuronal survival and maintenance and that it might be altered in ALS.

To follow up on this hypothesis, we first analyzed autopsy material from sALS and fALS patients. In motor neurons of ALS patients, the localization of SigR1 was significantly altered. The SigR1 protein accumulations were ubiquitinated and associated with the 20s proteasome subunit. They were not co-localized with p62 aggregates suggesting that the UPS pathway rather than macro-autophagy degrades such abnormal ER proteins. We confirmed and extended these results by immunohistochemistry of G93A SOD1 mouse á-motor neurons.

SigR1 is predominantly localized to cholinergic postsynaptic densities, also known as C terminals, and is co-localized with type-2 muscarinic receptors at these sites. We observed a focal sub-surface SigR1 immunoreactivity in normal human á-motor neurons which were significantly increased in size in ALS á-motor neurons.in ALS patient á-motor neurons. Consistent with the notion that increase in the size of C-terminals is due to an elevation in synaptic drive from parent interneurons to compensate for the substantial loss of synaptic input from other sources in order to protect the surviving neurons, our finding of elevated SigR1-immunoreactivity in C-terminal territories and altered localization suggests that the surviving motor neurons attempt to preserve functions of SigR1 at these sites. The defects in regulating ion channel conduction, synaptic transmission, axonal transport, lipid metabolism, IP3R-mediated Ca2+ signalling, and aspects of memory and learning, as a part of SigR1 function which deteriorates as ALS progresses, might reflect the consequences of altered localization and abnormal modifications of SigR1 over a long period of time.

Amyloid-ß-induced NMDA-receptor signaling to the nucleus

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Amyloid-ß oligomers (Aßo) are widely considered to cause synaptic disruption via alterations of NMDAR signaling leading to onset of Alzheimer's disease and cognitive impairment. Jacob, a protein messenger that encodes the synaptic vs. extrasynaptic origin of NMDAR signals, seems to play an important role in Aßo-caused synaptopathy. The phosphorylation state of Jacob in the nucleus determines whether it induces cell death or promotes cell survival. Upon the stimulation of synaptic NMDARs, Jacob is phosphorylated by ERK and translocates to the nucleus promoting expression of pro survival genes. On the contrary, upon stimulation of GluN2B-containing extrasynaptic NMDARs, non-phosphorylated Jacob translocates to the nucleus causing CREB shut-off, stripping of synaptic contacts and retraction of dendrites. Similarly, Aßo induces extrasynaptic GluN2B-activation, resulting in synaptic loss, CREB shut-off and Jacob accumulation in the nucleus. Therefore, the present study focuses on elucidating mechanisms related to Aßo-caused Jacob translocation to the nucleus. Firstly, we showed that shRNA knock-down of Jacob prevents Aß-induced CREB shut-off. Secondly, we compared the mechanisms of action of 2 different types of Aßo: conventional Aßo1-42 and Aß3(pE)-42 (truncated Aßo with an aminoterminal pyroglutamate), which was reported to be more toxic. We observed that Aß3(pE)-42 cause increased detrimental effects on neuronal cell morphology, CREB shut-off and Jacob accumulation only in the presence of astroglia. Furthermore, detailed analyses revealed that whereas Aßo1-42 accumulates on the neuronal surface, Aß3(pE)-42 accumulates in the astrocytes. In addition, Aß3(pE)-42 induces more prominent microglia activation in murine hippocampal slices. These results indicate different mechanisms of action of Aß-oligomers.

An animal mouse model for retinal degeneration reveals *Ccdc66* transgene expression outside the retina and expression profiling identifies retinal degeneration marker

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More than 200 genes are involved in retinitis pigmentosa (RP), a heterogeneous group of human retinal disorders characterized by retinal degeneration. Human RP can be investigated in canine and mouse models based on naturally occurring mutations. By that means, we were able to identify and characterize the previously unknown gene Ccdc66 and its respective protein product and link its function to retinal degeneration. In the herding breed of Schapendoes dogs, a mutation in *CCDC66*, as identified by linkage analysis, results in autosomal recessively inherited generalized progressive retinal atrophy (gPRA), the canine counterpart to RP in man (Dekomien et al., 2011). Based on this finding, a gene-trap based *Ccdc66*-deficient mouse model was generated in order to reveal the function of *Ccdc66* and the pathogenesis of this gPRA form of retinal degeneration (Gerding et al., 2011). This homozygous *Ccdc66*-deficient mouse model lacks retinal *Ccdc66* RNA and protein expression and an early initial degeneration is evident at postnatal day 13 (P13) followed by a slowly progressive degeneration preceding into adulthood as demonstrated by morphological studies and electroretinogram recordings. In order to gain insights into the molecular mechanisms that govern *Ccdc66*-deficient degeneration, a detailed evaluation is performed in order to reveal the key structures, cell types, pathways and gene expression patterns that are involved in *Ccdc66*-dependent organization in our mouse model during early postnatal development until adulthood. Several techniques are employed like RNA expression array analysis, quantitative real-time-PCR, reporter gene localization and *in situ* hybridization at several postnatal stages. Our results indicate that *Ccdc66* deficient mice reveal 1) multiple expression sites of *Ccdc66* reporter gene in retina as well as extraretinal expression including the hippocampal formation, olfactory bulb and rostral migratory stream in the brain as well as olfactory epithelium and testis during postnatal mouse development and 2) that early changes in gene expression (P10-P28) are limited to a small proportion of differentially expressed genes including retinal degeneration marker in *Ccdc66*-deficient mice but not in whole-brain expression profiles. The results can be expected to aid in future studies unrevealing the functional impact of *Ccdc66* in mouse retinal information processing and beyond - and possibly contribute to future studies in man and human disease.

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Attempt to prove the acetylcholine-dependent character of suppression of the apomorphine-induced rotation rate of hemiparkinsonian rats after intrastriatal treatment with botulinum neurotoxin-A and evidence for the possibility of repeated intrastriatal BoNT-A treatments of hemiparkinsonian rats during 6 months

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In Parkinson's disease (PD) a loss of dopaminergic neurons of the substantia nigra pars compacta leads to an overactivity of cholinergic interneurons in the striatum. The medical treatment of PD lacks therapy options of the striatal hypercholinism without massive peripheral adverse reactions.
Recently we showed that intrastriatal injections of 1 ng and 2 ng botulinum neurotoxin-A (BoNT-A) inhibits pathologic apomorphine-induced rotation behavior of unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats up to 3 months completely and inhibits them significantly up to 6 months after the BoNT-A injection. To prove that the apomorphine-induced rotation behavior after BoNT-A administration in hemiparkinsonian rats is eliminated due to an reduction of the pathologic increased cholinergic synaptic transmission in the striatum, we repeated the former experiment of unilateral 6-OHDA lesion followed by an ipsilateral striatal BoNT-A application. Assuming that the observed beneficial effect of BoNT-A results from a reduction of striatal acetylcholine content by blocking the transmitter vesicle fusion with the presynaptic membrane, it should be possible to abrogate this effect by application of a cholinesterase inhibitor, which increases the striatal acetylcholine content once again. Therefore, we performed at every monitoring point a classical apomorphine-induced rotation test and two days later we injected donepezil (2 mg/kg BW) 1 h prior to a second rotation test. The results of both tests were compared. Donepezil is a well known cholinesterase inhibitor for the treatment of Alzheimer's disease which passes the blood brain barrier. We used rats, which were 6-OHDA hemilesioned and whose pathologic apomorphine-induced rotation behavior was successfully treated by an intrastriatal administration of 1 ng BoNT-A. Indeed, subsequent donepezil injections led to an increase of apomorphine-induced rotation behavior in 6-OHDA lesioned, BoNT-A-treated rats. Nevertheless, also sham-donepezil injections led to increased apomorphine-induced rotation rates. This argues, therefore, for an increased rotation rate after injection of donepezil, respectively the sham substance (0.9% NaCl solution), being not a result of the donepezil influence, but rather an effect of the promptly (48 h) twofold performed apomorphine administration and rotation test. We draw the conclusion that systemic donepezil injection is not qualified to proof that the BoNT-A effects on the apomorphine-induced rotation behavior are caused by an reduction of striatal acetylcholine.
In the course of former experiments it could be shown that the reducing effect of intrastriatal BoNT-A treatment on the apomorphine-induced rotation behavior of 6-OHDA hemilesioned rats is limited to 3 - 6 months. In order to investigate whether it is possible to prolong the suppression of apomorphine-induced rotations of a single BoNT-A treatment, we injected 1 ng BoNT-A again into the right striatum 6 months after the first application. Indeed, the second BoNT-A injection led to a further reduction of the meanwhile reverted apopmorphine-induced rotation rate. This experiment supplied evidence for the theoretical possibility of intracerebral administration of BoNT-A for treatment of motor symptoms of PD especially bradykinesia of PD patients over long time.

Characterization of a human cell culture model system for studying Parkinson's disease

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Neurodegeneration in Parkinson's disease (PD) is characterized by a progressive loss of dopaminergic neurons in the *substantia nigra pars compacta*. The subsequent deficiency of dopamine results in characteristic motor symptoms namely bradykinesia, rigidity, resting tremor, and postural instability. Symptomatic treatment for PD alleviates motor deficits, but does not alter the course of the disease. The molecular mechanism underlying the degeneration of dopamine-producing cells is still unknown, although there is persuasive evidence suggesting the onset of pathogenic events within the axonal-dendritic compartment.

To study early PD-associated cellular changes, we use LUHMES (Lund human mesencephalic) cells as a human model system to mimic a human midbrain dopaminergic neuron. LUHMES cells are conditionally immortalized neurons derived from fetal midbrain. During proliferation, cells have characteristics of neuronal progenitors that can be differentiated into post-mitotic neurons.

We apply immunocytochemistry and electron-microscopy for a morphological and ultrastructural analysis of the differentiation process as well as electrophysiology including calcium-imaging for functional analysis.

During differentiation, LUHMES cells undergo a process of maturation such as the progressive outgrowth of neurites that contact each other. In addition, the expression of early progenitor markers decreases and the cells show an increased expression of mature markers such as NeuN and Tuj1. Proteins of the pre- and postsynaptic compartment like Bassoon and PSD95 are expressed and redistributed within neurites throughout differentiation; however there are no synaptic connections observed at the ultrastructural level. Patch clamp recordings from cultured LUHMES cells for assessing possible changes in excitability between early and late stages of differentiation revealed a constant excitability of the cells by evoked stimulation. When examining synaptic transmission by measuring spontaneous and evoked (paired recordings) synaptic events, the cells did not show synaptic activity. These findings confirm the data from our ultrastructural analysis suggesting that LUHMES cells do not build synaptic connections in culture after 20 days of differentiation.

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Characterization of newly identified regulators of protein homeostasis in mammalian cells

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The maintenance of protein homeostasis throughout life is of vital importance for the survival of cells. Cellular function and activity depends on an adaptive regulation of protein folding, stabilization and degradation, processes that prevent protein aggregation. This is of particular interest in postmitotic cells like neurons whose functional decline and degeneration leads to several late onset neurodegenerative diseases. Protein homeostasis, called proteostasis, is regulated by a fine-tuned system of chaperones that stabilizes proteins and direct instable proteins towards the proteasome or autophagy degradation pathway. We performed a RNAi-based screen in C. elegans following an acute heat stress paradigm to identify new components of the proteostasis network and identified, among others, RME-8. The human ortholog to RME-8 is called DNAJ homolog subfamily C member 13 (hRME-8) and is characterized by the presence of the J-domain that interacts with HSP70. hRME-8 was initially identified as a protein involved in receptor mediated endocytosis and has been implicated more generally in endosomal trafficking. It is of note that mutations in hRME-8 had recently been identified in familial forms of Parkinson's disease. To investigate the role of hRME-8 in proteostasis in human cells we established cell lines stably expressing GFP-tagged luciferase as a protein folding marker. We showed that the downregulation of hRME-8 in these cells induced the presence of protein aggregates upon heat stress. In addition, the overexpression of mutant, misfolded Cu/Zn superoxide dismutase (SOD1) resulted in an aggravated aggregation of mutant SOD1 in cells with reduced RME-8 protein levels. In line with these data is the observation that RME-8 protein levels were increased in transgenic mice expressing two different mutant SOD1 variants that represent models for amyotrophic lateral sclerosis. In summary, these data strongly suggest that RME-8 is part the proteostasis network. Further experiments will determine how RME-8 is involved in protein degradation pathways.

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Cyclodextrin mimics alteration of inhibitory synaptic transmission observed in CA1 pyramidal cells of NPC1 deficient mice

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NPC1 is a rare progressive neurodegenerative disease caused by mutations in the NPC1 gene. A mutation in the NPC1 gene leads to an impaired lipid transport resulting in an accumulation of cholesterol and gangliosides in the late endosome and lysosome. The pathogenic mechanisms ultimately leading to neurological manifestations caused by neuronal dysfunction and cell death are not exactly understood. Besides a described variety of morphological alterations of neurons, a detailed knowledge about the pathophysiological processes in neurons is still missing. Of special interest are studies about synaptic transmission and plasticity, as a disturbance of this functionality may be causative for clinical symptoms. Cholesterol is essential for a proper synaptic transmission as pre- and post-synaptic receptor clustering depends on cholesterol, as well as fusion and release of synaptic vesicles. In former studies, an increased excitatory synaptic transmission was observed in cultured hippocampal neurons from $NPC1^{-/-}$ mice and in hippocampal slices. In addition, alterations of pathways innervating the hippocampus, changed expression levels of glutamate and GABA transporters, and a disturbance in purinergic inhibition in the hippocampus were described.

Thus, we asked if any alterations of inhibitory transmission can be found in hippocampal CA1 pyramidal cells of NPC1 deficient mice ($NPC1^{-/-}$). Therefore we recorded post synaptic currents (PSCs) by means of the whole cell configuration of the patch clamp technique.

We found, that the frequency of inhibitory postsynaptic currents (IPSCs) is slightly increased in $NPC1^{-/-}$ mice compared to $NPC1^{+/+}$ mice. Inhibition of glycine receptors with strychnine had no effect on the frequency of $NPC1^{+/+}$ mice, but clearly decreased the frequency in $NPC1^{-/-}$ mice to the level of $NPC1^{+/+}$ mice. The application of the $GABA_A$ receptor antagonist gabazine nearly abolished all PSCs in both genotypes. Therefore, we assumed that the glycinergic inhibitory system is altered in $NPC1^{-/-}$, mice whereas the GABAergic system remains unchanged. Further, since there are no synaptic glycine receptors descript in hippocampal CA1 pyramidal cells, the higher frequency in $NPC1^{-/-}$ mice is likely caused by network alterations. Based on these results, we were interested if 2-hydroxypropyl-B-cyclodextrin (cyclodextrin), which is described to improve the symptoms in the $NPC1^{-/-}$ mouse model, can reduce the higher IPSC frequency to the frequency found in $NPC1^{+/+}$ mice. Surprisingly, we found that the administration of cyclodextrin elevated the IPSC frequency significantly and increased the influence of the glycinergic inhibitory system in $NPC1^{+/+}$ mice, mimicking the $NPC1^{-/-}$ phenotype. However, cyclodextrin treatment did not have any effect on the IPSC frequency of $NPC1^{-/-}$ mice. The mechanism, how cyclodextrin elevates the IPSC frequency in hippocampal CA pyramidal cells of $NPC1^{+/+}$ mice remains unclear. One can assume that the increased IPSC frequency is a secondary effect of an increased excitatory synaptic transmission, observed in former studies. On the other hand, since cyclodextrin treatment increased the influence of the glycinergic inhibitory system on the IPSC frequency, it could also interact directly with glycine receptors. Further studies are necessary to understand the effects of cyclodextrin on the central nervous system, especially as cyclodextrin is currently under investigation as a treatment for NPC1 patients.

De- and remyelination in Metachromatic Leukodystrophy

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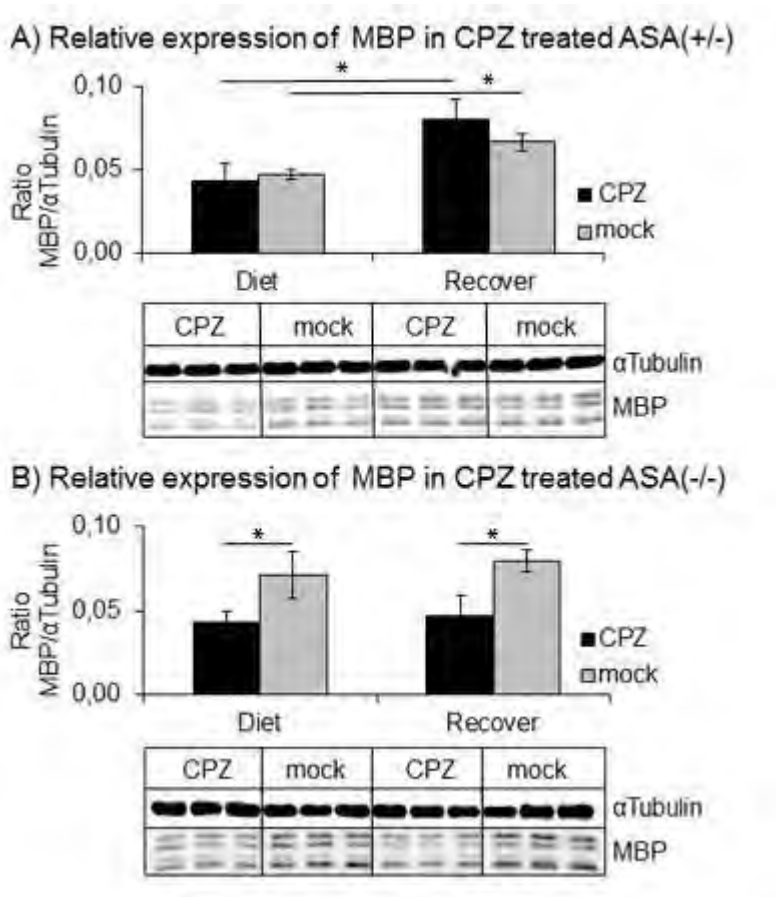
Metachromatic Leukodystrophy (MLD) is a lysosomal storage disorder caused by the deficiency of the lysosomal enzyme arylsulfatase A (ASA). ASA mediates degradation of sulfatide, a major myelin component. Lack of this enzyme leads to storage of sulfatide in central and peripheral nervous system, causing a progressive demyelination in MLD patients. It has been speculated, that demyelination is a result of a non sufficient remyelination due to a lack of oligodendrocytes or their precursor cells, a defect in myelin synthesis and others. As an animal model for MLD, ASA-deficient ASA(-/-) mice have previously been generated by disruption of the ASA gene. These mice show sulfatide storage, reminiscent to the human disease, but no demyelination.

Cuprizone-induced demyelination is a widely accepted method to study remyelination. Cuprizone (Bis(cyclohexanone)oxaldihydrazone) is a copper-chelator, that induces a highly reproducible demyelination in distinct brain areas, although the biochemical processes remain unknown. Subsequent feeding of common chow allows remyelination to occur. We fed seven week old ASA(-/-) mice with a diet containing 0.2% cuprizone.

Alcian Blue-staining showed a distinctive storage of sulfatide in microglia of white and grey matter of cerebrum and cerebellum after six weeks of diet.
Furthermore, treated ASA deficient mice showed reduced expression of the myelin markers myelin basic protein (MBP), proteolipid protein (PLP) and 2',3'-Cyclic-nucleotide 3'-phosphodiesterase (CNPase) suggesting a disturbance of remyelination in ASA deficient mice (shown in Figure 1) that did not recover on normal diet.
Histochemical staining and Western blotting showed a two-fold higher extent of astrogliosis after CPZ treatment in ASA(-/-) mice, but not after remyelination. Surprisingly, no treatment dependent difference could be shown in cytokine expression.

Our results show that remyelination is affected in ASA-deficient mice, suggesting impaired remyelination may, at least in part, be responsible for myelin loss in MLD patients. Whether this impairment is based on sulfatide storage will be answered by our ongoing double demyelination experiments: ASA-deficient mice are repeatedly fed with CPZ diet to increase amount of stored sulfatide. Effects of increased storage material on remyelination and immune response will give a more detailed insight into the relations between sulfatide storage and demyelination.


A) Relative expression of MBP in CPZ treated ASA(+/-)
Ratio MBP/αTubulin
0,10
0,05
0,00
*
*
CPZ
mock
Diet
Recover
CPZ
mock
CPZ
mock
αTubulin
MBP
B) Relative expression of MBP in CPZ treated ASA(-/-)
Ratio MBP/αTubulin
0,10
0,05
0,00
*
*
CPZ
mock
Diet
Recover
CPZ
mock
CPZ
mock
αTubulin
MBP

Deciphering the brain non-coding RNAome linked to cognitive aging and Alzheimer's disease using the mouse as model organisms

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Alzheimer's disease is the most common form of dementia in the elderly. A small number of AD cases are caused by mutations in amyloid precursor protein (APP) gene or the genes that mediate APP processing (familial AD). The majority of the AD cases (95%) are however sporadic and are characterized by late onset (loAD). Despite intense research there is still no effective treatment for AD or loAD.

There is now emerging evidence that loAD is caused by variable combinations of genetic and environmental risk factors. Epigenetic processes such as histone-modifications, DNA-methylation and the action of non-coding RNAs control gene-expression programs at a systems level and are key mechanisms regulating genome-environment interactions. Recent data suggest that epigenetic processes contribute to loAD and age-associated cognitive dysfunction, and may provide a novel therapeutic avenues to treat loAD. Of particular interest are non-coding RNA (NcRNA). Thus, we used an unbiased approach to study the brain non-coding RNAome in mouse models for age-associated memory decline. Selected candidates are currently under investigation.

Delayed feedback control of pathological network oscillations

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During the last two decades various methods such as high-frequency deep brain stimulation (DBS) have been developed for the treatment of oscillations associated with various pathological conditions, e.g. in Parkinson's disease [Perlmutter and Mink 2006]. These methods have been traditionally based on open-loop strategies, that is on fixed, predetermined stimulation parameters. They are highly effective in quenching the aberrant oscillations and, thus, in alleviating the clinical symptoms. At the same time, however, they introduce undesirable side effects and in most cases they do not restore the network transfer function. Recently, adaptive control strategies have emerged, which promise to increase the efficacy of the existing stimulation methods to control and correct the network activity dynamics [Priori et al. 2013].

Here, we investigate the effects of adaptive, closed-loop control schemes on networks of spiking neurons. Specifically, we design an appropriate control strategy, based on delayed state-feedback to quench sparse or stochastic oscillations, which closely resemble the pathological oscillations and are known to be robust compared to synchronous regular activity [Brunel and Hakim 2008]. Our control protocol is able to sufficiently suppress these stochastic oscillations and to drive the network in an asynchronous irregular regime. Importantly, the network transfer function, defined as the ratio of the population rate response to incoming stimuli, is also recovered. Our results thus suggest that adaptive state-feedback control is a promising strategy to design brain stimulation protocols to correct the aberrant dynamics without inducing strong side-effects.

Deletion of Myosin VI causes slow retinal optic neuropathy and age-related macular degeneration (AMD)-like retinal phenotype

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Retinal neurons degenerate in diseases like age-related macular degeneration (AMD) and glaucoma, leading to blindness. Yet, the lack of suitable mouse models hampers an efficient search for potential effector proteins that underlie these diseases and thereby progress in the development of effective treatments.
A potential new model for glaucoma and/or AMD may be the myosin VI-deficient mouse line. Myosin VI, a member of the actin-based myosin motor protein family, is expressed in the retina, and earlier ERG measurements pointed at a visual deficit in these knock-out mice (Kitamaro et al., Exp Eye Res 2005). Analyzing wild-type and myosin VI-deficient mice in more detail, we found myosin VI expression in the retinal pigment epithelium, the outer limiting membrane, and outer plexiform layer. A lack of myosin VI expression in myosin VI-deficient mice could be linked with differential progressing ocular deficits that mirrored an AMD and glaucoma phenotype. Data are discussed in the context of an identification of a new blindness disease-causing protein and introduction of a presumptive new putative glaucoma and/or AMD mouse model.

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Does the cytoplasmic membrane localized voltage-dependent anion channel 1 (VDAC-1) participate in Ha-Ras-mediated neuronal protection?

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Neuronal neurite growth, differentiation and survival are regulated by the proto-oncogene GTPase Ras. Our synRas mouse model was established to investigate Ras in the adult central nervous system. Therefore, the synapsin-1 promoter controls the expression of constitutively activated V12-Ha-Ras activating the MAPK-pathway and effecting selectively postmitotic neurons of cortex and hippocampus in transgenic mice. This mouse model is ideal for investigating neuroprotection, for example there is a great attenuation for neurotoxin-induced degeneration of dopaminergic *substantia nigra* neurons. Quantitative proteome analysis revealed altered expression levels of several energy metabolism proteins, especially a decrease of 70% for the voltage-dependent anion channel-1 (VDAC-1) in cortex and hippocampus of adult synRas mice. VDAC-1 is mainly expressed in the outer mitochondrial membrane (mt-VDAC-1), but also in other cellular locations, such as in the plasma membrane (pl-VDAC-1). Alternative splicing of VDAC-1 mRNA leads to mt-VDAC-1 and pl-VDAC-1 in mouse. Since the expression level of VDAC-1 has an effect on cellular survival in general, we investigated whether the decreased VDAC-1 expression participates on a molecular level to the neuroprotection in the synRas mouse model.
Whereas the mt-VDAC-1 mRNA is unchanged, the mRNA level of pl-VDAC-1 is selectively decreased in hippocampus and cortex in adult synRas mice. Using PC12 cells as a model system, the overexpression of either mt-VDAC-1 or pl-VDAC-1 in combination with a neurotoxic insult led to cell death, which was prevented by the co-expression of activated Ras. In primary cortical cultures, synRas mediated neuroprotection was confirmed by excitatory glutamate stimulation. In line with the data of adult mice, the selective decreased mRNA-level for pl-VDAC-1 was also demonstrated in synRas-derived cortical cultures. Since pl-VDAC-1 functions as a NADH-ferricyanide reductase, this enzymatic activity was selectively decreased in transgenic cortical cultures. Further experiments suggest that activated Ras and its MAPK-signalling influences the alternative splicing of VDAC-1 mRNA in primary cortical cultures.
In summary, there are experimental indications that activated Ras may alter the alternative splicing of VDAC-1 mRNA yielding to a specific reduced expression of pl-VDAC-1 protein which could contribute to the mechanism of neuroprotection in synRas mice.

Effects of alpha-synuclein on axonal transport of mitochondria.

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The protein alpha-synuclein (aSyn) is implicated in the pathology of Parkinson's disease (PD) due to genetic evidence and the fact that it accumulates in Lewy bodies, which are the pathological hallmarks of PD. However, the underlying pathomechanism is not known. Since the intracellular protein burden seems to be an important pathogenic factor, we use adeno-associated viral vectors (AAV) to overexpress aSyn in different model systems. We found previously, that overexpression of aSyn leads to an impairment of fast axonal transport of synaptophysin labelled with EGFP in rat primary midbrain neurons. This is in line with other reports showing an early reduction of the transport motor proteins kinesin and dynein in the striatum of AAV.aSyn injected rats and of human PD patients.

Here, we examined the effects of aSyn overexpression on axonal transport of mitochondria. We used a microfluidic chamber system to isolate axons of rat cortical neurons in vitro and studied axonal transport of mitochondria visualized with MitoTracker employing live-imaging. Three different groups were compared: untransfected axons, axons overexpressing EGFP and axons overexpressing EGFP and aSyn. The average speed of mitochondrial movements was 0.37 µm/s in all examined groups. Retrograde transport was significantly faster than anterograde transport and there were more anterograde than retrograde movements in all groups. Neither anterograde nor retrograde movements were slower in the aSyn overexpressing axons compared to the other groups. There was no significant difference in the percentage of mobile mitochondria between the three groups. We observed 30 % mobile and 70 % stationary mitochondria in all conditions.

Taken together, we did not detect any effects on axonal transport of mitochondria caused by the overexpression of aSyn in rat cortical neurons. We conclude that the pathogenic effects of aSyn on axonal transport depend on specific rate components of axonal transport.

Effects of STN-DBS on the Amphetamine-induced Turning Behavior in Hemi-Parkinsonian Rat Model

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Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is now established as an effective treatment of movement disorders such as Parkinson disease, and is increasingly tested for other neuropsychiatric disorders. Nevertheless, the mechanisms have not been understood in depth. In particular, the pulses used in STN-DBS are regular, whereas theoretical studies have suggested that pulse trains with more stochastic variability may allow for more effective treatment with lesser stimulus current. In this study, we investigate the effects of STN-DBS with stochastic pulse trains on turning behavior induced by Amphetamine injection in the 6-hydroxy dopamine hemi-Parkinsonian rat model, and quantify the effects.

Epigenetic profiling of APP/PS1 mice – a mouse model for Alzheimer's disease

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Age related neurodegenerative diseases are an increasing burden in our societies that will become even more severe due to increasing life expectancies. Alzheimer's disease is the most common neurodegenerative disorder in the elderly and - despite intensive research - no effective cure is available. While a small number of AD cases are genetically inherited, the majority of all cases is sporadic and arises due to variable combinations of genetic and environmental risk factors. Such genome-environment interactions are likely to be mediated by epigenetic mechanisms that orchestrate gene-expression programs on a systems level. To address this hypothesis, we performed RNA-Sequencing combined with epigenetic gene profiling in a mouse model for severe amyloid pathology. We investigated 3 different time points, namely early, intermediate and advanced stages of pathology. The corresponding data allowed for an unprecedented view of transcriptome plasticity including differential splicing and RNA editing in the diseased brain that allowed us to test new therapeutic strategies.

Evidence of nucleolar stress in genetic models of neurodegenerative disorders: focus on basal ganglia

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Decreased rRNA synthesis and nucleolar disruption - nucleolar stress - are signs of cellular stress and trigger context-specific neuroprotective/neurotoxic mechanisms. Nucleolar stress is associated with several neurodegenerative disorders including Parkinson´s and Huntington´s disease. The expansion of CAG repeats typical of polyglutaminopathies confers pathogenic property to these mutant RNAs by impairing rRNA transcription.

Here we characterized the link between nucleolar dysfunction and neurodegeneration, in particular in striatal and dopaminergic neurons of Huntington´s disease (HD) and Parkinson´s disease (PD) genetic mouse models, respectively. To this end, we optimized experimental approaches to detect and quantify rRNA synthesis and markers of nucleolar stress in tissue sections focusing on the basal ganglia. We showed that nucleolar stress is an early event in medium spiny neurons of the striatum in R6/2 mice, a transgenic model of HD but not in pre-symptomatic genetic models of PD. In addition, we found that PD recessive and dominant mutations lead to homeostatic and metabolic responses that alter rRNA transcription with different modalities during the disease progression.

These results reinforce the emerging role of the nucleolus in neuronal homeostasis and the identification of specific cellular and molecular pathways dependent on nucleolar stress to explain disease progression.

Exposures to daily social defeat lead to motor impairment and calreticulin upregulation. A risk factor for later-life onset of neurodegenerative disorders.

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Innumerable environmental insults such as chronic psychosocial stress enhanced glucocorticoid hormones release and alter protein-folding reactions in the endoplasmatic reticulum (ER) through mechanisms that include depletion of ER calcium, alteration in the redox status and energy deprivation. Misfolded proteins are either retained within the ER lumen in clomplex with molecular chaperones. Calreticulin is anchored to the lumen of ER, provides chaperone function to proteins and contributes to the regulation of intracellular calcium. Mitochondrial reactive oxygen species (ROS) can also be generated as a result of ER stress-induced Ca2+ release and depolarization of the inner mitochondrial membrane.

Chronic psychosocial stress can interfere with protein-folding homoeostasis and ROS production resulting in oligodendrocyte dysfunction and death.

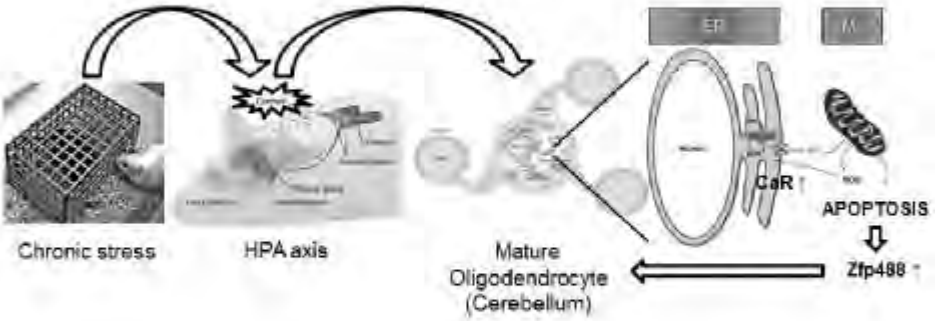
The cerebellum is a motor center structure which participates in emotional reactions, constant states of activity, and experience of rage, anger of fear. In the present study we hypothesized that chronic psychosocial stress activates HPA axis, leading to persistent glucocorticoid hormones release, ER stress, oxidative stress and in the last term the oligodendrocyte death.

Mice subjected to chronic psychosocial stress were tested by behavior paradigms and finally they were sacrificed. The Cerebellum was isolated for gene expression analyses.

Behavior: Stressed mice displayed worse righting reflex and less latency on the rota-rod.

Gene expression analyses: RNA seq revealed 7 genes deregulated by stress. From this pool, Calreticulin was validated. Nanostring nCounter revealed an upregulation of Calreticulin and Zfp488.

Findings from this study support the hypothesis that chronic social defeat affects motor function and may put individuals at risk for later–life onset of neurodegenerative disorders


Chronic stress
HPA axis
Mature
Oligodendrocyte
(Cerebellum)
CaR ↑
APOPTOSIS
Zfp488 ↑

Expression and function of inwardly rectifying potassium channel in ALS oligodendrocytes

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Amyotrophic lateral sclerosis (ALS) is devastating neurodegenerative disease affecting motor neurons. Although motor neurons are affected, alterations in glial physiology have profound implications in ALS onset and progression. Recent studies reported the abnormalities in morphology and function of oligodendrocytes in ALS indicating their involvement in disease pathogenesis. Essential function of oligodendrocytes is the regulation of extracellular potassium levels by means of prominent inwardly rectifying potassium current (Kir). The aim of this study was to examine the expression of Kir4.1 and characterize Kir currents in spinal cord oligodendrocytes in culture isolated from newborn rats. For that purpose, immunocytochemistry and whole-cell patch-clamp experiments were performed. Double immunolabeling of Kir4.1 and CNPase showed reduced immunoreactivity of Kir4.1 in ALS oligodendrocytes compared to WT. Furthermore, whole-cell patch-clamp recordings from cultured ALS oligodendrocytes showed a significantly lower Kir current density. Accordingly, addition of 100 µM $BaCl_2$ to the extracellular solution revealed statistically significant decrease of Ba^{2+}-sensitive Kir current density in ALS compared to WT oligodendrocytes. Challenging the Kir channels with an increased extracellular potassium concentration of 20 mM, we observed reduced potassium uptake current in ALS oligodendrocytes. Observed changes in expression and functional properties of Kir channels in oligodendrocytes could disturb the neuronal microenvironment and lead toward dysfunction and death of motor neurons.

Expression of the Cytoskeleton Protein Vimentin is Altered in Niemann-Pick Type C1 Patient-Specific iPSC Derived Cells

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Niemann-Pick type C1 (NPC1) is a rare progressive neurodegenerative disease, which is caused by a mutation in the NPC1 gene and is inherited in an autosomal recessive manner. In this lysosomal storage disorder the intracellular transport and sequestration of several lipids like cholesterol is severely impaired resulting in their accumulation in the late endosomes and lysosomes. The neurological manifestation of the disease is caused by dysfunction and death of neurons, astrocytes and microglia. However, the pathogenic mechanism is not completely understood. Recent publications suggest that fibroblasts of NPC1 patients undergo changes of the cytoskeleton, where vimentin seems to play a pivotal role. Vimentin is a member of the family of intermediate filaments and is tightly regulated during the maturation of cells. Vimentin can be phosphorylated by different protein kinases, where the phosphorylated form is soluble and non-bounded and the non-phosphorylated form builds insoluble filaments and bundles. Moreover, upregulation of vimentin is speculated as damage response mechanism in neurodegenerative diseases like Alzheimer disease. Here, we asked, if a regulation of vimentin takes place in cells derived from fibroblasts of Niemann-Pick Type C1 (NPC1) patients. We reprogrammed fibroblast of NPC1 patients into patient-specific induced pluripotent stem cells (iPSCs) and differentiated these subsequently into cells of the neural lineage. By means of western blot and immunocytochemistry we demonstrated an altered expression level of vimentin and changes in the intracellular aggregation of vimentin. Western blot data showed a 2 times higher level of phosphorylated (non-bounded) vimentin in control cells in comparison to NPC1-deficient cells but a 2 times higher level of non-phosphorylated (bounded) vimentin in NPC1-deficient cells compared to control cells. Immunocytochemical data elucidated changes in the aggregation of vimentin, where the control cells showed a mixture of punctuated long organised filaments and shorter bundles. On the other hand the NPC1-deficient cells displayed an aggregation of longer disorganised bundles. Further studies are aimed to elucidate the underlying mechanism and the impact on the cell morphology, structural organisation like lipid rafts or synaptic clustering of ion channels, which are discussed to contribute to the pathogenic mechanism in NPC1 disease.

Fear and fear extinction learning in APP/PS1 mice

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One of the most challenging topics in neuroscience research is the identification of novel treatment approaches for Alzheimer's disease (AD). It has been shown by several studies, that in AD patients emotional processing, e.g. the recognition of fearful faces or the learning of fear, is impaired. Interestingly, these impairments occur already at early stages of the AD etiopathology. Thus, an altered emotional processing might be regarded as an early symptom in the development of AD.
In the present study, we analyzed different aspects of fear learning and fear extinction in differently aged APP/PS1 mice. This AD mouse model combines the Swedish APP (KM670/671NL) mutation with the PS1-L166P mutation under control of the Thy1 promoter (Radde et al., 2006, EMBO), resulting in a rather mild but constant post-developmental expression of Aß and subsequent plaque formation. By testing amygdala-dependent cued fear learning, we observed only slight impairments in 12 months old but not in younger APP/PS1 mice. In the adjacent fear extinction training, we observed no impairments in the extinction of these cued fear memories, neither in short nor in long-term extinction memory. In contrast to the cued fear learning, we observed deficits in contextual fear learning in six months old APP/PS1 animals. Here, animals could not discriminate between the conditioned and a neutral context. However, the subsequent extinction of these contextual fear memories seemed to be unimpaired. As a non-emotional control experiment we also tested the object recognition memory of the APP/PS1 mice and observed no impairments in the short-term memory of these animals. Currently, we are analyzing the protein level of $Aß_{40/42}$ in the hippocampus, amygdala and medio-prefrontal cortex of the tested animals in order to correlate the local occurrence of these toxic Aß-species with the behavioral performance of the animals. In addition, we started to analyze long-term potentiation (LTP) in acute hippocampal slices from APP/PS1 mice. Here, first results indicate an impaired LTP in the CA1 region of six but not three months old APP/PS1 mice.
In conclusion, we could demonstrate selective impairments in contextual fear learning in middle-aged APP/PS1 mice. Experiments trying to further analyze the underlying mechanisms for this deficit by analyzing expression levels of soluble forms of Aß protein and altered hippocampal synaptic plasticity are in progress.

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Fibroblasts as an attractive model for the exploration of glucocerebrosidase deficiency in Parkinson's disease

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BACKGROUND:
Parkinson's disease is the most common movement neurodegenerative disorder. It is characterized pathologically by abnormal alpha-synuclein inclusions in neurons, mitochondrial dysfunction and endoplasmic reticulum stress. The presence of one mutant allele of GBA1, encoding lysosomal glucocerebrosidase, is the greatest genetic risk factor for sporadic Parkinson's disease. Furthermore, glucocerebrosidase deficiency in the substantia nigra of Parkinson's disease brains has been reported. However, the mechanisms involved in the interaction between glucocerebrosidase and Parkinson's disease remain largely unresolved. In this view, we investigated the potential deregulations linked to glucocebrosidase deficiency in fibroblasts from Parkinson's disease patients and controls.

METHODS:
Fibroblasts were established from skin biopsy from patients with sporadic Parkinson's disease carrying GBA1 mutation (n=3) or without mutation (n=7) and from control (n=4). Glucocerebrosidase protein level was measured using western blot and its enzymatic activity was evaluated using a fluorometric assay with 4-methylumbelliferyl b-D-glucopyranoside as the substrate. Markers of endoplasmic reticulum stress were measured using quantitative polymerase chain reaction and western-blotting and the mitochondrial membrane potential was evaluated following tunicamycin exposure by flow cytometry using the mitochondrial fluorochrome CMX-ROS.

RESULTS:
Preliminary results suggest that glucocerebrosidase activity appeared to be lower in fibroblasts from sporadic PD patients with GBA1 mutation and without GBA1 mutation compared to controls (30.74 ± 14.48% and 44.39 ± 13.49 %, respectively), although statistical significance was not reached (Kruskal-Wallis test p=0.069). This was accompanied by a decreased glucocerebrosidase protein level in fibroblasts carrying GBA1 mutation (60.93 ± 9.48% compared to control levels). While our data did not evidence significant changes in markers of endoplasmic reticulum stress (GRP78, ATF6 and XBP1 splicing), we observed that fibroblast mitochondrial membrane depolarization following tunicamycin exposure was more important in patients (with GBA mutation: -20.24% ± 4.94% ; without GBA1 mutation: -7.90 ± 6.80%) compared to controls (-1.73 ± 7.69%).

CONCLUSION: All together, these preliminary data suggest that patient-derived fibroblasts are a pertinent model to explore the relationship between glucocerebrosidase deficiency and mitochondrial dysfunction in Parkinson's disease. Ongoing inclusion of patient and control fibroblasts for such experiments will allow to increase the statistical power and to further analyze the underlying pathological mechanisms.

Functional Characterisation of Niemann-Pick Type C1 Neuronal Cells Derived From Patient-Specific Induced Pluripotent Stem Cells

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Niemann-Pick type C1 (NPC1) is a rare progressive neurodegenerative disease, which is caused by mutations in the NPC1 gene and is inherited in an autosomal recessive manner. In this lysosomal storage disorder the intracellular transport and sequestration of several lipids like cholesterol is severely impaired resulting in their accumulation in the late endosomes and lysosomes. The neurological manifestation of the disease is caused by dysfunction and death of neurons, astrocytes and microglia. However, the pathogenic mechanism is not completely understood. Several animal models like mouse, cat and fruit fly were used to analyse the impaired pathways but the loss of neurons is still unexplained and the genetic variability in humans cannot be reflected. Therefore, human models using patient-specific induced pluripotent stem cells (iPSCs) provide a promising approach. Recently, we reported about the successful reprogramming of human fibroblasts from NPC1-patients and the subsequently differentiation of these iPSCs into neuronal cells, providing a potential model to study the pathogenic mechanisms of NPC1 (Trilck et al., 2013). Here, as a next step, we functionally characterised control cells and NPC1-deficient cells by means of patch clamp recordings and Ca^{2+}-imaging experiments. The whole cell configuration of the patch clamp technique was used to study the expression of voltage-gated ion channels like Na_V and K_V and ligand-gated channels like $GABA_A$- or glutamate-receptors (Glu-Rs). For Ca^{2+}-imaging Fura2-AM was used to explore spontaneous Ca^{2+}-transients as well as the expression of Ca^{2+}-permeable ion channels. Independent of the genotype, neuronal differentiated cells expressed a wide range of voltage-gated as well as ligand-gated ion channels. Na_V and K_V were observed already after 2-3 weeks of differentiation. The expression increased slightly over time and after 5-7 weeks of differentiation higher current densities for e.g. voltage-gated Na_V were observed, indicating maturation of the cells. Regarding ligand-gated ion channels, both control and NPC1-deficient cells expressed inhibitory and excitatory ligand-gated ion channels, but we observed differences in the expression in Ca^{2+} permeable receptors like Glu-Rs and P2X-receptors. Although Glu-R mediated Ca^{2+}-influx was detected in similar amounts of NPC1-deficient and wildtype cells, the AMPA-mediated Ca^{2+}-influx was significantly smaller in NPC1-deficient cells. Furthermore, NPC1-deficient cells showed a reduced reaction towards the application of ATP. Interestingly, an altered glutamatergic synaptic transmission is described for neurons of a NPC1 mouse model suggesting a contribution to the pathogenic mechanism in NPC1. An altered functionality of Glu-Rs or P2X-Rs might lead to an injurious Ca^{2+}-homoeostasis in NPC1-deficient cells and thus contribute to a higher susceptibility of the cells for e.g. Ca^{2+}-based neurotoxicity or oxidative stress. Further studies will elucidate if such alterations contribute to the pathogenic mechanism of NPC1.

Functional properties of microglia in mouse models of Alzheimer's disease

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Microglia, resident macrophages of the central nervous system (CNS), survey and support neuronal functions. They participate in oligodendrogenesis and neurogenesis, learning and behavior, and are able to mount crucial innate immune responses upon CNS infection and damage. Ageing and associated neurodegenerative processes may impair these functions. Accordingly, in Alzheimer´s disease (AD), the microglial incapability to clear amyloid ß (Aß) may lead to massive accumulation and deposition of this peptide. On the other hand, microglia seem to be activated in such an environment, leading to excessive production of inflammatory mediators, including cytokines and chemokines, which can further damage the vulnerable CNS circuitry. Here, we investigated properties of microglia isolated from 5XFAD mice at 3 different ages compared to age-matched wild-types (WTs). We cultured the microglia isolated from brains of adult mice and determined their response to the Toll-Like receptors (TLRs) agonists including lipopolysaccharide (LPS) by cyto- and chemokine release and their phagocytotic activity using fluorescently labeled E.coli and myelin. We injected LPS into the brains of 6 and 9 months old mice to study microglia responses *in vivo*, regarding supported recruitment of neutrophils and monocytes from the periphery to the CNS, using flow cytometry analysis.

The current study shows no significant differences between microglia isolated from 5XFAD and WT mice in culture, suggesting that 5XFAD microglia outside of the tissue – and without influences by its environment – can behave like wild-type controls. *In vivo* studies, however, show significant differences between these two genotypes, indicating an impact of the diseased tissue.

Hsp22 mediates the differential degeneration of dopaminergic midbrain neurons

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Parkinson's disease (PD) is the second common neurodegenerative disease after Alzheimer's disease. PD results primarily from the death of dopaminergic (DA) neuron in the substantia nigra (SN) other than the neighboring ventral tegmental area (VTA). The mechanisms underlying this differential DA neuron vulnerability are largely unknown. Here, we identified small heat shock protein Hsp22 as a potential contributor to this process based on a previously published DNA microarray dataset. We found that Hsp22 displayed significantly higher expression levels in the SN compared to the VTA. Immunohistochemistry combined with *in situ* hybridization revealed that mesencephalic DA neurons that were positive for tyrosine hydroxylase (TH) Expressed Hsp22. Treatment of MES23.5 DA neuron-like cell line with neurotoxin 6-hydroxydopamine (6-OHDA) elevated the Hsp22 expression levels. Moreover, overexpression of Hsp22 enhanced the vulnerability of MES23.5 cells to 6-OHDA-induced neurotoxicity, while down-regulation of Hsp22, but not other differentially expressed genes in the SN examined, including Sox6 and GPCR5c, using siRNA significantly reduced 6-OHDA-induced MES23.5 DA neuronal cell death. Accordingly, knocking out of *hsp22* partially ameliorated SN dopaminergic neuronal loss from MPTP induced-neurotoxicity. To investigate the impact of Hsp22 overexpression on the vulnerability of nigral DA neurons *in vivo*, we generated transgenic mice selectively overexpressing Hsp22 in neurons, including midbrain DA neurons. The transgenic mice displayed exacerbated nigral DA neuron death after MPTP intoxication. Co-immunoprecipitation showed that flag-tagged Hsp22 strongly interacted with BCL2-associated athanogene 3 (Bag3) both in MES23.5 cells and transgenic mouse brain. Western blot analysis showed that Bag3 was down-regulated in *hsp22* knock out mice. In contrast, Bag3 was up-regulated in Hsp22 transgenic mice. Wild type mice intoxicated with MPTP showed marked increases in the expression levels of Bag3 in both the SN and striatum, and Bag3 overexpression delivered by AAV virus exacerbated DA neuron loss in both SN and VTA after MPTP intoxication. Simultaneous overexpression of Hsp22 and Bag3 led to aggresome formation and apoptosis of the MES23.5 cells. Taken together, we identified Hsp22 as an important regulator that mediates the differential degeneration of dopaminergic midbrain neurons through interaction with Bag3, and provide new insights into regulatory network controlling vulnerability of nigral DA neurons to environmental neurotoxins. This work was sponsored by the Chinese Academy of Sciences and the NSFC (31123002).

Influence of a-synuclein on intracellular levels of transition metals

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Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide.
PD patients' brains bear the presence of aggregates of proteins called Lewy Bodies (LB) whose major component is a-synuclein. Intriguingly, these patients have a higher concentration of iron (Fe) in some areas of the brain in respect to healthy controls. Furthermore there is a co-localization of LB and Fe.
It has been extensively investigated that a-synuclein can readily associate with many metals in vitro. When a-synuclein is bound to iron and copper, it is more prone to aggregation and this step is considered to be crucial in the etiopathology of the disease.
Therefore we wanted to investigate how the expression of a-synuclein might influence trace metal distribution in primary neuronal cultures.
In our model we utilized primary midbrain cultures transfected with adeno-associated viral (AAV) vectors in order to overexpress a-synuclein. After 3 days in vitro, cells were treated for three hours with Fe^{2+}. The treatment aimed to evaluate the effects of this metal on the distribution of elements in the cells, which was determined by particle induced X-ray emission (PIXE). This analysis enabled us to evaluate the elemental content of single cells at a high spatial resolution.
PIXE data of single cells overexpressing a-synuclein showed an intracellular copper concentration that was doubled compared to control-transfected cells.
This finding may reflect one of toxic mechanisms of a-synuclein in the progress of PD and suggests chelator therapy as a possible therapeutic strategy in this neurodegenerative disorder.

Label Free Quantitative Proteomics of Astrocytes Directly Converted to Neurons

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Meso-diencephalic dopaminergic (mdDA) neurons play a key role in motor control, cognition, arousal and motivation. Their dysfunction or loss in the *substantia nigra* is known to cause Parkinson's disease (PD). To date, the progression of PD can only be retarded by deep brain stimulation (DBS) and pharmacological treatment, which can cause serious *striatal* graft-induced dyskinesia. In future approaches, cell replacement therapies might be applied for the treatment of Parkinson's disease. Caiazzo *et al*. directly converted human and mouse fibroblasts into functional mdDA neurons by induced viral-expression of the transcription factors Lmx1a, Mash1 and Nurr1 [1]. However, proper reprogramming of fibroblasts to neurons is still under debate. Therefore, astrocytes have the advantage of being a predominant neural cell type in the central nervous system. In addition, they are more prone to mdDA neurons. Hence, it is an ideal population for direct conversion into patient-derived mdDA neurons for cell replacement therapies. Certainly, virus-based vectors are detrimental for therapeutic use in humans due to adverse effects. For this reason, protein transduction constitutes to be an alternative method to avoid any application of foreign nucleic acids. A protein transduction domain (PTD), which is fused to the protein of interest, allows the cellular uptake of the protein.

Here, we present a label free proteomic analysis of astrocytes directly converted to neurons. The mass spectrometry data were obtained from an LTQ-Orbitrap Velo coupled to Proxeon nano-HPLC. In embryonic B6J mouse astrocytes cultures transfected with the transcription factors Sox2, Mash1, Lmx1a and Nurr1 a number of proteins were identified which are newly expressed as neural and pro-neuronal specific proteins. Additionally, glial cell characteristic proteins were also regulated. The effect of the transfected transcription factors during the astrocyte conversion to neurons was verified by analysis of downstream target mRNA using quantitative real time PCR (qRT-PCR). Furthermore, we evaluated the expression of key candidate genes by immunocytochemistry and western blot. Moreover, we report the expression of human Mash1 and Lmx1a, which are fused to the protein transduction domain TAT derived from trans-activator of transcription. The cellular uptake ability of the fusion proteins HTN-Mash1 and HTN-Lmx1a was explored by Rhodamine-labelling. In addition, the biological activity was validated after transducing these factors into SH-SY5Y cells by analysis of downstream targets mRNA by qRT-PCR.

In summary, these results afford first insights into proteome profiles during the direct conversion of astrocytes to neurons towards patient-derived cell replacement therapies. Furthermore, the generation of the transducible transcription factors Mash1 and Lmx1a is shown which are involved in the conversion process towards dopaminergic neurons.

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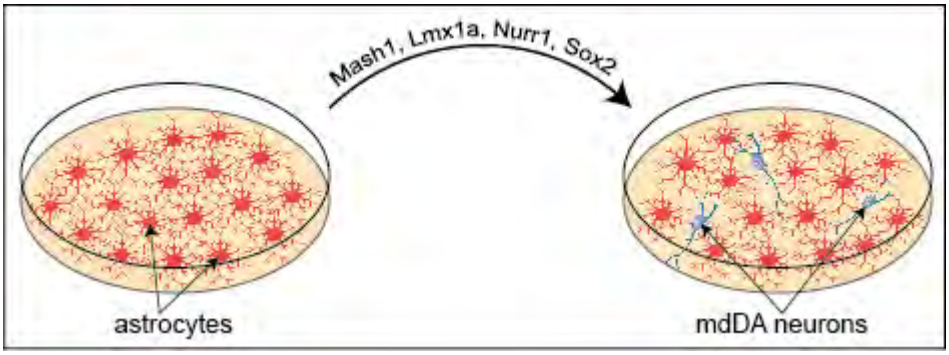
Mash1, Lmx1a, Nurr1, Sox2
astrocytes
mdDA neurons

miRNAs in neurite outgrowth and regeneration of midbrain neurons

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miRNAs are small non-coding RNAs that are important in post-transcriptional regulation of gene expression. The functions of miRNAs in neurons are just beginning to emerge, however there are indications that miRNAs drive neuronal differentiation and morphogenesis through specific expression patterns. Understanding these changes in the miRNAome during neurite outgrowth would lead to new insights into the molecular processes involved and might provide new therapeutic targets to treat diseases of the central nervous system. We employed massive parallel sequencing and present the complete and quantitative miRNAome of murine primary midbrain neurons (PMNs) at different time points of neuronal maturation and neurite outgrowth. Furthermore, we tested the influence of the pro-dopaminergic growthfactor GDNF on the miRNAome of PMNs. Small RNA sequencing revealed that 163 miRNAs are significantly regulated during neuronal maturation, whereas GDNF treatment lead only to minor changes in expression levels of single miRNAs. Using different bioinformatic platforms we identified the most promising miRNA candidates which are analyzed in detail for their potential influence on neurite outgrowth, neurite regeneration and their capacity to act neuroprotective in PMNs. Therefore we transfected PMNs with synthetic miRNA mimics and examined neurite growth, survival after 1-methyl-4-phenylpyridinium (MPP+) treatment and neurite regeneration after scratch lesion in vitro. As the midbrain is central target of neurodegenerative diseases such as Parkinson's disease and has limited regenerative capacities, detailed knowledge about miRNA-mediated regulation of molecular processes involved in neurite outgrowth may be useful for the identification of future therapeutic targets in degenerative CNS diseases.

Molecular imaging of CaV3.2-channel promoter regulation in hippocampi of living mice during epileptogenesis

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Background

Focal epilepsies often originate in the hippocampal formation of the temporal lobe (temporal lobe epilepsy; TLE) and generally not start at birth but are acquired later in life. In many patients, transient insults such as status epilepticus (SE) induce cellular and structural reorganization processes of the hippocampus, referred to as epileptogenesis that finally convert the brain spontaneous epileptic. A better understanding of the molecular basis of epileptogenesis will provide means to retard or even stop the conversion to chronic epilepsy. Recently, we identified the T-type calcium channel CaV3.2 to be differently expressed in the pilocarpine-SE model of epileptogenesis. However, established analyses from brain tissue of animal models naturally only allow observing expression at single time-points but not within an individual brain longitudinally. Here, we developed new molecular imaging strategies to provide insights in key epileptogenic mechanisms.

Materials and Methods

We combined recombinant adeno-associated virus (rAAV) gene delivery with in vivo bioluminescence imaging (rAAV-IVIS). We produced rAAVs bearing the previously characterized CaV3.2 promoter fused to a luciferase reporter. Viruses were stereotaxically injected into the CA1 region of the hippocampus of mice. Luciferase expression, corresponding with CaV3.2 expression, was assayed in the living mice 3 days before and 3, 10 and 25 days after sham/pilocarpine-SE treatment.

Results

Our results showed a specific above background bioluminescent signal over the skull, indicating that sufficient reporter gene activation can be detected. The bioluminescent signal of the CaV3.2 reporter construct increased significantly three days after SE and gradually decreased until 10 days after SE. In contrast, no significant activation was seen in the chronic epileptic stage. Neuropathological analysis of mice 30 days after pilocarpine-SE treatment showed loss of hippocampal CA1 neurons and considerable gliosis. No evident neuropathological damage was observed in the sham-injected animals, indicating that the hippocampal alterations were due to the pilocarpine-SE treatment and not the rAAV-injection.

Conclusion

Our data suggest that interfering with CaV3.2 promoter activation provides a promising anti-epileptogenic target mechanism. Notably, our molecular imaging tool will be ideally suited for CaV3.2 promoter-targeted experimental therapy monitoring. In more general terms, this strategy has great potential to study cerebral gene promoter dynamics longitudinally and correlate to behavioral parameters in the same mice. Furthermore, our rAAV-IVIS technique can be extrapolated into other fields of experimental neuropathology in the future.

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Neuronal Differentiation of Niemann-Pick Type C1 Patient-Specific Induced Pluripotent Stem Cells

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Niemann-Pick type C1 (NPC1) is a rare, progressive neurodegenerative disease, which is inherited in an autosomal recessive manner. Mutations in the NPC1 gene lead to an impaired cholesterol transport resulting in an accumulation of cholesterol in the late endosomes and lysosomes in general and an accumulation of the ganglioside GM2 in the brain in particular. The neurological manifestation of the disease is caused by dysfunction and death of neurons while the pathogenic mechanism is not completely understood. Animal models were used to analyze the impaired pathway, but the loss of neurons is still unexplained and the genetic variability in humans cannot be reflected. Our aim was to generate a human neuronal *in vitro*-model for NPC1, based on patient-specific induced pluripotent stem cells (iPSCs), providing a tool to study the pathogenic mechanism of the disease.

Therefore, iPSCs were generated by retroviral reprogramming of NPC1 patient-specific fibroblasts using the classical four transcription factors SOX2, KLF4, OCT4 and cMYC. Pluripotency of iPSCs was proofed by expression of stem cell markers and pluripotent differentiation potential *in vitro* and *in vivo*. iPSCs were differentiated into neural progenitor cells and neuronal cells, respectively and typical marker expression was immunocytochemical identified. The neuronal cells were analyzed regarding (1) cholesterol accumulation, (2) total cholesterol amount, (3) expression of GM2, and (4) electrophysiological functionality.

After neural induction and 6 weeks of terminal differentiation, cells were positive for the neuronal markers beta III-Tubulin and MAP2 and also for the glial marker GFAP. The proportion of glia cells was much higher in the cell lines with NPC1 mutation hinting at a disease-caused gliosis, which was just described in the NPC1 mouse model so far. In addition, a genotype-dependent storage of cholesterol and GM2 was found displaying specific hallmarks of the disease. Furthermore, the electrophysiological analysis revealed a maturation of neural progenitor cells into functional neurons demonstrated by patch clamp recordings and calcium imaging experiments.

In summary, the here generated human neuronal *in vitro*-model for NPC1 displays disease-specific hallmarks like cholesterol and GM2 accumulation together with a disease associated gliosis. Thus, we are convinced that the generated NPC1 model is not only appropriate to analyze the pathogenic mechanism of the disease, but also provides a platform for drug discovery regarding the treatment of NPC1.

Posttranslational modification and mutation of histidine 50 trigger alpha-synuclein aggregation and toxicity

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Accumulation of aggregated alpha-synuclein (aSyn) in intraneuronal inclusions called Lewy bodies is associated with sporadic and monogenic forms of Parkinson's disease (PD). Recently, a putative novel PD-causing aSyn mutation has been reported separately in two PD kindred, resulting in the substitution of histidine 50 (H50) by a glutamine residue (H50Q). While aSyn mutations are linked to monogenic PD, increasing evidence suggests that oxidative stress-related posttranslational modification contributes to aSyn aggregation and toxicity in sporadic PD. Oxidative stress is accompanied by an increase in reactive oxygen and nitrogen species, as well as in lipid peroxidation leading to the formation of reactive aldehydes, such as 4-hydroxy-2-nonenal (HNE). We have recently shown that HNE modification of aSyn enhances its oligomerization and specifically promotes the toxicity of aSyn to human dopaminergic neurons. Interestingly, our previous results revealed that H50 is one of the target residues for HNE modification, suggesting that H50 alterations play an essential role in both sporadic and monogenic PD.
Thus, we aimed to elucidate the relevance of H50 for HNE-induced aSyn oligomerization and toxicity. Moreover, we investigated whether the novel H50Q mutation has a similar impact on aSyn pathology as HNE modification. Besides the PD-related H50Q mutation, we analyzed a PD-unrelated control mutation, in which H50 was replaced by arginine (H50R).
We show that H50 of aSyn is the residue with the strongest reactivity to HNE addition. Furthermore, H50 is essential for HNE-mediated oligomerization of recombinant aSyn. Overexpression of aSyn with substituted H50 in H4 neuroglioma cells reduces HNE-induced cell damage, indicating a pivotal role of H50 in HNE modification-induced aSyn toxicity. *In vitro* experiments reveal that H50Q/R mutations substantially increase the formation of high density and fibrillar aSyn species, and potentiate the oligomerization propensity of aSyn in the presence of an oxidizing/nitrating agent. Additionally, cell-based experiments indicate that the overexpression of H50Q aSyn in H4 cells promotes aSyn oligomerization. Importantly, overexpression of both H50Q/R aSyn mutants in H4 cells significantly increases apoptosis compared to wild type aSyn and furthermore exacerbates the toxic effect of H_2O_2, suggesting that H50 mutation results in an increased susceptibility to oxidative stress.
In conclusion, we show that both H50 HNE modification and mutation trigger aSyn aggregation and toxicity, supporting a crucial role of aSyn H50 alterations in the pathology of sporadic and monogenic PD.

Reading the code: The role chromatin readers in the healthy and diseased brain.

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Neurodegenerative diseases, such as Alzheimer's Disease (AD), are a huge emotional and economical burden to our societies. Unfortunately, no effective treatment to ameliorate or cure these impairments is yet available. Sporadic AD is a multifactorial disease that is influenced by genetic and environmental factors. Genome-environment interactions critically depend on epigenetic mechanisms and there is emerging evidence for a deregulated histone-acetylation in AD. The histone code hypothesis states that the post translational modifications (PTMs), are added to the histone n-terminus by a diverse group of enzymes often considered as "writers" and removed by another group termed "erasers", resulting in alteration of the electrostatic properties of chromatin. In addition to this chromatin readers bind to a specific combination of PTMS and mediate various biological functions. Here we employ stable isotope labelling by amino acids (SILAC) followed by MS/MS-LC to identify chromatin readers that bind to histone-modifications altered in AD. We identify a number of proteins that bind to combinatorial patterns of histone marks and are currently further analysing them.

RETINAL NEURODEGENERATION, REACTIVE GLIOSIS AND COMPLEMENT ACTIVATION IN PROTEIN TYROSINE PHOSPHATASE MEG2 DEFICIENT MICE

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Purpose: Retinal neurodegeneration is one of the leading causes of blindness worldwide. In the present study we report retinal ganglion cell loss, reactive gliosis and complement activation in transgenic PTP-Meg2 heterozygous (HET) mice. Methods: Intraocular pressure was evaluated in aging PTP-Meg2 HET and control wildtype (WT) animals (n>6/group). Immunohistochemistry, electron microscopy, molecular biological analyses (n>3) and scotopic electroretinogram (ERG) recordings (n=4) were used to characterize degeneration in PTP-Meg2 mice. Results: PTP-Meg2 HET mice develop progressive intraocular pressure elevation upon 10 weeks of age ($p<0.001$). At one year of age, HET animals exhibit a significant loss of Brn3a+ retinal ganglion cells ($p<0.01$) and optic nerve degeneration. ERG recordings reveal that PTP-Meg2 HET mice exhibit reduced a-wave ($p<0.05$ at 0.3, 1 and 25 cd*s/m^2) and b-wave ($p<0.01$ at 0.1 and 1 cd*s/m^2; $p<0.05$ at 0.3 and 3 cd*s/m^2) amplitudes as well as implicit time-to-peak prolongation ($p<0.01$ at 25 cd*s/m^2; $p<0.05$ at 1 and 10 cd*s/m^2). Immunohistochemistry verifies a retinal upregulation of GFAP ($p<0.01$ central; $p<0.001$ peripheral) and vimentin ($p<0.05$ central; $p<0.01$ peripheral). Moreover, an increased number of Iba1+ microglia ($p<0.01$) and an upregulation of C1q and C3 complement components was noted in HET retinae. Conclusion: In conclusion, PTP-Meg2 HET mice may serve as a powerful animal model to study the pathomechanisms involved in onset and progression of retinal neurodegeneration, a hallmark of several retinal diseases.

ROCK-inhibition as a therapeutic approach in models of Parkinson´s disease

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Parkinson´s disease (PD) is the most common neurodegenerative movement disorder and accounts for more than 12,000 new cases per year in Germany. Since the underlying pathogenic molecular mechanisms mostly remain unsolved, disease-modifying therapies which attenuate the pathology and foster regeneration are urgently needed.
Rho-associated kinase (ROCK) has been shown to be involved in the regulation of cellular pathways mediating neuronal survival as well as axonal degeneration, making it a highly interesting target for the treatment of neurodegenerative disorders.
In cell culture and animal models of PD we could show that pharmacological inhibition of ROCK improved neuronal survival and fostered the regenerative potential of axons. In toxin-induced mouse models of PD using 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridin (MPTP) or 6-hydroxydopamine (6-OHDA), oral application of the ROCK inhibitor Fasudil increased the number of nigral dopaminergic neurons, regenerative sprouting to the striatum and striatal dopamine metabolites. Small-molecule ROCK inhibitors like Fasudil, however, are limited in target-specificity and can affect other kinases. We therefore used adeno-associated viral vectors (AAV) expressing short-hairpin-RNA (shRNA) against ROCK2 to knockdown the brain-specific isoform exclusively in neurons. Silencing of ROCK2 protected primary dopaminergic midbrain neurons against 1-methyl-4-phenylpyridinium (MPP+) toxicity and enhanced neurite regeneration in a traumatic scratch model in vitro. In an in vivo mouse model neuron-specific downregulation of ROCK2 protected dopaminergic neurons in the substantia nigra from 6-OHDA-induced degeneration, resulting in significantly increased TH-positive neuron numbers and also improving motor behavior.
Our data demonstrate that neuron-specific inhibition of ROCK2 promotes survival of lesioned dopaminergic neurons and thus confirm ROCK2 as a promising therapeutic target for Parkinson´s disease.

Significant effect of L-DOPA treatment on the transcriptome of a brain area relevant for Parkinson's Disease: induction of TH expression in the striatum

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The loss of dopamine (DA) neurons in the substantia nigra is a hallmark of Parkinson's Disease (PD) and leads to non-motor and motor symptoms. The most commonly used therapy is treatment with L-DOPA, a precursor converted into DA by specific enzymes in the brain, thereby replacing partially the function of the lost cells. Following a period of effective alleviation of PD symptoms, L-DOPA treatment often induces side effects, attributed at least in part to cellular, molecular and functional alterations in the striatum, the brain region with the highest density of DA innervation.

To study if L-DOPA treatment induces changes on the transcription levels or RNA editing in the striatum, we used RNA-Seq and MitoPark mice, a genetic model of PD with slow but progressive degeneration of DA neurons. MitoPark and littermate control mice were treated daily for 3 weeks with either L-DOPA or saline. The comparison of the transcriptome of the four groups revealed that almost 2000 genes were differentially expressed in the two genotypes and that genes higher expressed in MitoPark mice show enrichment in phosphorylation and signaling pathways. Only a few genes showed significant p values for the interaction between genotype and treatment, and top of the list was tyrosine hydroxylase (TH). The rate-limiting enzyme in DA synthesis, TH is highly expressed in all neurons of the DA and noradrenalin system in the brain stem and hypothalamus. A small number of scattered TH positive neurons have previously been identified in the human striatum. The existence and nature of these striatal TH neurons has also been studied in toxin-induced rodent PD models, with partially conflicting results. In MitoPark mice we show the significant induction of TH mRNA expression following L-DOPA treatment with an unbiased RNA-Seq method. We confirmed the TH induction also on the protein level and studied the time line of TH induction and the properties of these cells in our mouse model. In MitoPark mice of all ages analyzed (12 to 32 weeks), modeling progressive stages of PD, and in littermate control mice we found very occasional a TH immunoreactive cell in the striatum. Following L-DOPA treatment the number of striatal TH cells increased sharply only in MitoPark mice in an age and L-DOPA dose dependent way. A detailed characterization of these cells revealed that the expression of TH co-localized with markers of interneurons, including GAD67 or calretinin, whereas DARPP-32, a marker of medium spiny neurons was not detected. To carry out functional studies on the striatal TH neurons, we crossed the MitoPark mouse with a mouse expressing enhanced green fluorescent protein (eGFP) under the control of the TH promoter. The functional analysis confirmed that these eGFP-TH neurons have interneuron-like electrophysiological properties and are certainly not striatal projection neurons.

Finally, we also analyzed TH expression in human brain tissue and confirmed the increased number of TH immunoreactive neurons in the striatum of PD patients, together with significantly increased levels of TH mRNA in both PD caudate and putamen.

Taken together our study demonstrates the induction of a subpopulation of TH expressing neurons in the DA depleted striatum following L-DOPA treatment in a PD mouse model and in PD patients.

Na^+/K^+ pump and Kir channel functional relationship in spinal cord oligodendrocytes in Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by dysfunction of motor neurons in the spinal cord, brainstem and cortex. Accumulating studies have indicated that glial cells contribute to the neuronal degeneration in ALS. Previous research on astrocytes showed an altered expression of the Na^+/K^+ pump as well as the change in the expression and functional properties of inwardly rectifying potassium channels (Kir) in ALS. Glial Na^+/K^+ pump and Kir channels are functionally connected in maintaining the extracellular potassium concentration. The Na^+/K^+ pump is responsible for active potassium uptake while Kir channels mediate potassium spatial buffering, and their combined activity in glia is important for proper neuronal functioning. In the present study, we used whole-cell patch-clamp to investigate the influence of the Na^+/K^+ pump activity on the Kir currents in cultured spinal cord oligodendrocytes of the $hSOD1^{G93A}$ rat model. Experiments were performed on differentiated oligodendrocytes with detectable Kir currents. As oligodendrocytes express alpha1 and alpha2 isoforms of the Na^+/K^+ pump catalytic subunit and Kir4.1 as a major channel subtype, immunofluorescence was used to examine cellular expression of the Na^+/K^+ pump alpha1-subunit and Kir4.1. Data obtained showed that after the inhibition of the Na^+/K^+ pump by ouabain, the Kir currents were completely reduced in both, non-transgenic and ALS oligodendrocytes, but the effect of ouabain was significantly lower in ALS. To further explore the relationship between oligodendrocyte Na^+/K^+ pump and Kir channels, we examined the Na^+/K^+ alpha1-subunit/Kir4.1 colocalization and found that it was not significantly changed in ALS. However, the Na^+/K^+ alpha1-subunit was almost completely colocalized with the Kir4.1 in non-transgenic oligodendrocytes, while in ALS we could observe the portion of the Na^+/K^+ alpha1-subunit signal not colocalized with Kir4.1. By further examination we found that Na^+/K^+ pump alpha1-subunit expression is significantly higher, while expression of Kir4.1 channel is significantly lower in ALS oligodendrocytes. Collectively, our findings indicate that lower magnitude of ouabain effect on Kir currents in ALS is most probably related to the decreased Kir4.1 expression in ALS spinal cord oligodendrocytes. Furthermore, obtained immunofluorescence data suggest that combined role of Na^+/K^+ pump and Kir channels is perturbed in ALS which could cause oligodendrocyte dysfunction and increase vulnerability of spinal cord motor neurons in this neurodegenerative disease.

Sphingosine-1-phosphate Lyase Deficiency in the Brain: Possible Link to Alzheimer's Disease?

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Mice with systemic deletion of sphingosine-1-phosphate lyase (S1PL) exhibit a rather severe phenotype including a retarded growth and reduced body weight to about half of their age-matched littermates. In addition, their life expectancy is limited to no more than 6-8 weeks (1). Neuroanatomical and biochemical studies revealed significant changes in the hippocampus as well as in the cerebellum of these rather young mice when compared with their age-matched wild type littermates (2). Based on these findings we hypothesized that impairment of S1PL activity might be involved in the process of neurodegeneration (3-6). The latter is usually a rather subtle process that progresses gradually over time. The restricted lifetime of S1PL-KO mice, however, prevents age-related studies. To overcome this essential limitation posed by systemic deletion of S1PL in mice, we generated a conditional knockout mouse model with a specific deletion of S1PL in neural tissue. The phenotype of these mice is unremarkable. However, we saw a decreased expression of presynaptic marker proteins and also behavioural changes of animals with neural directed ablation of S1PL. These changes were accompanied by an increased ubiquitination of presynaptic proteins and an increase in proteasomal activity in the S1PL conditional KO mouse brain. Furthermore the autophagy was impaired followed by an age-dependent increase of amyloid precursor protein (APP) expression in brains lacking S1PL. The potential molecular mechanism underlying our observations will be discussed.

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Syt10 is a novel downstream target of NPAS4 and plays a role in synaptic activity-induced neuroprotection

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The neurotransmitter glutamate mediates excitatory synaptic transmission and plays a key role in synaptic plasticity, learning and memory as well as in other cognitive functions. On the other hand excessive stimulation by glutamate causes neuronal dysfunction and degeneration, a pathological process termed excitotoxicity. Glutamate excitotoxcitiy has been suggested as a major mechanism in several human diseases like epilepsy, ischemia, and trauma as well as in neurodegenerative diseases such as Huntington's disease, Alzheimer disease, and Parkinson's disease. In response to excitotoxic insults neurons activate specific regulatory transcription factors in order to up-regulate expression of endogenous protective factors. Synaptotagmin 10 (Syt10) was originally identified as a seizure-induced gene in the hippocampus and was recently shown to control activity-dependent insulin-like growth factor (IGF1) exocytosis from olfactory bulb neurons. However, its role in injury responses induced by seizures has so far remained elusive. Here, we describe a role for Syt10 in protection against cell death of hippocampal neurons triggered by excitotoxic stimuli. Syt10 expression is selectively up-regulated in hippocampal neurons in response to stimuli inducing pathophysiological synaptic activity in vitro and in vivo. Transcript levels of IGF1 are also induced in cultured hippocampal neurons by excessive glutamate release. Notably, we found that Syt10 deficiency renders hippocampal neurons but not glial cells more susceptible to kainic acid induced cell death. At the functional level, our results showed that as reported for the pro-survival growth factor, IGF1, Syt10 was observed to be important for SE-induced progenitor cell proliferation. Mechanistically, we demonstrated that Syt10 gene transcription is regulated by the synaptic activity regulated transcription factor and "activity-regulated inhibitor of death" (AID) gene neuronal PAS domain protein 4 (NPAS4) and the circadian clock gene Period 1 (Per1), both of which are also up-regulated by excitotoxic stimuli. Thus, we have shown that Syt10 is a novel neuroprotective effector downstream of NPAS4.

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The effect of cognitive and motor impairments on the P300 sources in Parkinson's disease patients

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Main symptom of Parkinson's disease (PD) is movement disorder, but different other symptoms may be found in patients. Specific emphasis is directed at cognitive impairment and harbingers of dementia. Cognitive potentials P300 are associated with information processing, memory, attention, and there is an objective method for testing cognitive functions. 3D localization of P300 sources provide data about specific areas of the cortex involved in activity generation.

The aim of the present study was to determine characteristics of P300 generators in PD patients with different laterality of motor symptoms and with varying degrees of severity of cognitive and motor impairments.

78 PD patients (age 46-74, without dementia) and 22 healthy volunteers (age 46-74) were observed. PD patients with right (D, n=36) and left (S, n=42) side of predominance of motor impairments were divided into groups based on the degree of manifestation of cognitive (UPDRS-1 scale, 0-1 and 2-4 points) and movement symptoms (Hoehn-Yahr scale, 2-2.5 and 3 stages). Cognitive potentials P300 were recorded in oddball paradigm task with auditory stimuli using NeuroCom Pro EEG system (XAI-Medica, Ukraine). On the next stage we used sLORETA (standardized low resolution brain electromagnetic tomography) to identify the sources of P300 activity.

Greater severity of motor dysfunction was associated with decreased activity of P300 sources in the right frontal cortex in PD patients (compared to control group). Also the progression of motor symptoms lead to increasing of the area of the cortex involved to generating P300 (mainly parietal and central regions), but the activity of P300 sources decreased. At the same time, between-group comparison by sLORETA showed an increased activity of P300 sources in the right hemisphere in patients with earlier stages of cognitive impairment as compared to control. Also decreased activity of P300 generators in left prefrontal area was shown for these PD patients relatively to healthy people. Higher level of cognitive impairments were associated with increased (compared to controls) activity of P300 sources in the right hemisphere in patients with left-sided motor symptoms and symmetrical changes in patients with right-sided symptoms. This differences between effect of cognitive and movement disorders on P300 sources may confirm the hypothesis of independence of these dysfunctions in PD.

As a conclusion, our study revealed that common feature of P300 generators in PD is the increased activity in the right frontal cortex. Depending on the side of predominance of motor symptoms these differences are mainly related to contralateral hemisphere. The changes in P300 generators have no external symptoms, but may serve as a background of successive dementia in PD patients.

The effect of Curcumin against acute Aluminum intoxication on the dopaminergic system in rat

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Aluminum (Al) is a potent neurotoxic and has been associated to several neurodegenerative diseases such as Parkinson disease, this metal can include changes in many neurotransmitter levels,However, studies of Al effects on brain neurotransmitters are limited. Curcumin is a polyphenol extracted from the rhizome of Curcuma longa and well known as a multi-functional drug with antioxidative activities, the present study was designed to evaluate by means of immunohistochemistry using antibodies against tyrosine hydroxylase (TH; the rate-limiting enzyme of dopamine synthesis) the effect of acute Al exposure and curcumin against Al intoxication.

Experiments were carried out on male wistar rats intoxicated acutely with an intraperitoneal injection of Al (25mg/Kg B.W), (50mg/Kg B.W) and (100mg/Kg B.W),the two Al intoxicated groups (25mg/Kg B.W and 100mg/Kg B.W) received concomitantly

Curcumin by oral gavage (30mg/kg) for 3 days. Our results showed, a significant increase of TH immunolabelling in both of the substantia nigra (SN) and the ventral tegmental area (VTA), this increase of TH immunolabelling has been remedied with daily curcumin administration .

The functional link between autophagy and the trans-cellular spread of alpha-synuclein

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The term synucleinopathy describes the intracellular accumulation of alpha-synuclein (aSyn), which is a major pathological hallmark of several neurodegenerative diseases of the central nervous system, including Parkinson's disease (PD). Synucleinopathy has mainly been studied as an intracellular condition, attributed to the dysfunction of autophagy[1]. However, recent evidence has shown that aSyn is encountered extracellularly, and as such supports the cell-to-cell propagation concept in PD development. aSyn was shown to be secreted partly via exosomes[2] –vesicles generated in the endosomal multivesicular body compartment, which constitutively mediate intercellular communication. Most recent findings from our group have highlighted a connection between autophagy, intracellular aggregation of aSyn and an extracellular aSyn-related microenvironmental response1, *in vitro* and *in vivo* (*Poehler et al., 2014, accepted in Autophagy*).

In the present study, we further focus on this link at a molecular level in our *in vitro* models of synucleinopathy i.e. human neuroglioma (H4) and dopaminergic (LUHMES) cells stably overexpressing WT or aggregating-prone aSyn. Using modulators of the autophagy-lysosome pathway (ALP) we are showing that dysfunction of autophagy directly affects aSyn-related toxicity by affecting exosome secretion. Importantly, WT and aggregating-prone aSyn appears to differentially influence the link between autophagy and exosome secretion. In addition, we examine how alterations in the abundance and lipid composition of autophagosomes and multivescicular bodies under ALP dysfunction, affect aSyn secretion. It appears that both factors are important for aSyn secretion and extracellular aSyn-related toxicity. We are currently in the process of validating our findings in primary cultures from PD-related mouse models and human iPSC-derived neurons from sporadic PD patients.

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The hippocampal CA2 region in temporal lobe epilepsy

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Temporal lobe epilepsy (TLE) is characterized by recurrent epileptic seizures of hippocampal origin and structural changes in the hippocampus. These include the loss of principal cells and interneurons in the Ammon's horn (CA3, CA1) and hilus, reactive gliosis and dispersion of the granule cell layer. Furthermore, mossy fibers (MF) sprout to the dentate molecular layer and form pathologic recurrent circuits. In contrast, CA2 is one of the few hippocampal regions that seem mostly resistant to the pathological alterations of TLE. Yet, the role of CA2 in TLE has not been thoroughly investigated up to now which might be due to limited methods to specifically distinguish CA2 neurons from their neighbors. Recent studies led to a better understanding of the structure and function of CA2 in the healthy hippocampus and, in particular, showed that CA2 cells receive MF inputs (Kohara et al., 2013, Nature Neuroscience; Hitti and Siegelbaum, 2014, Nature). This finding was contrary to the classical definition of CA2 as the region adjacent to CA3 but which does not receive MF input.
To analyze structure, function and synaptic connectivity of CA2 in epilepsy we use the intrahippocampal kainate mouse model for TLE. Here, we ask (i) whether CA2 neurons survive in this model and contribute to epileptic activity and (ii) whether the MF input to CA2 is altered in TLE. To this end we inject kainate (saline in controls) into the septal hippocampus of adult male mice and implant electrodes into the CA2 region and the dentate gyrus at several septotemporal positions for in vivo recordings. Subsequently, we perform immunocytochemistry with CA2-specific antibodies (regulator of G-protein signaling 14 (RGS14) and Purkinje-cell protein 4 (PCP4)). To display MF inputs to CA2 we use Thy1-eGFP mice (M-line, Feng et al., 2008, Neuron) in which in particular adult granule cells, their dendrites and axons express GFP.
We show that kainate-injected mice develop epileptic activity which reaches its maximum ~21 days after injection and involves the dentate gyrus and the CA2 region. Nevertheless, apparently most CA2 pyramidal cells survive after kainate injection, even in areas close to the injection site as revealed by PCP4 and RGS14 immunostaining. These cells receive mossy fiber connections which we will characterize in detail.
We conclude that the survival of CA2 cells in the TLE model is comparable to the resistivity of the CA2 region in human TLE and thus offers an easily accessible system to investigate the role of CA2 in epilepsy.

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The Role of *Drosophila* APPL (Amyloid Precursor Protein Like) Protein in Brain Function and Behaviour.

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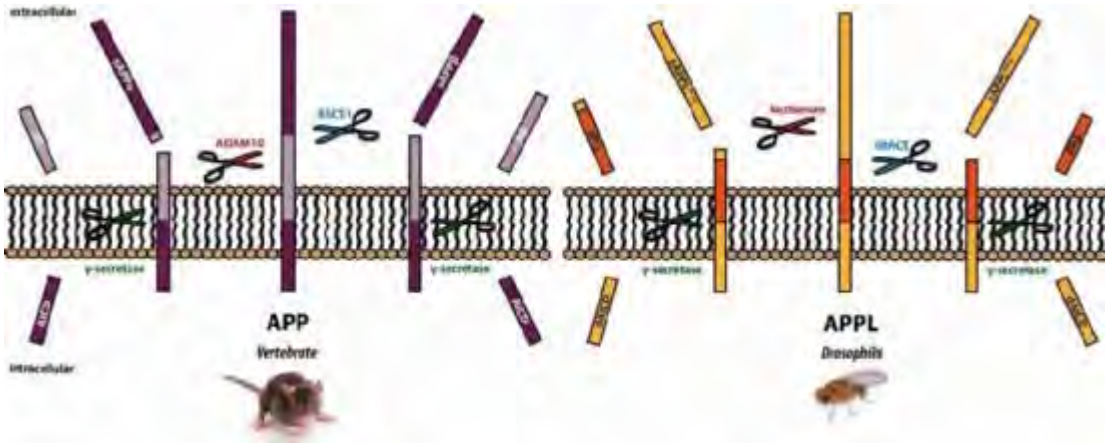
The amyloid precursor protein (APP) has been identified in the search for the causative agent of Alzheimer's disease. Altered proteolytic processing of APP in the brain, leading to increased production of the neurotoxic Aß-amyloid peptide and subsequent plaque formation is a hallmark of Alzheimer's. The APP gene is conserved from nematodes to man but its endogenous function remains elusive by large. The evolutionary conservation of APP genes suggests a beneficial endogenous role for brain integrity and function. The central hypothesis of this work is that the balanced differential processing of the *Drosophila* APP orthologue protein APPL has a crucial role for the function of the central nervous system.

The genome of *Drosophila* encodes orthologues for all three main types of secretases [*kuzbanian* (kuz) = a-secretase; *dBace* = ß-secretase, and *Presenilin* (Psn) = γ-secretase] and APPL is processed in a similar way as human APP. Altered ratios of full-length APPL and its proteolytic fragments may differentially affect brain connectivity and synaptic efficiency. Changes in synaptic function and connectivity of neuronal networks are most sensitively monitored by changes in behaviour. To assess how a change in *Drosophila* APPL processing affects behaviour we have initiated an age-dependent comparative study on heterozygous mutants for always one of the genes that encode these three secretases in different behavioural paradigms. Walking and visual orientation abilities are being tested in Buridan's Paradigm and different forms of memory in a courtship-suppression paradigm and in the detour paradigm. By this means we identify beneficial or detrimental roles of individual APPL fragments for brain integrity and function.

For instance, analysis of a visual working memory revealed that wild-type flies lose the ability to memorize target objects that disappeared from sight at the age of four weeks. Surprisingly, heterozygous flies for any of the three secretases display an improved orientation memory when compared to age-matched controls of up to six weeks. Interestingly, aged heterozygous *Notch* mutants (like APPL a substrate for KUZ and PSN) behaved like wild-type flies. This result suggests that a reduction in APPL processing is beneficial for the working memory and it also excludes a simple heterosis effect in heterozygous secretase mutations. Moreover, analysis of hypomorphic and null-mutants for the *APPL* gene revealed an essential role of APPL for the visual working memory.


extracellular
APP
ADAM10
BACE1
γ-secretase
AICD
intracellular
APP
Vertebrate
APPL
Drosophila

The role of intracellular Ca^{2+} stores for neuronal dysfunction in a mouse model of Alzheimer's disease

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Alzheimer's disease (AD) is a progressive and fatal neurodegenerative disorder that slowly destroys neurons and cognitive abilities. Familial AD is linked to mutations in a specific set of genes, most often in the genes encoding amyloid precursor protein (APP) and the presenilins 1 and 2 (PS1, PS2). Several studies demonstrated that the missense mutations in PS genes perturb endoplasmic reticulum (ER) Ca^{2+} homeostasis. *In vitro*, such perturbations result in an increase in Ca^{2+} level within the ER Ca^{2+} stores and in a reduction of the capacitive Ca^{2+} entry. However, contribution of intracellular Ca^{2+} stores to neuronal Ca^{2+} signaling *in vivo* and their role for AD-related neuronal dysfunction remain unclear.

We studied the *in vivo* function of intracellular Ca^{2+} stores in cortical neurons by using two-photon Ca^{2+} imaging in 10-14 months old transgenic APP23xPS45 mice (AD mice; Busche et al. 2008) and age-matched wild type (WT) littermates. The Ca^{2+} release from stores was activated using an agonist of ryanodine receptors (RyRs) caffeine. Under our experimental conditions, brief (40-50 ms-long) pressure applications of caffeine induced large Ca^{2+} transients in amyloid depositing and WT mice. The amplitudes of these Ca^{2+} transients were similar in both mouse strains but the duration of the transients at a half amplitude (T/2), decay time constant tau, and normalized area under the transient were significantly higher in amyloid depositing mice compared to WT littermates ($p=0.04$, $p=0.04$, and $p=0.01$ respectively, Mann-Whitney test). Next, we monitored spontaneous activity of layer 2/3 neurons in AD and WT mice and classified cells as silent, normal or hyperactive based on the frequency of their Ca^{2+} transients (according to criteria listed in Busche et al. 2008). Interestingly, a substantial fraction of hyperactive cells (21.6%) was found in WT mice of this age group. In mutant mice the fraction of hyperactive cells (40%) increased further indicating that disease progression aggravates neuronal hyperactivity. To decipher the contribution of Ca^{2+} release from the intracellular Ca^{2+} stores to spontaneous neuronal activity, the stores were emptied by the reversible inhibitor of SERCA pumps Cyclopiazonic acid (CPA). Topical CPA application significantly reduced the frequency of spontaneous Ca^{2+} transients in hyperactive and normal cells of mutant mice ($p<0.01$). In addition, the fraction of hyperactive neurons decreased dramatically in the presence of CPA, suggesting that hyperactivity is selectively reduced upon store depletion.

Thus, *in vivo* function of intracellular Ca^{2+} stores is altered in amyloid depositing mice as suggested by prolonged RyR-mediated Ca^{2+} release signals. Furthermore, intracellular Ca^{2+} stores are critically involved in controlling the frequency of spontaneous Ca^{2+} transients in AD mice. Our findings pave the way for the development of new therapeutic strategies based on normalizing Ca^{2+} release from the intracellular Ca^{2+} stores.

*a*Abnormal modification of ALS-associated mutant E102Q Sigma receptor-1leads to ER stress-mediated defects in protein degradation and endosomal trafficking.

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AA major pathological hallmark of amyotrophic lateral sclerosis (ALS) are abnormal accumulations of misfolded proteins as intracellular inclusion bodies. Recently, mutations in Sigma receptor 1 (SigR1) have been found to cause a form of ALS and frontotemporal lobar degeneration (FTLD). But how mutations in SigR1 lead to the ALS pathogenesis is unclear thus far.

AMutant proteins exert neuronal toxicity either by loss of function mechanism or by toxic gain of function . We previously described the neuropathological consequences of loss of SigR1 protein in neuronal cell lines. In the present study we focused on the neuropathology induced by the ALS-causing mutant SigR1 (E102Q) and the underlying molecular mechanisms. We showed that the E102Q SigR1 protein is highly unstable and accumulates in the ER, which induces ER stress and cellular toxicity. Beside with the accumulation of mutant SigR1 and several other proteins involved in protein quality control, deranged calcium signaling and abnormalities in mitochondria, endoplasmic reticulum and Golgi structures were observed in cells over expressing mutant SigR1. Along with alterations of ER-Golgi structure, disturbances in vesicular trafficking and endo-lysosomal pathways were also evident in cell lines expressing mutant SigR1 protein. Data obtained from primary lymphoblast cultures derived from ALS patients harboring the same mutation confirmed these findings. Our data suggestthat mutant SigR1 induces neuronal toxicity by inducing ER stress, deranged calcium homeostasis and defective endo-lysosomal pathways.

Deletion of the amyloid precursor protein family members APP and APLP2 results in aberrant changes of the mouse hippocampal presynaptic active zone proteome

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The amyloid precursor protein (APP) belongs to a conserved gene family that comprises in mammals the amyloid precursor like proteins 1 and 2 (APLP1 and APLP2). Interestingly, double knockout mice, lacking APP and APLP2 die shortly after birth due to impairments in neuromuscular transmission. To circumvent the lethality, the NexCreAPP/APLP2 mouse line (NexCre-cDKO) was recently designed allowing the analysis of the APP/APLP2 deletion in excitatory hippocampal neurons at any age. By analyzing NexCre-cDKO knockout mice we evaluated the impact of APP/APLP2 deletion at the hippocampal presynaptic active zone proteome derived from mouse brain. Following immunopurification of synaptic vesicles attached to the presynaptic plasma membrane, individual proteins were subjected to quantitative proteomic approached based on an isobaric labeling (TMT, tandem mass tag). Hippocampal PAZ derived from three individual NexCre-cDKO mice and three individual control mice (APLP2-KO) were labeled with an isobaric tandem mass tag that allows the identification and quantification of immunopurifed proteins. The chemical mechanism is based on a substitution of the NHS-reactive group by the free e-amino-group of lysine residues. In the course of LC-separation, labeled peptides co-elute due to the identical structure and mass of the TMT reagents and can further be co-isolated during MS/MS analysis. The fragmentation of the labeled peptides is induced by a CID (collision-induced dissociation) leading to unique reported ion mass that is finally used for protein identification and quantification. Quantification of proteins is performed by comparing the relative intensities of the six reporter ions in the MS/MS spectra. We demonstrate for the first time that APP/APLP2 deletion affects the abundance of proteins involved in Ca2+-homeostasis (calmodulin, neurogranin, neuromodulin), and two members of the synuclein family (a-synuclein and ß-synuclein). Interestingly, a-synuclein is an important key player in SNARE-complex fusion and the severe downregulation at presynaptic release sites may account for the reported impairments in LTP associated with deficits in learning and memory (Hick et al., 2014). Furthermore, the additional deletion of APLP2 in NexCre-cDKO may be responsible for differentially regulation in PAZ protein abundance (e. g. a-synuclein) as compared to the PAZ hippocampal proteome derived from APP-KO mice. Taken together, the deletion of the active zone constituents APP and APLP2 brought to light numerous differences in abundance for hippocampal PAZ constituents pointing to a crucial role for APP and APLP2 in synaptic transmission.

Deletion of the amyloid precursor protein results in aberrant changes of the hippocampal presynaptic active zone proteome in mouse brain

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The amyloid precursor protein (APP) has been allocated to an organellar pool residing in the Golgi apparatus and in endosomal compartments, and in its mature form to the presynaptic active zone proteome (PAZ). By analyzing homozygous APP knockout mice we evaluated the impact of APP deletion at the hippocampal presynaptic active zone proteome derived from mouse brain. Following immunopurification of synaptic vesicles attached to the presynaptic plasma membrane, individual proteins were subjected to quantitative proteomic approached based on an isobaric labeling (TMT, tandem mass tag). Hippocampal PAZ derived from three individual APP-KO mice and three individual wildtype control mice were labeled with an isobaric tandem mass tag that allows the identification and quantification of immunopurifed proteins. The chemical mechanism is based on a substitution of the NHS-reactive group by the free e-amino-group of lysine residues. In the course of LC-separation, labeled peptides co-elute due to the identical structure and mass of the TMT reagents and can further be co-isolated during MS/MS analysis. The fragmentation of the labeled peptides is induced by a CID (collision-induced dissociation) leading to unique reported ion mass that is finally used for protein identification and quantification. Quantification of proteins is performed by comparing the relative intensities of the six reporter ions in the MS/MS spectra. We demonstrate for the first time that APP deletion affects the abundance of proteins involved in Ca2+-binding (calmodulin, annexin A5) and Ca2+-regulation (neuromodulin, neurogranin), the endocytotic machinery (clathrin light chain A, epsin-2), the extracellular matrix (nidogen-2, laminin), the synaptic vesicle compartment (synaptogyrin, synaptophysin) and a member of the solute carrier family (the glucose transporter 1). Interestingly, a common feature of the majority of these proteins is their dysregulation in Alzheimer`s disease (AD). Our data suggest that APP has an impact on the abundance of proteins at the presynaptic release sites and thus impact synaptic physiology.

Poster Topic

T12: Neuroimmunology, Inflammation, and Neuroprotection

T12-1A Activated microglia induce deficits in excitatory synapses through IL-1ß: Implications for cognitive impairment in sepsis
Carolina A Moraes, Gabriel Santos, Tania Cristina Leite de Sampaio Spohr, Joana D'Avila, Flávia Regina Souza Lima, Claudia Farias Benjamim, Fernando A Bozza, Flávia CA Gomes

T12-2A Anti-DPPX encephalitis: Pathogenic effects of antibodies on gut and brain neurons
Johannes Piepgras, Markus Höltje, Klaus Michel, Qin Li, Carolin Otto, Christoph Drenckhahn, Christian Probst, Ralph Buchert, Michael Schemann, Winfried Stöcker, Josep Dalmau, Gudrun Ahnert-Hilger, Klemens Ruprecht

T12-3A Anti-inflammatory role of heme oxygenase-1 / carbon monoxide in functional assays using co-cultures of microglia and human model neurons
Hannah Scheiblich, Gerd Bicker

T12-4A Brain endothelial specific knockout of NEMO causes blood brain-barrier disruption and mimics neurological symptoms of incontinentia pigmenti
Jan Wenzel, Dirk A. Ridder, Kristin Müller, Kathrin Töllner, Hartwig Wolburg, Stijn Stroobants, Xin-Kang Tong, Rudi D´Hooge, Detlef Balschun, Wolfgang Löscher, Edith Hamel, Markus Schwaninger

T12-5A CD14 control over microglial TLR4 functions involves an IFNß-mediated feedback mechanism
Christin Fritsche, Hana Janova, Uwe-Karsten Hanisch

T12-6A Changes of BDNF-mediated signaling mechanisms during the formation of neural networks and in the acute normobaric hypoxia in vitro
Tatiana Alexandrovna Sakharnova, Maria Valerievna Vedunova, Elena Vladimirovna Mitroshina, Irina Vasilievna Mukhina

T12-7A Comparative analysis of stem-cell-markers in matched primary and recurrent glioblastomas
Charlotte Flüh, Kirsten Hattermann, H. Maximilian Mehdorn, Rolf Mentlein, Janka Held-Feindt

T12-8A Crossreactivity of antibodies to Neisseria gonorrhoeae with the heat shock protein Hsp60 correlates with reduced mitochondrial activity in the human choroid plexus papilloma cell line HIBCPP
Bernhard Reuss, Horst Schroten, Hiroshi Ishikawa, Abdul Rahman Asif

T12-9A Decompressive craniectomy for prevention of secondary brain damage in patients with traumatic intracranial hematomas.
Jamshid Kuzibaev, Kozim Makhkamov

T12-1B Erythropoietin promotes survival of insect neurons via receptor-dependent signalling
Natasa Miljus, Jan Vincent Rison, Ralf Heinrich

T12-2B Euphorbia Resinefera extract induced pain decrease on mice
Ouassil EL KHERCHI, Hanane Khalki, Maryam Mountassir, Abdelmajid ZYAD, Abderrahman Chait

T12-3B Evaluation of inflammatory and synaptic protein alterations during acute, sub-acute, and chronic inflammatory response following status epilepticus (SE)
Una Avdic, Idrish Ali, Christine T Ekdahl

T12-4B Gut microbiota influences Lipopolysaccharide-induced depressive-like behaviors by inflammatory mechanism: involvement of adult hippocampal neurogenesis and serotonergic neurotransmission.
Alline Cristina Campos, Natalia Pessoa Rocha, Jacques R Nicoli, Leda Q Vieira, Mauro M Teixeira, Antonio L Teixeira

T12-5B Interactions between signaling mechanisms of neurotrophic factors BDNF and GDNF during normobaric hypoxia in vitro
Maria Valerievna Vedunova, Tatiana Alexandrovna Sakharnova, Elena Vladimirovna Mitroshina, Alexey Alexandrovich Babaev, Tatiana Victorovna Shishkina, Natalia Alexandrovna Shchelchkova, Irina Vasilievna Mukhina

T12-6B Intralesional transplantation of mesenchymal stem cells in the toxic demyelinating cuprizone model.
Laura Salinas Tejedor, Kristin Jacobsen, Gabriel Berner, Viktoria Gudi, Nicole Jungwirth, Florian Hansmann, Wolfgang Baumgärtner, Thomas Skripuletz, Martin Stangel

T12-7B Lose and use your head! Inverse signaling of transmembrane chemokines in gliomas
Kirsten Hattermann, Henrike Gebhardt, Ralf Lucius, Andreas Ludwig, Rolf Mentlein, Janka Held-Feindt

T12-8B Microglia activation in mice showing depressive-like behavior after chronic Interferon-alpha treatment
Simone Wachholz, Manuela Eßlinger, Jennifer Plümper, Marie-Pierre Manitz, Georg Juckel, Astrid Friebe

T12-1C Neurogenesis in organotypic hippocampal slice cultures is strongly affected by glial cell activation and inflammatory processes
Johannes Gerlach, Catharina Donkels, Gert Münzner, Carola A. Haas

T12-2C Neurotrophins BDNF and NGF in patients with affective disorders
Lyudmila Levchuk, Natalya Vyalova, German Simutkin, Svetlana Ivanova, Nikolay Bokhan

T12-3C Polymeric nanoparticles as drug carriers across the blood-brain barrier: an effective and non-toxic system
Petra Henrich-Noack, Nadine Voigt, Sarah Kockentiedt, Werner Hintz, Jürgen Tomas, Bernhard A. Sabel

T12-4C PRENATAL IMMUNE CHALLENGE INDUCES CHANGES OF MICROGLIAL SURFACE MARKERS IN AN ANIMAL MODEL OF SCHIZOPHRENIA
Manuela Eßlinger, Marie Pierre Manitz, Simone Wachholz, Rainer Sommer, Jennifer Plümper, Awatef Esshili, Georg Juckel, Astrid Friebe

T12-5C Primary brain cell cultures of *Tribolium castaneum* as a model to study erythropoietin signaling pathways
Nina Hahn, Ralf Heinrich

T12-6C QUANTITATIVE ASSESSMENT OF BLOOD-BRAIN BARRIER PERMEABILITY AND CELL DAMAGE AFTER CORTICAL ISCHEMIA - ROLE OF FREE RADICALS
Karl Schoknecht, Ofer Prager, Udi Vazana, Lyn Kamintsky, Yoash Chassidim, Uwe Heinemann, Alon Friedman

T12-7C Responses of mouse retinal ganglion cells to experimentally induced hypoxia / ischemia
Gabriel Christian Knop, Anneka Göppner, Bianca Assmann, Andreas Feigenspan

T12-8C Role of p75 neurotrophin receptor in mediating neuronal alterations in *Toxoplasma gondii* chronically infected mice
Jan Alexander Kleveman, Marianna Weller, Alexandru Parlog, Ildiko Dunay, Marta Zagrebelsky, Martin Korte

T12-9C Alterations of the glial activation markers in rat chronic mild stress model
Weronika Dominika Duda, Katarzyna Curzytek, Marta Kubera, Eimear Fagan, Thomas J. Connor

T12-1D Systemic inflammation is associated with a reduction in Synaptopodin expression in the mouse hippocampus
Andreas Strehl, Maximilian Lenz, Ze'ev Itsekson-Hayosh, Denise Becker, Joab Chapman, Thomas Deller, Nicola Maggio, Andreas Vlachos

T12-2D THE EFFECT OF METHYLENE BLUE ADMINISTRATION ON CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY
Teodora Alexa, Andrei Luca, Catalina Roxana Bohotin

T12-3D The effect of N-arachidonoyldopamin (N-ADA) on functional homeostasis of neural networks in normal conditions and in modeling of acute hypoxia
Elena Vladimirovna Mitroshina, Maria Valerievna Vedunova, Tatiana Alexandrovna Sakharnova, Mikhail Yurievich Bobrov, Leonid Georgievich Khaspekov, Irina Vasilievna Mukhina

T12-4D The Effect of Neuroinflammation Induced by Influenza A virus infection on Hippocampal Neuron Morphology
Shirin Hosseini, Kristin Michaelsen-Preusse, Esther Wilk, Klaus Schughart, Martin Korte

T12-5D The influence of patients glutamate receptor 2 antibodies on AMPA receptor mediated transmission
Holger Haselmann, Benedikt Grünewald, Christian Geis

T12-6D The transmembrane chemokine CXCL16 transduces “inverse signaling” effects in human

meningiomas
Janka Held-Feindt, Kareen Bartsch, Henrike Gebhardt, Maximilian Mehdorn, Rolf Mentlein, Kirsten Hattermann

T12-7D Thermal Impact of Optogenetic Laser Light Stimulation on Neural Tissue
Gonzalo Arias Gil, Frank Ohl, Kentaroh Takagaki, Michael T. Lippert

T12-8D Comparing neuroprotective actions in a rat model of retinal degeneration
Mattia Di Paolo, Darin Zerti

Activated microglia induce deficits in excitatory synapses through IL-1ß: Implications for cognitive impairment in sepsis

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Recent clinical studies have shown that sepsis survivors may develop long-term cognitive impairments. The cellular and molecular mechanisms involved in these events are not well understood. This study investigated synaptic deficits in sepsis and the involvement of glial cells in this process. Septic animals showed memory impairment and reduced numbers of hippocampal and cortical excitatory synapses, identified by synaptophysin/PSD-95 co-localization, nine days after disease onset. The behavioral deficits and synaptophysin/PSD-95 co-localization were rescued to normal levels within 30 days post-sepsis. Septic mice presented activation of microglia and reactive astrogliosis, which are hallmarks of brain injury and could be involved in the associated synaptic deficits. We treated neuronal cultures with conditioned medium derived from cultured astrocytes (ACM) and microglia (MCM) that were either nonstimulated or stimulated with lipopolysaccharide (LPS) to investigate the molecular mechanisms underlying synaptic deficits in sepsis. ACM and MCM increased the number of synapses between cortical neurons in vitro, and these effects were antagonized by LPS stimulation. LPS-MCM reduced the number of synapses by 50%, but LPS-ACM increased the number of synapses by 500%. Analysis of the composition of these conditioned media revealed increased levels of IL-1ß in LPS-MCM. Furthermore, inhibition of IL-1ß signaling through the addition of a soluble IL-1ß receptor antagonist (IL-1 Ra) fully prevented the synaptic deficit induced by LPS-MCM. These results suggest that sepsis induces a transient synaptic deficit associated with memory impairments mediated by IL-1ß secreted by activated microglia.

Key words: sepsis, microglia, astrocyte, synapse, and cognitive deficit

Anti-DPPX encephalitis: Pathogenic effects of antibodies on gut and brain neurons

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Limbic encephalitis (LE) is an autoantibody mediated neurological disease which affects the central nervous system, predominantly the hippocampal formation. Seizures, short-term memory dysfunction, and topographical disorientation due to hippocampal malfunction are just some of the possible symptoms that patients suffering from LE may present. The recently discovered anti-DPPX encephalitis is a novel form of LE with antibodies targeting the dipeptidylpeptidase-like protein 6 (DPPX), a regulatory protein associated with voltage gated potassium channels Kv4.2 and Kv4.3, playing a pivotal role in establishing dendritic electrophysiological properties. In these patients, massive diarrhea precedes the onset of neurological symptoms. We here report a novel case of anti-DPPX encephalitis. Additionally, we studied effects of DPPX antibodies collected from four patients on neuronal function. We show immunocytological and –histological stainings of enteric (ENS) and central nervous system (CNS) neurons using patients' sera. Brief application of patients' sera led to increased activity of ENS neurons evaluated by neuroimaging studies. Incubation of cultured murine hippocampal neurons resulted in a reduction of DPPX and Kv4.2 expression shown by biochemical approaches. Taken together, we here present first experimental proof of antibody effects on neuronal function in anti-DPPX encephalitis suggesting that antibodies cause the symptoms in this novel form of LE.

Anti-inflammatory role of heme oxygenase-1 / carbon monoxide in functional assays using co-cultures of microglia and human model neurons

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Clearance of infected and apoptotic neuronal corpses during acute inflammatory conditions within the central nervous system (CNS) is a fundamental process for creating a favorable environment for neuronal regeneration. Microglia are the resident immune cells and the predominant phagocytic cells of the CNS playing a crucial role during neuroinflammation. There is growing evidence that excessive activation of microglia in response to CNS infections or spinal cord injury (SCI) constitutes a critical event causing neurodegenerative processes. The gaseous messenger carbon monoxide (CO) has been postulated to have neuroprotective and anti-inflammatory properties. CO is generated by the enzyme heme oxygenase (HO) during the degradation of heme to biliverdin-IX, ferrous iron (Fe2+), and CO. In microglia the HO-1 isoform has been found to be induced by various oxidative agents e.g. lipopolysaccharide (LPS). Here, we developed an in vitro approach to test for a neuroprotective function of CO during an inflammatory process. We used LPS as stimulator of acute inflammatory processes to induce alterations from resting to reactive microglia of cells from the mouse microglial cell line BV-2. LPS-induced activation of microglia was accompanied by an increased nitric oxide (NO) release (Scheiblich et al. 2014). Co-application of the CO-releasing molecule CORM-II prevented NO production of microglia to control level. In a phagocytosis assay using apoptosis-induced neurons from the human NT2 cell line we showed the ability of BV-2 microglia to engulf neuronal remnants. End points of phagocytosis were defined by time-lapse video analysis and confocal immunodetection. LPS stimulation of microglia highly increased engulfment of apoptotic neuronal remnants in both resting and reactive microglia. However, induction of HO-1 or application of exogenous CO attenuated phagocytic activity of microglia significantly. Moreover, we highlighted the harmful effects of activated microglia on neurite outgrowth of developing human model neurons. LPS activated microglia inhibited neurite elongation significantly - even without direct cell-cell contact. Inhibition of neurite outgrowth was totally reversed by the induction of HO-1 or the application of exogenous CO. Taking together, our findings suggest that HO-1 induction and application of CO can modulate microglial activity upon immunostimulation with LPS and can provide neuroprotective effects during inflammatory processes.

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Brain endothelial specific knockout of NEMO causes blood brain-barrier disruption and mimics neurological symptoms of incontinentia pigmenti

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Incontinentia pigmenti (IP) is a hereditary disease caused by mutations in the NF-κB essential modifier (NEMO). In addition to skin pathologies, patients often suffer from epileptic seizures and other neurological symptoms. As NEMO deletion in different cell types of the brain does not result in an IP-like phenotype, the aim of this study was to examine the effects of a NEMO knockout specifically in brain endothelial cells.

Therefore, we used mice carrying an inducible Cre recombinase which is expressed only in brain endothelial cells (Slco1c1-ER^{T2}) and crossbred these mice with a mouse line carrying the NEMO gene flanked by loxP sites. The resulting NEMO^{beKO} mice developed a disruption of the blood-brain barrier as shown by increased brain weight, IgG extravasation and increased permeability to fluorescent tracers. These effects were accompanied by cerebral hypoperfusion and apoptosis of endothelial cells. The alterations in the structure and function of the blood-brain barrier resulted in a change of behavior. NEMO^{beKO} mice were less active in the running wheel, lost body weight and were more anxious. Additionally, these mice developed epileptic seizures with generalized as well as focal activities.

Our data demonstrate an important role of NEMO in brain endothelial cells for protecting the blood-brain barrier and preventing cell death. These functions could be an explanation for the neurological symptoms of patients suffering from IP and might be a therapeutic target in the future.

CD14 control over microglial TLR4 functions involves an IFNß-mediated feedback mechanism

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Microglia are the innate immune cells of the central nervous system (CNS). They are capable of sensing infection and damage through various receptors and consequently trigger an appropriate immune response. In this regard, Toll-like receptor 4 (TLR4) can signal upon recognition of both pathogen-associated molecular patterns (PAMPs), like bacterial lipopolysaccharide (LPS), and damage-associated molecular patterns (DAMPs), such as fibronectin. We had previously shown that microglial TLR4 signaling in response to PAMPs and DAMPs is distinctly organized and that the TLR4 co-receptor CD14 has important regulatory functions. CD14 is mandatory for TLR4-mediated microglial responses to CNS damage, as demonstrated by the lack of microglial reactions to fibronectin in the absence of CD14. Additionally, CD14 keeps microglial responses to infectious challenges in a moderate range. It increases cell-specifically the sensitivity of microglia to low amounts of LPS and protects against overshooting responses to high amounts of LPS. CD14 especially suppresses overproduction of the chemokine CXCL1, thereby preventing excessive neutrophil infiltration into the brain. This regulation of CXCL1 depends on an interferon ß (IFNß)-mediated feedback mechanism, which involves the interferon-a/ß receptor (IFNAR) and downstream components of the JAK-STAT signaling pathway. In the absence of CD14, the production of IFNß is impaired leading to loss of the negative regulation. In the presence of CD14, IFNß prevents CXCL1 overproduction in response to PAMPs and DAMPs. IFNß could thereby prove to be a key factor in regulating TLR4-mediated microglial reactions to CNS infection and damage.

Changes of BDNF-mediated signaling mechanisms during the formation of neural networks and in the acute normobaric hypoxia in vitro

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Brain-derived neurotrophic factor (BDNF) is an important member of the neurotrophin family of growth factors, abundant in the brain and the periphery nervous system. Various effects that it exerts on the nervous system have been recognized, such as neuronal outgrowth, differentiation and formation of synaptic contacts in the brain development. In addition to its classical neurotrophic effects BDNF may be considered as one of the potential substances to control cell metabolic rate under hypoxemia. But not all mechanisms have discovered yet. In this study we have investigated the fast effect of BDNF action on the spontaneous bioelectrical activity of hippocampal dissociated culture networks in different stages of development and during acute normobaric hypoxia in vitro.

Dissociated hippocampal cells were taken from the brain of CBA mice embryos (E18) and cultured by the previously developed protocol on the multielectrode arrays (MED64, Alpha Med Sciences; MEA60, Multichannel Systems). A single application of BDNF (0,1 ng/ml, 1 ng/ml, 10 ng/ml) was conducted into culture medium on 7th, 14th and 21 days in vitro (DIV). The modeling of hypoxia was performed after 21 day by replacing the normoxic culture medium by a medium with low oxygen for 10 minutes. The main characteristics of spontaneous neural network activity were investigated: a number of small bursts; a number of spikes per burst, a burst duration. For the immunostaining principal cytoskeleton protein of neurons (MAP2) and astrocytes (GFAP), and the tropomyosin-related kinase B (TrkB) receptor were marked in dissociated hippocampal cultures on the 7th day after acute normobaric hypoxia.

It was shown the single application of different BDNF concentrations on 14th and 21 DIV to modify the spontaneous bioelectrical activity by changing the duration of bursts mainly. On the 7th DIV this effect was not observed. The acute normobaric hypoxia to cause the negative effect on the viability of primary cultures. Immunocytochemical staining of TrkB receptor showed the presence of changes in the distribution and the expression of the receptor on the membrane of neurons after hypoxia. Preventive application of BDNF (1 ng/ml) reduced the consequences of oxygen deficiency by partial preservation of the functionally active neurons. Using the selective antagonist of TrkB receptor - k252a (150 nM) partially offset the positive effect of BDNF during hypoxia and in the posthypoxic period.

We suppose antihypoxic and neuroprotective properties of BDNF were mainly caused by the activity of TrkB receptor and the subsequent launching of signaling mechanisms.

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Comparative analysis of stem-cell-markers in matched primary and recurrent glioblastomas

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Embryonal stem cell (ESC) markers play a crucial role in the development of many malignancies. Studies revealed that so called neural stem cell (NSC) markers might play a role in brain tumors, where the NSC expression levels are correlated with the tumor grading. Nevertheless, comparative studies on primary WHO °IV glioblastomas versus relapses are currently not available. Therefore, our study investigates the expression profile of different neural and embryonal stem cell markers in matched primary and recurrent glioblastomas, analyzes their coexpression with markers of tumor differentiation in situ and evaluates the effect of chemotherapy on transcriptional gene regulation in vitro.

Quantitative qPCR of homogenates from matched surgical primary and relapse samples of 11 individual patients revealed, that in comparison to primary tumors, the NCS markers c-Myc, CXCR7, Musashi-1, Oct-4 and Nanog were downregulated in relapses. In contrast, Sox-2, Klf-4 and CXCR4 were not differentially transcribed in primary versus reccurent glioblastomas. These results were partly in line with quantitative analysis in situ: analysis revealed that the number of CXCR4 positive cells increases in recurrent tumors, whereas the number of CXCR7 positive cells decreases. The number of cells costained with one of the markers (e.g. Oct-4, Klf-4 or Sox-2) and CXCR4 increases in recurrent tumors whereas the number of cells costained with CXCR7 decreases. The number of Klf-4 positive cells decreases in recurrent glioblastomas. No effect was seen concerning Sox-2.

Additionally, glioma cell lines were stimulated with the chemotherapeutic drugs temozolomide (400µg/ml; 6, 24, 48h) or camptothecin (100µg/ml; 6, 24, 48h), and transcriptional regulation of NSC-markers was determined by qPCR. Temozolomide was able to induce upregulation of Klf-4 and Oct-4 in T98G cells. There was no effect seen for Sox-2, Nanog, c-myc, and Musashi-1. Camptothecin was able to induce upregulation of Klf-4 in T98G and A172 cells. Preliminary results show that some of the markers stimulate each other.

Summarized, the expression of stem cell markers and their regulation by temozolomide and camptothecin is complex. It seems that especially Klf-4 and Oct-4 play a pivotal role in recurrent glioblastomas and in the interplay with the chemokine receptors CXCR4 and CXCR7.

Crossreactivity of antibodies to Neisseria gonorrhoeae with the heat shock protein Hsp60 correlates with reduced mitochondrial activity in the human choroid plexus papilloma cell line HIBCPP

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Maternal infection with the Gram negative bacterium Neisseria gonorrhoeae (NG) during the first trimester of pregnancy, increases the risk for the offspring to develop schizophrenic psychosis in later life[§,&]. We investigated here interactions of a polyclonal antiserum directed to NG (a-NG) with HIBCPP-cells, a human choroid plexus papilloma cell line, and an established *in vitro* model for the human choroid plexus epithelium. As shown by immunocytochemistry, in HIBCPP-cells a-NG labels antigens within an intracellular organelle. As revealed by Western blot analysis, a distinct band of around 60kDa could be detected in HIBCPP cells, together with several weaker bands of higher and lower molecular weights. Two dimensional gel electrophoresis followed by Western transfer and immune incubation revealed crossreactivity of a-NG with a set of distinct protein spots the most prominent could be identified by LC-Q-TOF mass spectrometric analysis as mitochondrial heat shock protein Hsp60. This result could be confirmed by Western blot analysis for interaction of a-NG with a commercial Hsp60 protein sample. Finally, a 24h incubation with 10µg/ml a-NG was able to reduce mitochondrial activity in HIBCPP-cells significantly, as revealed by the fluorescent mitochondrial activity indicator JC-1. To our knowledge, this is the first report that cross-reactivity of a-NG specific anti-bacterial antibodies with the heat shock protein Hsp60 is able to impair mitochondrial activity in human choroid plexus papilloma cells. Further experiments will have to clarify the importance of this interference for choroid plexus functioning *in vivo*.

§ Babulas et al., 2006, Am. J. Psychiatry 163, 927-929

& Sørensen et al., 2009, Schizophrenia Bull. 35, 631-637

Decompressive craniectomy for prevention of secondary brain damage in patients with traumatic intracranial hematomas.

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Introduction: Traumatic intracranial hematomas represent a challenge for neurosurgeons due to their high mortality and morbidity. Secondary brain damage is an important factor that influences the outcome of traumatic intracranial hematomas. In the event of severe traumatic brain injury the object of surgery is not only to remove intracranial hematomas, but also to preform decompressive craniectomy for prevention of secondary brain damage. To evaluate the effect of decompressive craniectomy in prevention of secondary brain injury we compared the effect of decompressive craniectomy with that of non- decompressive craniectomy on the outcome of patients with secondary brain damage following traumatic intracranial hemorrhage.

Method: A retrospective review was conducted of 127 consecutive patients who presented with secondary brain damage following isolated severe head injury with intracranial hematomas. Early decompressive craniectomy after hematoma removal (mean time from injury: 5.8±2.5 h) was carried out in 82 patients (mean age: 45.7±5.6 years), whereas 45 patients (mean age: 43.4±4.1 years) were underwent only hematoma removal without decompressive craniectomy (mean time from injury: 6.1±3.1 h). All patients in two groups were comparable due to the level of consciousness of patients, volume and localization of hematoma, severity of secondary brain injury and axial dislocation of middle brain structures.

Results: Due to postoperative CT results volume of secondary brain injury zone was reduced 2.4 times more in patients who underwent early decompressive craniectomy compared with the patients without decompressive craniectomy. Axial dislocation of middle brain structures was decreased from 11.4±3.7 mm to 1.8±0.8 mm in the early decompressive craniectomy group, and from 8.9±4.5 mm to 4.4±2.5 mm in non-decompressive craniectomy group.

Conclusions: Early decompressive craniectomy, employed prior to the onset of irreversible ischemic changes, may be an effective method of prevention of secondary brain damage following traumatic intracranial hematomas.

Erythropoietin promotes survival of insect neurons via receptor-dependent signalling

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Erythropoietin (Epo), apart from regulating vertebrate erythropoiesis, has been recognized as a tissue protective cytokine that initiates adaptive cellular responses against diverse harmful stimuli. Beneficial functions of Epo in mammalian nervous systems are mediated through its binding to a cell surface membrane receptor and subsequent activation of intracellular signalling cascades. A dimer consisting of the classical hematopoietic Epo receptor (EpoR) monomer associated with an unidentified, and probably tissue-specific, partner has been suggested to initiate Epo's neuroprotective functions.

We hypothesize that tissue-protective functions of Epo evolved before its hematopoietic role in vertebrates and study neuroprotective and neuroregenerative effects of recombinant human Epo (rhEpo) in insects. We have previously demonstrated that rhEpo promotes the survival of primary cultured brain neurons from *Locusta migratoria* exposed to apoptosis-inducing conditions including hypoxia and H7-exposure. This neuroprotective effect depends on the activation of a Janus Kinase (JAK)-associated receptor and phosphorylation of signal transducers and activators of transcription (STAT). Both, the type of receptor and the activated intracellular signalling pathway seem to be similar to those described in the mammalian nervous system. Mammalian Epo receptors are endocytosed following ligand binding and receptor activation. Current studies with the styryl dye FM1-43 revealed that rhEpo stimulation also induces receptor endocytosis in Epo-responsive locust brain neurons. The results of our studies indicate the presence of a neuroprotective ligand/receptor system in insects that shares high structural and functional similarities with the mammalian non-hematopoietic Epo signalling system.

Euphorbia Resinefera extract induced pain decrease on mice

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Euphorbia resinifera is a native species of Morocco, where it occurs on the slopes of the Atlas Mountains. It is a shrub growing to 60 cm tall, with a rapidly clumping columnar with unique, four-sided almost square stems (erect, quadrangular) and brown thorns. It will mound, branch and spread.
This euphorb is one of the oldest documented medicinal plants. The plant extract is used for drastic, purgative and emetic properties. The plant extract is used as a novel treatment of pain.
The goal of our study is to evaluate the anti-nociceptive activity of this extract on mice by using different behavioral tests such as the hot plate test, writhing test and formalin test.
Our results show that treated mice with plant extract, showed a noticeable decrease of pain by using pharmacological tests (hot plate, formalin and writhing tests).
Data may clarify the possible involvement of the Euphgorbia resinifera extract on the future treatment of some diseases such as cancer.

Evaluation of inflammatory and synaptic protein alterations during acute, sub-acute, and chronic inflammatory response following status epilepticus (SE)

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Today there are no biomarkers for epilepsy and the current anti–epileptic treatment is successful in less than 70%. Few of the current anti–epileptic treatments target the immune system or synaptic adhesion molecules. Synaptic adhesion molecules are known to control the over all activity within a synapse, both inhibitory and excitatory. In animal models of epilepsy and brain inflammation, altered synaptic protein expression is evident (Jackson et al 2012, Chugh et al 2013). Modulating adhesion molecules may therefore be a new way to “tune” the synaptic activity (Ekdahl 2012) and dampen hyperexcitability in epilepsy. However, it is afflicted by several side effects. Selective immune–modulation is more attractive. But recent evidence suggests that brain inflammation is not only detrimental but also favorable for brain repair (Ekdahl et al 2009). Therefore it is of vital importance to establish and characterize the nature of the inflammatory response and to do this in not only the long term perspective but also to characterize the acute inflammatory patterns in the epileptic brain and learn more about the alterations in protein expression during and after seizure activity.

Our objective in this study is to evaluate the inflammatory and synaptic protein changes that occur during the acute, subacute, and chronic inflammatory response following status epilepticus (SE) and evaluate their potential as biomarkers for epilepsy and epileptogenesis. We study a panel of synaptic proteins (PSD-95, gephyrin, NL-1, NL-2 etc.) and cytokines (IL-10, IL-6, TNF-a etc.) during epileptogenesis in different brain areas in a rat model of SE. The SE is electrically-induced in the hippocampus of adult male Sprague –Dawley rats. Electrode-implanted rats with no stimulation are used as controls for the SE- stimulated rats. Animals are sacrificed at different time points post-SE induction. Subsequently, brains are removed for immunohistochemistry, western blot (WB) and ELISA analysis. Cardiac blood is also collected. Our results show an acute up-regulation of the pro-inflammatory cytokines in the cortex and hippocampus at 6 hours following SE, as well as increased levels of the mitogenic cytokine KC/GRO (or CXCL1) in cortex, hippocampus and subcortex, Conversely, we found decreased levels of anti-inflammatory cytokines.

The results implicate that there is a substantial increase in pro-inflammatory markers in the brain 6h after status epilepticus. Since changes in cytokine levels can also be detected in the serum, the alterations we detect may work as combinatorial biomarkers, which would have great relevance in the clinical setting in terms of diagnostics/prognosis and possibly even characterization of seizure-type.

Gut microbiota influences Lipopolysaccharide-induced depressive-like behaviors by inflammatory mechanism: involvement of adult hippocampal neurogenesis and serotonergic neurotransmission.

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Changes in the microbiota composition in GI tract are emerging as potential players in the physiopathology of neuropsychiatric disorders. In the present work we suggest that gut microbiota affects depressive-like behaviors by modulating neuroinflammatory mechanisms. Methods: Germ-free (GF) or control (Crtl) male mice were treated or not with E. coli lipopolysaccharide (LPS i.p.; 0.83mg/Kg). Twenty-four hours later animals were submitted to the forced swimming test (FST), Tail Suspension Test (TS) or Sucrose Preference Test (SP). After behavioral evaluation, prefrontal cortex and hippocampus (HC) of mice were dissected for cytokines and neurotrophins (BDNF and NGF) determination. In an independent group of animals doublecortin (DCX) and IBA-1 expression was determined in the hippocampus, while ΔFosB was analyzed in the dorsal raphe nucleus. Results: LPS injection induced increase in the immobility time in FST and TS in Crtl mice as compared to vehicle-treated and GF groups. The same panorama was found in SP. Behavioral alterations were followed by an up-regulation in the expression of TNF-a, IFN-γ and the microglial activation marker, IBA-1 and a reduction in the expression of DCX in the HC of LPS-treated Ctrl group. These effects were not observed in LPS-treated GF mice. GF mice exhibited an increase in the number of DCX positive cells in the HC and ΔFosB positive cells in the dorsal raphe nucleus, events not influenced by LPS. Conclusions: Our results suggest that gut-microbiota interactions influence depressive-like behavior, HC neurogenesis and raphe nucleus activation, which is, partly, dependent on the involvement of inflammatory mediators.

Interactions between signaling mechanisms of neurotrophic factors BDNF and GDNF during normobaric hypoxia in vitro

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Hypoxia is considered as the main factor of ischemic tissue damage. Modified oxygen metabolism of neurons leads to changes in the processes of synaptic transmission, cell death and destruction of neural networks in the brain. Brain-derived neurotrophic factor (BDNF) and Glial cell line-derived neurotrophic factor (GDNF) as neurotrophic factors participate not only in the differentiation of neurons and the formation of synaptic contacts in the brain development, but also can be active correctors of mature neuron's metabolism. Despite the fact that the previous data have shown the role of neurotrophic factors in brain cells protection during hypoxia, interactions between these factors remain unclear. In this regard, the aim of investigation was to study the influence of interrelations between molecular effects of BDNF и GDNF in the modeling of normobaric hypoxia in vitro.

Dissociated hippocampal cells were taken from the brain of mice embryos (E18, CBA line) and cultured by the previously developed protocol on the multielectrode arrays (MED64, Alpha Med Sciences; MEA60, Multichannel Systems). The modeling of hypoxia was performed by replacing the normoxic culture medium by a medium with low oxygen for 10 minutes. The main characteristics of spontaneous neural and calcium activity as well as the viability of cells were investigated. Also commercial RNA detection probes (SmartFlareTM, Millipore) allowed us to evaluate the RNA expression of NFkB1 and BDNF. Moreover, for enhancing the expression of endogenous BDNF the viral vector based on the adeno-associated virus were used.

The carried out experiments revealed the application of BDNF (1ng/ml) and GDNF (1ng/ml) during hypoxia to preserve the neural and the functional calcium activity and the viability of cells within 7 days of the posthypoxic period. The combination of BDNF and GDNF reduced the protective effects each of them. In addition, the antihypoxic effect of BDNF implemented at the TrkB-related activation of NFkB1. Blockade of TrkB-receptor (k252a, 150nM) reduced the level of NFkB1 mRNA expression. The level of BDNF expression in hypoxia-exposed cells were investigated. It was evaluated the possibility of enhancing the synthesis of BDNF in the posthypoxic period using GDNF and BDNF applications. Also it was shown the role of TrkB receptors in the protective effect of GDNF during normobaric hypoxia in vitro. Possible molecular mechanisms of BDNF and GDNF interaction in correction of disorders caused by normobaric hypoxia were identified.

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Intralesional transplantation of mesenchymal stem cells in the toxic demyelinating cuprizone model.

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Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system that leads to demyelination and progressive axonal damage and subsequently, to loss of neurological functions. Remyelination is the natural repair mechanism, but it is often incomplete or fails in MS lesions. To date, therapeutical treatments to enhance remyelination are not available. Recently, the transplantation of exogenous mesenchymal stem cells (MSC) has emerged as a promising tool to enhance repair processes. MSC has been reported to exert beneficial effects on the course of experimental autoimmune encephalomyelitis (EAE), which presents a widely used inflammatory model of MS. Here, we investigated the role of MSC on remyelination using the toxic cuprizone model of demyelination.

To induce demyelination C57BL/6 male mice were treated with cuprizone for up to 5 weeks. At the peak of disease (week 4) single injections containing 1 million human, murine, or canine MSC were applied into the corpus callosum via a stereotactic procedure. Mouse brains were investigated using histological and immunohistochemical methods.

Our results show that MSC did not exert any effects on cuprizone induced de- and remyelination. Immunohistochemical analyses revealed no changes in myelination and glial reactions including oligodendrocytes, microglia and astrocytes.

In conclusion, local application of MSC was not able to be identified as a new strategy to enhance remyelination in mice in our model of toxic induced demyelination.

Lose and use your head! Inverse signaling of transmembrane chemokines in gliomas

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Objectives: Chemokines, small chemotactic cytokines, and their receptors play a decisive role in tumor progression, dissemination, invasion and metastasis. Most of the 50 human chemokines are secreted 7-10 kDa peptides. Two exceptions, CXCL16 and CX3CL1=fractalkine, are synthesized as large, highly glycosylated transmembrane (tm) proteins comprising a chemokine domain, an extracellular stalk region a single membrane-spanning domain and an intracellular tail. The chemokine domain can be released from by constitutive or induced “shedding” through action of cell surface proteases of the ADAM family (a disintegrin and metalloproteinase). Whereas both transmembrane chemokines are abundantly produced in gliomas (and other types of tumors), their G protein-coupled receptors are strictly limited to either microglia/macrophages (CX3CR1) or very small subpopulation of tumor cells (CXCR6). We here report about new receptor-independent signaling mechanism of the transmembrane chemokines CXCL16 and CX3CL1.

Methods and Results: As shown by quantitative RT-PCR and immunohistochemistry CXCL16 and CX3CL1 are heavily expressed by glioma cells in situ and in vitro. In contrast, their receptors cannot be detected on glioma cells (or other tumor cells) in vitro. These cells however respond to stimulation with sCXCL16 or sCX3CL1 by activation of intracellular kinases, proliferation and rescue from apoptosis. Searching for alternative receptors we detected that the expression of the tm-chemokines is mandatory for binding of and responsiveness to s-chemokines. We concluded that transmembrane ligands themselves act as receptors for the shed soluble chemokines and thereafter generate auto- or paracrine signals which is depending on their intact intracellular tail. We term this novel mechanism “inverse signaling” in homology to “reverse signaling” where a transmembrane ligand transduces signals after binding to its receptor (or antibody) that was also observed.

Conclusions: Transmembrane chemokines play an important para- and autocrine roles in gliomas. Paracrine actions via classical receptors support microglial or stem-cell attraction and homing. Auto-/paracrine “inverse signaling” is a novel feedback and fine-tuning system in gliomas between tumor cells which supports tumor stabilization and proliferation.

Microglia activation in mice showing depressive-like behavior after chronic Interferon-alpha treatment

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Microglia activation in mice showing depressive-like behavior after chronic Interferon-alpha treatment
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Introduction:
It is increasingly recognized that inflammatory immune processes are associated with a higher prevalence of depressive symptoms. Immune stimulatory treatment with Interferon (IFN) alpha in patients with malignant melanoma or chronic hepatitis c frequently induces major depressive syndroms. An understanding of exact mechanisms is currently lacking. The aim of this study was to investigate the role of microglia in an established Interferon-alpha mouse model of immune mediated depression.

Methods:
Male Balb/c mice were treated with daily injections of IFN-alpha for two weeks. Anxious and depressive-like behavior was analyzed by behavioral tests such as open field (OF), novel object (NO), light dark (LD), forced swim (FST), and tail suspension (TST) test. Activation of microglial cells was analyzed by expression of cell surface markers by flow cytometry.

Results:
IFN-alpha treated mice showed significantly increased immobility time in the FST and TST indicating presence of depressive-like behavior, while anxious behavior did not differ statistically significant. Interestingly, we could clearly separate IFN-alpha treated mice in non vulnerable and vulnerable individuals. Only the latter reacted with an increase of depressive-like behavior and also a significant increase of anxious behavior in the OF and LD test. In whole brain, IFN-alpha treatment led to a Th1 type I activation of microglial cells as indicated by a significantly increased percentage of cells expressing MHC-II, CD86, and CD54. Moreover, only the IFN-alpha vulnerable subgroup was specifically characterized by a higher percentage of CD200R+ microglia.

Conclusions:
Repeated IFN-alpha stimulation induces an anxious and depressive-like behavioral phenotype only in vulnerable BALB/c mice. Reasons for high or low vulnerability are currently unknown, but a dose dependent effect of IFN-alpha might be considered. Th1 type activation of microglial cells seemed to be independent from the behavioral response. Only CD200R interacting with CD200 on neurons was specifically enhanced in IFN-alpha vulnerable mice. Its relevance for the development of behavioral changes needs to be tested by future studies.

Neurogenesis in organotypic hippocampal slice cultures is strongly affected by glial cell activation and inflammatory processes

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In the adult mammalian brain the generation of new neurons is restricted to two distinct and highly specified areas (so-called neurogenic niches): the subventricular zone and the subgranular zone (SGZ) of the hippocampus (HC). SGZ neurogenesis comprises a sophisticated sequence of proliferation, differentiation and migration, which is affected by a plenty of physiological and pathological factors.
In order to study pathological conditions and factors, leading to disturbances of HC neurogenesis, we made use of organotypic hippocampal slice cultures (OHSC), which contain the SGZ, but nevertheless gradually lose their neurogenic capacity for so far unknown reasons. Because tissue is damaged by the slice preparation process, glial cells become strongly activated and start to synthesize inflammatory signaling molecules, which is also a key feature of many CNS pathologies. We therefore hypothesized that overshooting activation of glial cells could be the main cause of the neurogenic decline.
To address this question, we quantified the extent of neurogenesis in OHSC by using a transgenic mouse model (POMC-EGFP), in which EGFP is expressed under the proopiomelanocortin promotor exclusively in newborn granule cells. EGFP signal intensity was measured daily during the in vitro course and shows a fast and continuing decrease during the first week of culturing. In addition, we analyzed the gene expression profile of OHSC by qRT-PCR and found that important neurogenic markers like Hes5 and doublecortin (DCX) also decrease in their relative gene expression, while markers of glial cell activation (e.g. nestin and MHCII) are strongly induced.
We therefore tried to interfere with gliosis by different pharmacological treatments. To enhance glial activation, we applied CNTF (ciliary neurotrophic factor), which is released by activated astrocytes and in turn further stimulates astroglial functionality. CNTF treatment accelerated the decline of the EGFP signal and lowered the expression of DCX. To counteract the glial reactivity, we applied a P2Y12 receptor antagonist and indomethacin. Both treatments exhibited protective effects on the neurogenic outcome and delayed the in vitro decline of neurogenesis. While indomethacin significantly increased the EGFP signal intensity as well as the gene expression of Hes5, treatment with the P2Y12 receptor antagonist significantly raised neuroD and doublecortin expression levels. Therefore, strong inflammation seems to be one of the most detrimental events affecting neurogenesis and anti-inflammatory treatments seem to provide a potent tool to protect neurogenesis under pathological conditions. This could be of particular medical interest, as in most CNS diseases strong glial cell activation and disturbance of their physiological functionality is centrally involved.

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Neurotrophins BDNF and NGF in patients with affective disorders

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The results of modern biological research of affective disorders are very ambiguous, there are many hypotheses of depressive disorders with contribution of psychological, social, genetic, biochemical, immunological, endocrine and other factors. However, there is still no clear idea about the causes and mechanisms of affective disorders. Classical monoamine hypothesis of depression does not fully explain the pathogenesis of affective disorders, suggesting involvement of other mechanisms in the pathogenesis of these disorders. Currently new biological models of the pathogenesis of affective disorders are developed, which give crucial irregularities in neurogenesis and neuronal plasticity.

Objectives Purpose of the study was to study the content of brain-derived neurotrophic factor and nerve growth factor in the serum of patients with a current depressive episode according to the nosologycal forms of affective disorders and mentally healthy persons.

Methods We studied the concentration of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in the serum of 97 patients with a current depressive episode (F32, F33, ICD-10) and 54 mentally healthy persons. The concentrations of neurotrophins was determined by enzyme immunoassay using reagent kits Human BDNF Immunoassay (R&D Systems) and Human Beta-NGF Elisa Kit Protocol (Ray Biotech, Inc) and automatic microplate spectrophotometer Epoch BioTek Instruments (USA). Statistical analysis was performed using the program SPSS, version 20.0.

Results and discussion The severity of the current depressive episode in Hamilton Depression Rating Scale (HDRS-17) in the group of patients was 23,50 (16,25-30,50) points, which corresponds to moderate depression. The study of BDNF in the total group of patients showed significantly reduced level of concentration in patients compared with healthy individuals (4852,02 (4052,7-4985,15) and 4950,33 (4625,03-5003,58) pg/ml). The study of the concentration of NGF in patients showed a slight decrease in the content of NGF in patients compared with control (10,38 (9,87-11,31) and 10,94 (10,01-11,73) pg/ml).

Patients with affective disorders were presented by 2 nosological forms: recurrent depressive disorder and single depressive episode. The study of BDNF and NGF in patients depending on the nosology of affective disorder found that level of BDNF in patients with a single depressive episode was significantly lower than concentration of BDNF in mentally healthy individuals (4758,32 (3997,10-4969,28) and 4950, 33 (4625,03-5003,58) pg/ml, p=0,006). Concentration of BDNF in patients with recurrent depressive disorder is slightly below compared with control values (4826,74 (4005,65-4989,25) and 4950,33 (4625,03-5003,58) pg/ml, p=0,068). The content of NGF in patients with a single depressive episode is different from the values of healthy individuals at the level of trend (10,38 (9,64-11,31) and 10,94 (10,01-11,73) pg/ml, p=0,064), whereas patients with recurrent depressive disorder characterized by reduced content of nerve growth factor compared with control (10,38 (10,01-11,68) and 10,94 (10,01-11,73) pg/ml).

Conclusions Thus, patients with current depressive episode characterized by changes in the neuroprotective and neurotrophic system, reduced content of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in serum.

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Polymeric nanoparticles as drug carriers across the blood-brain barrier: an effective and non-toxic system

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Polybutylcyanoacrylate nanoparticles (PBCA NP) have been suggested as a drug delivery system which can cross the blood-brain barrier (BBB). However, the evaluation of their possible toxicity has been controversial. We therefore investigated the potential neurotoxicity of PBCA NP not only with in vitro test systems but also by long-term in vivo assessment of neuronal survival after repeated injection of different PBCA NP variants (different coatings, different zeta-potential: Tween80; Tween80-SDS; Tween80-Dextran; Lutrol SDS; Lutol-Dextran; DEAE-Dextran-Lutrol).

PBCA NP were fabricated with various surfactants and viability of HeLa and HEK293 cells after NP incubation was quantified by analysing cellular metabolic activity (MTT test). In addition, we repetitively injected i.v. rhodamine-labelled PBCA NP variants into rats and monitored the survival and morphology of retrogradely labelled neurons in the retina by in vivo confocal neuroimaging (ICON) for five weeks. To test for carrier-efficacy and safety, PBCA NP loaded with Kyotorphin were injected in rats and a hot plate test was used to quantify analgesic effects as compared to controls.

In vitro we detected dose-dependent induction of cell death which was, however, only present at very high doses and mainly seen in the cultures incubated with NP fabricated with the tensids SDS and Tween (not dextran). However, the in vivo experiments did not show any obvious changes in the rat's behaviour and no NP-induced neuronal death, even with particles which were toxic at high dose in vitro, i.e. NP with Tween and SDS. More detailed analysis of the neuronal morphology revealed some effects of NP treatment, though; e.g. a transient cellular shrinkage after repeated injection of NP coated with Tween80-SDS. However, the interpretation whether this is due to cellular changes or due to effects of the NP on the fluorescent dye distribution still awaits further investigations. Additional experiments indicated that also the integrity of the BBB is not compromised after NP application as a fluorescent dye injected shortly after NP application was not detected in the parenchyma but only in the vessels. The histological analysis done on peripheral organs (heart, liver, kidneys) did not show any abnormalities, too. An increased pain threshold at the hot plate test in animals injected with Kyotorphin-loaded NP (in contrast to animals injected with unbound Kyotorphin) demonstrated that PBCA NP can effectively carry compounds across the BBB and thus comprise a useful tool for drug delivery into the central nervous system (CNS).

Our findings indicated for the first time that chronic treatment with polymeric NP which cross the BBB does not lead to neuronal death or degeneration and that there is no induction of general BBB leakage. The particles can transport a sufficient amount of an analgesic drug into the CNS to induce measurable behavioural effects. These results may encourage further research to exploit the potential of polymeric NP as carriers for investigational new medicines for the treatment of damage and diseases of the CNS.

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PRENATAL IMMUNE CHALLENGE INDUCES CHANGES OF MICROGLIAL SURFACE MARKERS IN AN ANIMAL MODEL OF SCHIZOPHRENIA

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Background: Epidemiological studies indicate that maternal infection during pregnancy (e.g. influenza) is associated with a higher incidence of schizophrenia and related disorders in the offspring. It is hypothesized that an inflammatory immune response interferes with normal fetal brain development. Long-lasting changes of this developmental disruption might provide a neural basis for enhanced vulnerability which might manifest in schizophrenia in the adult descendants. We suggest microglial cells as possible initiator for this enhanced vulnerability. In previous studies we showed a significant alteration of microglial cell numbers in the adolescent offspring of Poly(I:C) injected mice compared to controls. Here we suggest a long term effect on microglia properties manifested in an altered expression pattern of pro- and anti-inflammatory surface markers due to prenatal immune challenge.
To test this hypothesis, we used the Poly(I:C) mouse model of maternal immune activation which is a valid and reliable animal model of schizophrenia.

Methods: We analysed pro- and anti-inflammatory surface markers on microglia of descendants from Poly(I:C) vs. saline exposed BALB/c mice at postnatal days 30 and 100 by flow cytometric analysis.

Results: Analysis showed clear sex dependent differences in microglia surface marker expression between treatment groups. At day 30 (puberty), female offspring from Poly(I:C) exposed mice showed significant microglia activation by up-regulation of M1 related activation markers (CD54, CCR2) and strong down-regulation of regulatory M2 associated markers (CD124, CD206). Microglia activation was not detected in adult female mice (day 100). In contrast, male descendants did not show any signs of activation neither in adolescence (day 30) nor in adulthood (day 100).

Conclusion: The alteration of microglia surface marker expression in our animal model corresponds with our prior study, in which we showed an increased microglial cell number in the brains of the adolescent offspring from Poly(I:C) exposed mice. Here we show significant changes in M1 and M2 associated surface markers which indicate modified activation patterns of microglia populations based on prenatal infection. We detected a shift towards a M1 phenotype of the microglial cells of Poly(I:C) descendants regarding our screened surface markers. This points to the significance of immunological processes in schizophrenia.

Primary brain cell cultures of *Tribolium castaneum* as a model to study erythropoietin signaling pathways

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The cytokine erythropoietin (Epo) is a trophic factor which was first known for its function in the vertebrate hematopoietic system regulating erythropoiesis in dependency of pathological stressors. However, Epo and Epo receptor (EpoR) activity has also been demonstrated in non-hematopoetic tissue including the nervous system. We assume that tissue protection might have been the ancient beneficial function that shares structural and functional similarities between vertebrates and invertebrates. This hypothesis is further supported by the presence of Epo genes in non-mammalia including puffer fish and zebra fish. Whereas hematopoiesis is mediated via a homodimeric EpoR, tissue protective effects depend on a different receptor. At least for some cell types, this receptor seems to be a heteromer of on classical EpoR protein and a second unknown partner. As to the lack of hematopoiesis, insects appear to be promising model systems to study the Epo/EpoR signaling via the heterodimeric EpoR in neural tissue. We used *Tribolium castaneum* as a model system since it is amenable to functional gene analysis via RNAi and expresses a orthologue of the putatively involved cytokine receptor like factor 3 (CRLF3). CRLF3 is an orphan class I cytokine receptor and, like EpoR, a member of group 1. In humans, CRLF3 and EpoR share intracellular signaling pathways including signal transducers and activators of transcription (STAT). The CRLF3 orthologue in *Tribolium* is au3.g2971.t1.

We established *Tribolium* primary brain cell cultures and investigated the effect of recombinant human Epo (rhEpo) showing that rhEpo dose-dependently reduces overall cell viability. Furthermore, we performed RNAi by letting cultured cells soak dsRNA that targets the CRLF3 orthologue from the growth medium. After application of dsRNA, the deleterious effect of rhEpo was reduced indicating that the CRLF3 orthologue could be involved in Epo-signaling. Moreover, we aim to elucidate the involved intracellular signaling pathways using specific inhibitors. Our study yielded evidence that *Tribolium castaneum* is a suitable model organism to study Epo signaling pathways in primary brain cell cultures using RNAi.

QUANTITATIVE ASSESSMENT OF BLOOD-BRAIN BARRIER PERMEABILITY AND CELL DAMAGE AFTER CORTICAL ISCHEMIA - ROLE OF FREE RADICALS

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Stroke is one of the leading causes of death and morbidity worldwide and its treatment remains a clinically unmet challenge. The ischemic brain is typically characterized by an ischemic core and a peri-ischemic zone prone to undergo cell damage. The peri-ischemic brain may either recover or deteriorate, leading to secondary stroke progression involving complications, e.g. increasing intracranial pressure, hemorrhagic transformation, delayed cognitive decline and epileptogenesis. Recently, blood-brain barrier (BBB) dysfunction has been indicated as a potential common denominator for post-stroke complications. However, to date methods to quantify BBB permeability and cell damage in-vivo are limited. Here we introduce a novel imaging technique designed to investigate the spatial and temporal correlation of BBB dysfunction and cell damage in-vivo in the peri-ischemic region of rose bengal-induced neocortical photothrombosis.

BBB permeability and cell damage were assessed in rats through pial surface imaging (open cranial window method) following the peripheral injection of the tracer molecules fluorescein sodium salt (BBB permeability) and propidium iodide (PI, cell damage). We demonstrate that BBB permeability increases most prominently in the perfused region surrounding the ischemic core within minutes after thrombus formation. The region of augmented vascular permeability gradually expands and is associated with increasing uptake of PI into cells, suggesting progressive cellular damage within the peri-ischemic brain. In addition, we give preliminary evidence that inhibition of free radical signaling reduced the progression of cell damage, however did not affect BBB permeability in the peri-ischemic brain.

This implicates that BBB dysfunction is not driven by parenchymal cell damage and the debate whether BBB dysfunction increases cell damage remains open and demands future studies.

Responses of mouse retinal ganglion cells to experimentally induced hypoxia / ischemia

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Retinal hypoxia / ischemia occurs due to insufficient blood supply causing lack of oxygen and nutrients, respectively, to the retinal tissue. This situation may arise directly through local defects of the circulatory system, like in central retinal artery occlusion (CRAO) (Hattenbach, 2008). Additionally, chronic conditions of hypoxia / ischemia are thought to contribute to pathogenesis in many neurodegenerative diseases of the retina, e.g. in diabetic retinopathy or retinopathy of prematurity. Here, reduced oxygen supply also leads to neovascularization and to severe secondary effects (for review: Caprara & Grimm 2012).

On the cellular level, neuronal damage is thought to be the consequence of an acute cellular energy loss, i.e. depletion of ATP stores. As a result, membrane-bound ion pumps fail, leading to a pathological rise in membrane potential (MP) and an increase in intracellular calcium. These effects are part of a complex cascade of biochemical reactions (neurotoxic response) with cell death as the final consequence (Osborne, 2004).

In this study, we utilize a dark-adapted explant of the mouse retina to gain detailed insight into pathophysiological processes under hypoxia / ischemia. Hypoxia was induced by omitting oxygenation of the extracellular solution and adding 2 mM sodium dithionite (oxygenation reduced to ~3 % of control conditions). Ischemic conditions were mimicked by using glucose-free ringer solution. Solutions were perfused at a flow rate of 8 ml / min and heated to 35 °C to provide physiological temperature. The effects of hypoxic / ischemic conditions on retinal ganglion cell light responses (1 s full-field stimulus, 10 s interval, 0.5 lux) were studied with extracellular and whole-cell patch-clamp recordings.

In a first set of experiments we performed extracellular recordings to look for changes in light responses under ischemic conditions. We observed a very fast decrease of light responses, resulting in complete failure of action potentials after 4 minutes following ischemic insult. Howerver, we did not observe an expected increase in basic action potential frequency due to a neurotoxic depolarization.

We therefore changed to the whole-cell-configuration to directly measure changes in MP. The typical reaction to hypoxia / ischemia was characterized by a rapid depolarization (31 +/- 17 mV / min; n=9) after a very short delay (< 2 min). After reoxygenation, cells repolarized after a similar delay and even more rapidly (n=6, see figure below). Comparing effects between hypoxia and ischemia, we found similar results, except for the repolarization rate being faster under hypoxia (-159 +/- 18 mV / min) than under ischemia (-74 +/- 37 mV / min; n=3). Additionally, postsynaptic potentials decreased during the early depolarization phase leading to complete loss of light responses.

In a further set of experiments we tested two experimental treatments to reduce pathophysiologic effects of hypoxia / ischemia: hyperglycemia (pretreatment with glucose-enriched ringer solution) and hypothermia (lowering retinal energy demand by lowering temperature). While hyperglycemia could not reliably avoid the pathophysiological response, we found hypothermia effectively reduced depolarization. Therefore our experimental paradigm may be suitable to test for effects of new neuroprotective treatments in the retina.

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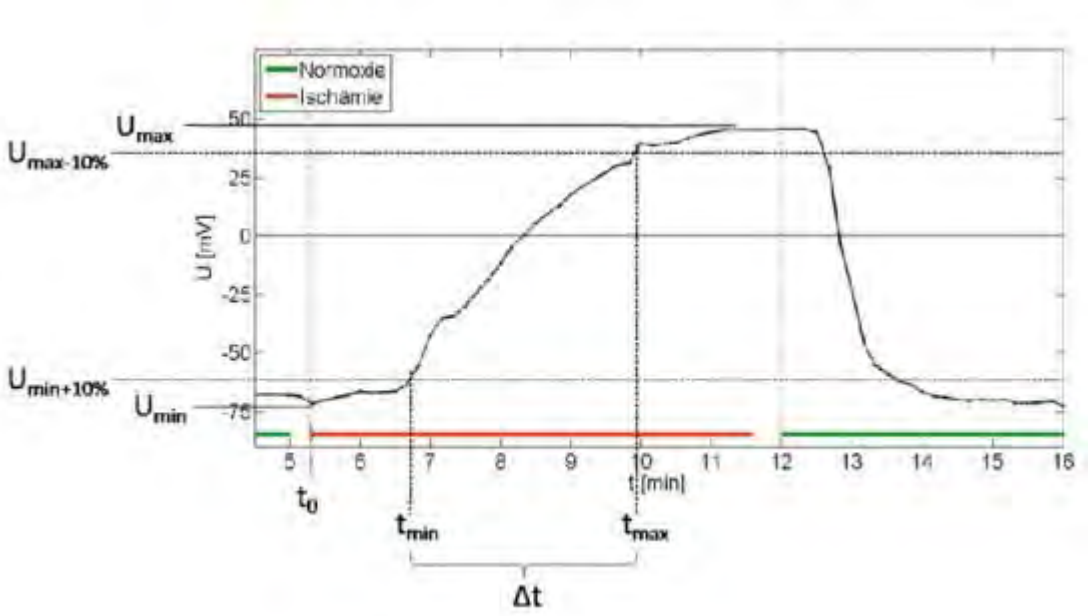

Role of p75 neurotrophin receptor in mediating neuronal alterations in *Toxoplasma gondii* chronically infected mice

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It is estimated that approximately 30% of the world population is infected with *Toxoplasma gondii* during lifetime. After an acute phase of infection the parasite forms persistent cysts within different tissues of its host – resulting in a latent infection. The lifelong presence of inactive cysts within the central nervous system (CNS) has long been judged to be harmless for immunocompetent individuals. Indeed cysts found within the brain of healthy people are not accompanied by obvious neurological symptoms. In the past decade independent studies, however, have shown that *T. gondii* is not the silent passenger previously thought. A number of these studies have demonstrated the ability of *T. gondii* to specifically manipulate the behavior of rodents to increase the probability of its transmission. Other studies strongly associated latent toxoplasmosis with the development of neuropsychiatric diseases in humans, e.g. schizophrenia. However a clear connection between latent *T. gondii* infection and certain neurological disorders has yet to be established, as the underlying neurobiological mechanisms by which *T. gondii* alters brain functions remain largely unknown. In this context the p75 neurotrophin receptor ($p75^{NTR}$) is of special interest. Indeed $p75^{NTR}$ is expressed both by neurons and by cells of the immune system and has been shown to regulate neuronal cell survival and to negatively influence dendritic complexity, spine density and synaptic plasticity of mature hippocampal and cortical neurons.

To address this issue, we first analyzed the dendritic architecture of hippocampal and cortical neurons in a well described murine model for chronic toxoplasmosis. When compared to non-infected wild type (WT) littermate controls, in *T. gondii* infected WT mice a significant reduction in dendritic complexity could be observed for hippocampal CA1 pyramidal neurons and granule cells in the dentate gyrus as well as of layer II/III cortical pyramidal neurons. Additionally, our results show that the spine density of layer II/III cortical pyramidal neurons is reduced and the spines were shorter than in non-infected mice. Current experiments are aimed at analyzing the dendritic architecture of neurons in the hippocampus and cortex of T. gondii infected and not infected $p75^{NTR}$ knockout mice. Furthermore, the inflammatory response elicited within the CNS by a *T. gondii* infection is being analyzed and correlated with observed the morphological phenotype.

Alterations of the glial activation markers in rat chronic mild stress model

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Major depressive disorder (MDD) affects up to 20% of the population. Research suggests that dysregulation within the hypothalamic-pituitary-adrenal axis and increased inflammatory response are associated with depression.

The aim of this study was to determine if chronic mild stress (CMS) causes alterations of the glial activation markers and if imipramine reverses these changes.

Male Wistar rats were individually housed. Animals were first trained to consume a 1% sucrose solution. The training lasted for two weeks and consisted of seven 1h-long baseline tests in which sucrose was presented in the home cage. Animals were food and water deprived 14 h before the test. The tests were performed twice a week for two weeks, and then at weekly intervals throughout the whole CMS experiment. Sucrose intake was measured by weighting preweighed bottles containing the sucrose solution at the end of the test. Only animals with stabile high sucrose intake in the three final baseline tests were subjected into chronic mild stress procedure for a period of two consecutive weeks.

Each week of the stress regime consisted of food or water deprivation, cage tilt, intermittent illumination, soiled cage, paired housing, low intensity stroboscopic illumination.

After three weeks of stress both stressed animals and controls were further divided into two matched subgroups and for the subsequent five weeks they received daily intraperitoneal injection of imipramine (10 mg/kg) or saline (1 mg/kg). Stress was applied throughout the treatment period. Twenty four hours after the last imipramine injection animals were sacrificed.

To measure neuroendocrine and immune parameters in the cortex and hipoccampus, ELISA (enzyme-linked immunosorbent assay) and Real-Time PCR (Polymerase Chain Reaction) were used. Data were statistically analysed with use of ANOVA followed by post hoc Newman-Keul's test. All statistical analyses were considered to be significant if $p<0.05$.

In the present study a significant decrease in GFAP (glial fibrillary acidic protein) and an increase in CD11b were observed in CMS-treated rats. These data demonstrate that CMS causes alterations in microglial activation.

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Systemic inflammation is associated with a reduction in Synaptopodin expression in the mouse hippocampus

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Systemic inflammation can affect memory function and behavior through the activation of immune cells and the release of inflammatory cytokines. However, the neuronal targets by which inflammatory signaling pathways affect synaptic plasticity remain incompletely understood. Here, we aimed at assessing whether systemic lipopolysaccharide (LPS)-induced inflammation influences the expression of synaptopodin (SP), a neuronal actin-binding protein and essential component of the spine apparatus organelle, which is considered to control the ability of neurons to express different forms of synaptic plasticity. Using quantitative PCR-analysis and immunohistochemistry we demonstrate that intraperitoneal LPS-injection in two-month-old male Balb/c mice leads to a reduction in hippocampal SP-levels (area CA1; 24 h after injection). These changes are accompanied by a deficit in the ability to induce long-term potentiation (LTP) of Schaffer collateral-CA1 synapses, similar to what is observed in SP-deficient mice. Hence, we propose that systemic inflammation may assert its effects on neural plasticity, at least in part, through the down-regulation of SP in vivo (supported by DFG and GIF).

THE EFFECT OF METHYLENE BLUE ADMINISTRATION ON CHEMOTHERAPY-INDUCED PERIPHERAL NEUROPATHY

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INTRODUCTION: Paclitaxel (P) is an antineoplasic drug whose benefits are counterbalanced by peripheral neuropathy (PIPN), a limiting side effect. Although PIPN mechanisms are still a subject of controversy, recent studies have indicated the presence of abnormal mitochondria in neurons exposed to P. Methylene blue (MB) is a mitochondrial enhancer that acts as an alternative electron carrier, transferring electrons from complex I directly to complex IV. In vivo studies have indicated that it improves cognitive function, has antidepressant proprieties and improves the outcome of several neurodegenerative disorders.

AIM: to assess the effect of MB preconditioning and MB concurrent administration on CIPN in mice

MATERIAL AND METHODS: PIPN was induced by daily i.p. administration of P 2 mg/kg b.w. for seven consecutive days. Adult BALB/c male mice were divided into 5 groups (n=8) as follows: group A received daily i.p. MB injections (5 mg/kg b.w.) during P administration (7 days); group B received daily i.p. injections of MB (5 mg/kg b.w.) for two weeks before P administration; group C received i.p. P alone; group D received daily i.p. injections of MB (5 mg/kg b.w.) for 7 days; group E received equivalent volume of saline for seven consecutive days. All animals were evaluated by means of dynamic plantar (von Frey) and Hargreaves tests in order to assess mechanical and thermal allodynia at baseline and weekly afterwards until the end of the experiment (24 days after the last P dose). Data was analyzed by ANOVA repeated-measures; unpaired and paired Student's t test were used when appropriate.

RESULTS: Paclitaxel alone induced persistent mechanical and thermal allodynia in group C; the decrease in response latency was significant starting with the 10th day after the last Paclitaxel dose; mechanical allodynia had a tendency to improve towards the end of the experiment, whereas thermal allodynia persisted until the end of the experiment. MB alone had no influence on thermal/mechanical thresholds after 7 doses, with no statistically significant differences when compared with its own baseline or with control group. Concurrent MB and P administration did not influence response latency for either mechanical or thermal stimuli. Paw withdrawal thresholds remained constant throughout the experiment. When chemotherapy was preconditioned by MB administration, a significant decrease ($p = 0.003$) in response latency to mechanical stimuli was present in the 8th day after the last P dose. No modifications were recorded in latency response to thermal stimuli. All latencies in group C were significantly lower when compared to either of the methylene groups (A and B) ($p<0.05$).

CONCLUSIONS: Concordant with literature data, Paclitaxel induced a persistent PIPN in mice; MB administration has different effects depending on administration schedule. Concurrent MB-P administration prevented PIPN in mice; 2 weeks of MB preconditioning had a protective effect against PIPN, but was also associated with a transitory mechanical allodynia. We can conclude that concurrent MB administration might be effective for preventing PIPN. Further studies must be conducted in order to elucidate the mechanism by which MB protects against CIPN.

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The effect of N-arachidonoyldopamin (N-ADA) on functional homeostasis of neural networks in normal conditions and in modeling of acute hypoxia

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The endogenous cannabinoid system regulates many brain functions through modulation of synaptic transmission and plasticity. Retrograde signaling by endocannabinoids modulate release of neurotransmitters and the activity of neural networks. In recent studies neuroprotective and antihypoxic effects of N-ADA have been shown. Hypoxia is one of the pivotal factors of neuron damage during ischemia injury, and endocannabinoids can realize their protective effects during hypoxia through cannabinoid receptor-dependent and -independent mechanisms, for example endocannabinoid interactions with neurotrophins, such as Brain-derived neurotrophic factor (BDNF).

We have studied the effect of N-ADA on the spontaneous neural network activity and CB1 and CB2 expression in dissociated hippocampal cultures in normal condition, during hypoxia and the mechanisms of N-ADA protective effects in the posthypoxic period. Hippocampal cells were dissociated from embryonic C57Bl6 mice (E18) and plated on multielectrode arrays (MEA60, Multichannel Systems) and glasses. A single application of N-ADA(2,5 and 10 mcM) was conducted into culture medium on 14th, 21 and 28 days in vitro (DIV). The main characteristics of spontaneous neural and calcium activity as well as the viability of cells were investigated. For the detection of patterns of spontaneous calcium oscillations we used fluorescent calcium dye Oregon Green 488 BAPTA-1 AM (Invitrogen) and confocal microscopy. The immunostained cultures were examined under a laser confocal scanning microscope (Zeiss LSM510, Germany). The modeling of hypoxia was performed on 21 DIV by replacing the normoxic culture medium by a medium with low oxygen for 10 minutes. In experimental groups N-ADA (2,5 and 10 mcM) or N-ADA with antagonists of cannabinoid and vanilloid receptors was added into culture medium during acute hypoxia and by the first day of posthypoxic period. We used SR151716 1 mcM (Sanofi) as antagonist of CB1 receptors, SR 141716A 1 mcM as antagonist of CB2 receptors and Capsazepine 1 mcM (Sanofi) as antagonist TRPV1. We studied the effects of N-ADA on the mRNA BDNF expression in cells using SmartFlareTM Detection Probes for mRNA BDNF with Cy3 fluorophore (Merck Millipore).

It was shown that application of 10 mcM N-ADA modified spontaneous bioelectrical activity in dissociated hippocampal cultures on 14 DIV (decrease number spikes in burst) but not on the 21 and 28 DIV. Also the modification of spontaneous calcium activity of neuronal network under the action of NADA was detected. Application of N-ADA during hypoxia and in the posthypoxic period prevented suppression of bioelectrical and calcium activity, the viability of neurons and normalized distribution of CB1 and CB2 receptors. It was shown the influence of N-ADA application on mRNA BDNF distribution. Therefore we have shown that protective properties of N-ADA primarily implemented through the CB1.

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The Effect of Neuroinflammation Induced by Influenza A virus infection on Hippocampal Neuron Morphology

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Influenza is an infectious viral disease which can take a severe course even resulting in death especially in children and older people. Despite the fact that some viral strains are reported to cause neuroinflammation, the general long term side effects of influenza infection on neurons in the central nervous system are only poorly understood.

The hippocampus has been shown to be an especially vulnerable brain region affected by inflammatory mediators and furthermore plays a very important role for cognitive functions. Therefore, this study is aimed to investigate the effects of neuroinflammation induced by various neurotropic and non-neurotropic subtypes of influenza viruses on hippocampal neuron morphology with a focus on dendritic spine density.

For this purpose, C57BL/6J mice 2 months of age were infected intranasally with influenza A virus (IAV) and sacrificed 4 weeks after infection. One hemisphere was prepared for Golgi Cox staining whereas the other hemisphere was fixed in 4% PFA for further immunohistochemical staining to quantify the number and activation status of microglia and astrocytes. Dendritic spine density was analyzed for principal cells of all hippocampal sub-regions: pyramidal neurons of the CA1 and CA3 region as well as granule cells in the dentate gyrus.

The results revealed that while some viral strains as PR8 (H1N1) had no effect on hippocampal neurons, a decrease in spine density could be shown for other subtypes as for instance maHK68 (H3N2) (both strains are non-neurotropic). Interestingly, the severity of the phenotype differed between the hippocampal sub-regions as CA1 pyramidal neurons seemed to be the most vulnerable cell type whereas granular cells of the dentate gyrus were not affected by an influenza infection. Previous studies demonstrated that in the acute phase of an influenza infection, neuroinflammation can lead to alterations in hippocampal neuronal morphology as well as cognitive deficits. The results of this study now also provide evidence that peripheral infection with IAV can induce longer lasting alterations in neuronal connectivity. Future experiments using different viral strains and following different time courses as well as young versus old mice will contribute to a better understanding of how peripheral viral infection alters the structure and function of neurons in the CNS.

The influence of patients glutamate receptor 2 antibodies on AMPA receptor mediated transmission

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Autoantibodies to a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) were recently described in the sera of patients with limbic encephalitis. In this study we purified autoantibodies from plasmapheresis material of a patient with limbic encephalitis. The patient IgG fraction showed high specificity to glutamate receptor 2 (GluR2) subunits which are involved in excitatory synaptic transmission and long-term-potentiation (LTP).
We used a combination of electrophysiological measurements and imaging methods to reveal the influence of autoantibodies against GluR2 subunits on a molecular basis.
Electrophysiological measurements showed impairments in plasticity of dendate gyrus granule cells of hippocampal brain slices after incubation with patients antibodies. Furthermore the effects of autoantibodies on synaptic transmission of cultured hippocampal neurons were investigated by single synapse stimulation by glutamate iontophoresis.
Incubation experiments with patients IgG showed a decrease in GluR2 containing synaptic boutons in cultured hippocampal neurons after 24 hours.
In this study we examined possible pathological mechanisms underlying the binding of GluR2-autoantibodies in limbic encephalitis.

The transmembrane chemokine CXCL16 transduces “inverse signaling” effects in human meningiomas

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Objectives: Meningiomas are slowly growing benign tumors, however, anaplastic meningiomas (WHO grade III) have an aggressive biological and clinical behavior, and also benign meningiomas (WHO I) can be life-threatening if they are inoperable. Since the molecular mechanisms involved in progression of meningiomas are not yet fully understood and recent investigations have suggested a possible role of chemokines in tumor biology, the aim of the study was to investigate the expression and functional role of the transmembrane chemokine CXCL16 and its receptor CXCR6 in human meningiomas.
Methods and results: Quantitative RT-PCR revealed a distinct expression in solid human meningioma samples, and double-immunostaining showed a predominant expression of the chemokine/-receptor pair in the tumor cells themselves, in infiltrating microglia cells/macrophages and endothelial cells of blood vessels. Interestingly, cultured human meningioma cells were characterized only by the expression of the chemokine ligand CXCL16, lacking the corresponding receptor. Nevertheless, cultured human CXCL16-positive meningiomas bound soluble CXCL16 and responded after stimulation with the chemokine by phosphorylation of the extracellular signal-regulated kinases (ERK) p42/44 and Akt in a time-dependent manner. Same results were observed when using a CXCL16-specific antibody. Additionally, enhanced proliferation and rescue from apoptosis were measurable in human CXCL16-positive meningioma cells after stimulation with soluble CXCL16. Since intracellular signaling effects and binding experiments were repressed after specific CXCL16siRNA transfection of human meningioma cells, we concluded that the transmembrane ligand itself acts as a receptor and generates auto- /paracrine signals (“inverse signaling”). In this view, our results provide an interesting basis for further investigations on the functional roles of chemokines and their receptors in human meningiomas.

Thermal Impact of Optogenetic Laser Light Stimulation on Neural Tissue

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Optogenetics is increasingly used in basic neuroscience and its potentials in clinical medicine are speculated. Despite the explosive growth and vast potential of the field, the potential drawbacks of the technique have been investigated less rigorously. Every component of the technique, from viral transduction, transgene expression, and light exposure, has the potential to be harmful. Here, we focus on thermal damage caused by heat from high-intensity laser stimulation in vivo.

High intensity laser light, as it is used for optogenetic stimulation, poses an inherent risk of thermal tissue damage. In cell culture or slice experiments, the bath medium disperses heat and the lack of blood limits the amount of absorbed light, thereby limiting thermal damage. Inside an intact brain, however, heat can only be dispersed by passive conduction and brain perfusion, and hemoglobin in blood adds significantly to heat absorption. Depending on the choice of opsin, target area size and wavelength, potentially dangerous levels of light exposure are required and reached.

To address the challenge of determining safe levels for optogenetic stimulation intensity, we measured temperature rises in phantom brain, dead tissue and live mouse brain with thermal imaging during laser illumination. We illuminated the cortical surface through an optical fiber with typical optogenetic wavelengths (473, 532, 589, 630 nm) and intensities. The measured temperature rises followed a simple logarithmic model with linear response to illumination intensity. Complicating factors which affected model coefficients included fiber diameter and blood vessel illumination. Our model can accurately predict the temperature rise caused by arbitrary pulse paradigms, and can therefore be used to estimate thermal damage in optogenetic experiments.

Comparing neuroprotective actions in a rat model of retinal degeneration

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Retinal neurodegenerative diseases are a group of clinically and genetically heterogeneous disorders characterized by progressive loss of vision due to neuronal death. The retina is a highly specialized tissue
with an unique architecture and it is crucial for healthy vision to maintain homeostasis into cellular network function. The retina can be exposed to a variety of environmental and genetical insults,
including light-induced damage and inherited mutations that can lead to photoreceptor apoptosis.
Neuroprotective strategies might reduce photoreceptors death and thus prolong the period of useful visual function. Whereas, both oxidative stress and neuro-inflammation play a significant role in the retinal pathogenesis, until now, it does not exist an ideal neuroprotective agent actives efficiently in all possible fields.
In an effort to select effective protections we tested and compared four different agents: two natural spices (e.g. Saffron, Curcumin) and two physical application (e.g. Cerium nanoparticles , Photobiomodulation).
Albino rats (Sprague Dawley, SD) were exposed to high light intensity (1000 lux for 24 hours, LD) to induced retinal degeneration. Animals were divided into six experimental groups: 1) normal control, 2) LD control 3) Saffron, 4) Curcumin 5) cerium nanoparticles and 6) Photobiomodulation.
Animals treated with natural spices were prefed for a week, cerium nanoparticles were intreavitreally introduced with single injection while Photobiomodulation exposure was daily applied for ten days.
In all the experimental procedures we used, treatment is followed by exposure to damaging light and comparison was performed among treated LD, untreated LD and normal control.
We analyzed treated and untreated retinae using functional and morphological techniques to evaluate the degree of damage in the different experimental conditions.
We observed that each treatment can modulated signaling pathways implicated in neurodegenerative as well as neuroprotective mechanisms. In the light-treated retinae proteins such as GFAP, Iba-1 and FGF2 were activated or inhibited, suggesting that cell death and cell survival pathways were regulated by treatment. All tested agents reduced neuronal death and maintained visual function although a careful analysis of selected parameters showed only a partial overlap of actions suggesting that a combination of different neuroprotectants might increase the protective outcome.

Poster Topic

T13: Cognitive, Emotional, Behavioral State Disorders and Addiction

T13-1A Accumbal CART Peptide 55-102 blocks amphetamine-induced locomotor activity by regulating Akt-GSK3ß signaling pathway and accompanied interaction with GluA1
Bo Ram Cho, Wha Young Kim, Ju Kyong Jang, Jeong-Hoon Kim

T13-2A Acute and chronic exposure to cannabinoid agonist modifies neuronal activity and coherence of local field potentials in sub-cortical limbic and somato-sensory cortical regions
Kerstin Schwabe, Mesbah Alam, Christof v. Wrangel, Joachim K. Krauss, Nadine John

T13-3A Animal model for Coffin-Lowry syndrome: function of RSK2/Rsk2 in neuronal plasticity and behaviour
Victoria Lily Cabello Gonzalez, Michaela Groma, Angelika Schmitt, Matthias Fischer

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T13-9A Differential regulation of cocaine-induced locomotor activity by leptin in the nucleus accumbens
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Andreas Görlich, Jessica L Ables, Inés Ibañez-Tallon

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T13-3B Effect of Cadherin-13 inactivation on the GABAergic system in the mouse hippocampus
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T13-4B Effects of different pharmacological manipulations on cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm
Rafal Rygula, Jakub Kregiel, Joanna Golebiowska, Jakub Kubik, Piotr Popik

T13-5B Effects of *Nymphaea lotus* Linn. aqueous extract on chronic mild stress-induced depression in rats
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T13-6B Retracted

T13-7B Electroconvulsive stimulation alleviates a breeding-induced prepulse inhibition deficit in rats
Nadine John, Wiebke Theilmann, Helge Frieling, Joachim, K. Krauss, Kerstin Schwabe, Claudia Brandt

T13-8B Enhanced excitability of granule cells after electroconvulsive seizures might be linked to concomitant up-regulation of activin signaling
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T13-9B Expression inflammasome in neuronal and astrocytic cells in neurodegeneration
Yulia Komleva, Olga Lopatina, Iana Gorina, Victoria Volkova, Alla Salmina

T13-1C Entorhinal Input Contributes to an Aberrant Hippocampal Circuitry in Mesial Temporal Lobe Epilepsy
Philipp Janz, Ute Häussler, Antje Kilias, Oliver Kretz, Matthias Kirsch, Ulrich Egert, Carola A. Haas

T13-2C Retracted

T13-3C Ghrelin diminishes the neuronal output of the dorsal raphe nucleus and its responses to food-predicting cues in freely behaving rats
Christoph Lindemann, Brian I. Hyland

T13-4C Inter-individual differences in behavioral inhibition and behavioral activation are reflected in human brain structure
Yadan Li, Lei Qiao, Jiang Qiu, Qinglin Zhang

T13-5C Modeling the dynamics of disease states in depression
Selver Demic, Sen Cheng

T13-6C Neuronal correlates of sustained fear in the anterolateral part of the bed nucleus of the stria terminalis
Jörg Lesting, Thiemo Daldrup, Patrick Meuth, Thomas Seidenbecher, Hans-Christian Pape

T13-7C Phasic dopamine activity in the dorsal striatum during variable interval responding to alcohol and sucrose
Tatiana Shnitko, Donita Robinson

T13-8C Positive allosteric modulation of the a7 nicotinic acetylcholine receptors enhances recognition memory in rats
Agnieszka Teresa Potasiewicz

T13-9C Social behavior at the molecular level
Olga Lopatina, Yulia Komleva, Yana Gorina, Natalia Kuvacheva, Alla Salmina

T13-1D Programming of promoter DNA methylation in heterozygous serotonin transporter deficient mice by prenatal stress
Magdalena Theodora Weidner, Karla-Gerlinde Schraut, Sissi B Jakob, Angelika G Schmitt, Klaus J Scholz, Tatyana Strekalova, Nady El Hajj, Thomas Haaf, Gabriela Ortega, Harry WM Steinbusch, Klaus-Peter Lesch, Daniel L van den Hove

T13-2D Responsiveness of activin A and its novel target gene *Pmepa1* to environmental stimulation and antidepressant treatment
Andrea Stephanie Link, Svitlana Kurinna, Steven Havlicek, Sandra Lehnert, Beate Winner, Tobias Huth, Fang Zheng, Sabine Werner, Christian Alzheimer

T13-3D Study hydrolysis myelin basic protein by IgG of schizophrenic patient according to the extension of the disease
Daria Parshukova, Ludmila Smirnova, Valentina Buneva, Svetlana Ivanova, Arkady Semke, Yulia Borodyuk

T13-4D The blockade of NMDA receptors reduced the extinction period on morphine-conditioned place preference in the rat
Ali Siahposht- Khachaki

T13-5D The effects of the positive allosteric modulator of a7-nAChRs in cognitive tasks in rats.
Agnieszka Nikiforuk, Agnieszka Potasiewicz, Piotr Popik

T13-6D The endocannabinoid system in the extended amygdala network modulates mechanisms of sustained fear.
Thiemo Daldrup, Maren Denise Lange, Hanna Szkudlarek, Thomas Seidenbecher, Joerg Lesting, Stephan Guggenhuber, Sabine Ruehle, Floortje Remmers, Sebastian Kuegler, Beat Lutz, Hans-Christian Pape

T13-7D THE TPH2 KNOCKOUT RAT: PHYSIOLOGICAL AND BEHAVIORAL ANALYSIS OF A SEROTONIN DEFICIENT RAT MODEL
Yasmine Graf, Daniel Beis, Cornelia Hainer, Michael Bader, Markus Wöhr, Natalia Alenina

T13-8D Variability of Cerebral Lateralization for Perception of Speech Emotional Prosody in the Course of Perceptual Learning (in different acoustic environment)
Elena Dmitrieva, Victor Gelman

T13-9D Impaired fast-spiking interneuron function in a genetic mouse model of depression.

Accumbal CART Peptide 55-102 blocks amphetamine-induced locomotor activity by regulating Akt-GSK3ß signaling pathway and accompanied interaction with GluA1

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It has been previously shown that microinjections of the biologically active cocaine- and amphetamine-regulated transcript (CART) 55-102 peptide into the nucleus accumbens (NAcc) significantly attenuated the locomotor effects of psychostimulants, suggesting that CART peptides exert an antagonistic effect on the generation of locomotion by these drugs in the NAcc. However, it has not been identified what signal pathway might be involved in this process. In the present study, the phosphorylation level of GluA1 was examined in the rats which were microinjected into the NAcc of either saline or CART 55-102 (2.5 µg/0.5 µl/side) followed by either systemic saline or amphetamine (AMPH) (1 mg/kg, i.p.). Microinjection into the NAcc of CART 55-102 recovered the AMPH-induced increase of pGluA1 back to saline levels. Next, we also examined the effect of CART 55-102 microinjection on AMPH-induced decreases of GSK3ß and Akt phosphorylation levels in the NAcc. Accumbal CART 55-102 also recovered the AMPH-induced decreases of pGSK3ß and pAkt back to saline levels. By performing immunoblot assay, we found that both GluA1 and GSK3ß co-immunoprecipitated from the tissue obtained in the NAcc. These results suggest that CART 55-102 peptide in the NAcc may play a compensatory inhibitory role in AMPH-induced locomotor activity by recovering the Akt-GSK3ß signaling pathway modified by AMPH and accompanied normalization of pGluA1 levels in this site.

Acute and chronic exposure to cannabinoid agonist modifies neuronal activity and coherence of local field potentials in sub-cortical limbic and somato-sensory cortical regions

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Cannabis application during puberty is an established rat model for experimental psychosis. It has been shown that intake of cannabinoid agonists during pubertal cerebral maturation lead to long lasting behavioral impairments like disturbed sensorimotor gating and memory, which are attributed to the effect of cannabinoids within the neuronal mesocorticolimbic circuitry. So far, the underling changes in neuronal function are poorly understood. We here examined the neuronal activity of certain regions of the mesocorticolimbic circuitry in adult rats after chronic pubertal treatment.

Wistar rats were chronically treated with WIN55212.2 (WIN; 1.2 mg/kg) during puberty, i.e., for 25 days from postnatal day 40 to 65, vehicle treated rats served as controls. After at least 3 month of intermission rats were anaesthetized with urethane (1.4 g/kg) and single neuronal activity (SU) and oscillatory local field potentials (LFPs) were recorded from the medial prefrontal cortex (mPFC), the nucleus accumbens (NAc) and the ventral pallidum (VP) before and after acute WIN administration (1.2 mg/kg). Additionally, an electocorticogram was recorded from the somato-sensory cortex.

Chronic pubertal WIN treatment led to higher neuronal firing rates in the mPFC and reduced firing rate in the VP, acute WIN administration hat no further effect. Neuronal activity in the NAc was not affected by pubertal WIN treatment, but acute challenge enhanced firing rate in these rats ($P<0.001$). Acute administration of WIN in pubertal WIN treated rats increased number of bursts in the mPFC but decreased number of bursts in the NAc ($P<0.001$), while in the VP bursts were not affected. Overall, coherence of low oscillatory frequency bands (delta and theta) between subcortical regions and the somato-sensory cortex was enhanced by chronic and acute WIN administration, while coherence of gamma activity was decreased.

This study demonstrates that pubertal cannabinoid intake has complex and long-lasting effects on neuronal activity in certain regions of the mesocorticolimbic circuitry in adult rats and affects the neuronal response to acute cannabinoid exposure, which may underlie the behavioral alterations previously reported for this model.

Animal model for Coffin-Lowry syndrome: function of RSK2/Rsk2 in neuronal plasticity and behaviour

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Loss-of-function mutations in the RPS6KA3 gene, which encodes Ribosomal s6 kinase 2 (Rsk2), cause Coffin-Lowry syndrome (CLS), an X-linked mental retardation syndrome. Rsk2 knockout (KO) mice have been created as an animal model to study human CLS and present spatial learning and fear conditioning deficits. The Rsk2 protein has been shown to be relevant for the birth of new neurons in the developing brain. Hippocampal adult neurogenesis (AN), generation of new neurons in the adult hippocampus, influences learning and memory processing. Our main goal is to investigate how Rsk2 deficiency affects hippocampal AN, anxiety- and depression-like behaviour, locomotor activity and impulsivity in this mouse model.

For the AN study, quantitative immunohistochemistry with antibodies detecting DCX, MCM2 and BrdU followed by stereological analysis using the StereoInvestigator software was performed. Using these three different AN markers we could not detect significant differences in stem cell proliferation, immature neurons and 28 days survived cells in the dentate gyrus of the two Rsk2 genotypes.

Applying different behavioural tests we found similar performances of Rsk2 KO and wildtype mice in Open Field Test and Elevated Plus Maze. But, in Light/Dark Box and Porsolt Swim Test, Rsk2 KO mice showed increased locomotor activity. Following these results, we decided to explore more in detail the behaviour of these mice by using the IntelliCage (NewBehaviour), device designed for long term investigations of mouse behaviour in a social context. Ongoing experiments in the IntelliCage focus on anxiety-like behaviour, on behavioural control/impulsivity and on depression-like behaviour. A sucrose preference test using sacharine as well as sucrose to reveal anhedonia-like behaviour is just ongoing. Moreover, we are planning to analyse neurotransmitters like Serotonin, Dopamine, Adrenalin, GABA and their metabolites in various brain regions.

Association of (N251S)-PIP5K2A with positive symptoms in schizophrenia

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Background: Phosphatidylinositol-4-phosphate 5-kinase IIa (PIP5K2A) is a key enzyme in the biosynthesis of phosphatidylinositol-4,5-biphosphate, and plays an important role in membrane transduction of neurotransmitter signals and in intracellular signaling (Gamper N., Shapiro M. S., 2007). The PIP5K2A gene is located in the schizophrenia candidate region on chromosome 10p14-11. Polymorphisms of this gene have been shown to be associated with schizophrenia in European and Chinese populations (Schwab S. G. et al., 2006; He Z. et al., 2007).

Objective: The aim of our research was the study of the association of (N251S)-PIP5K2A (rs10828317) polymorphic variant with clinical polymorphism in Russian patients with schizophrenia.

Materials and Metods: Blood samples were taken from 362 Russian Caucasian patients with clinically established schizophrenia (aged 41±15 years) in four different psychiatric departments in West Siberia. The Positive And Negative Syndrome Scale (PANSS) was used to assess the leading symptoms of schizophrenia. The control group consisted of 100 healthy volunteers. Genotyping of (N251S)-PIP5K2A (rs10828317) performed on ABI StepOne Plus (Applied Biosystems). The program SPSS17.0 was used for statistical analysis. Hardy-Weinberg equilibrium (HWE) and differences in genotype frequencies were tested using a chi-square test.

Results: The genotype distribution of (N251S)-PIP5K2A (rs10828317) polymorphism was in agreement with HWE (χ^2=0.32, p=0.648) in the control group, but not in the group of schizophrenic patients (χ^2=14.13, p=0.0023). 41.2% of patients and 45% of healthy volunteers were homozygous for the T-allele, 38.4% of patients and 46% of control persons were heterozygous, and 20.4% of patients and 9% of healthy volunteers were homozygous for the C-allele (χ^2=7.116, p=0.028). We found an association of rs10828317 with schizophrenia (p=0.028, Odds ratio [OR] = 2.60, 95%Cl= 1.25 – 5.40 for the CC genotype). In addition the frequency of CC carriers was significantly higher in schizophrenic patients with leading positive symptoms in comparison with schizophrenic patients with leading negative symptoms (34.8% and 17.6% respectively, χ^2=11.815, p=0.003, and OR=2.51, 95%Cl=1.43 – 4.38 for the CC genotype, p=0.003).

Discussion: (N251S)-PIP5K2A (rs10828317) is known to be a functional mutation. Previous studies have shown that the mutant kinase is ineffective in activating KCNQ channels, which may lead to lack of dopaminergic control in schizophrenic patients. Moreover (N251S)-PIP5K2A decreased the membrane abundance of the excitatory amino acid transporter EEAT3 in a study on EAAT3-expressing oocytes and human embryonic kidney cells (Fedorenko O. et al., 2008, 2009). Taken together, these facts may provide a biological explanation of the association of (N251S)-PIP5K2A (rs10828317) with schizophrenia and leading positive symptoms.

Conclusions: A significant association of (N251S)-PIP5K2A polymorphism with leading symptoms of schizophrenia has been found. CC carriers with schizophrenia had more severe positive symptoms as evaluated by PANSS. Further studies are needed to support our findings.

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Behavioural analysis of Flinders Sensitive Line rodent model of depression: An appropriate model for Medial Forebrain Bundle Deep Brain Stimulation?

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Major depressive disorder (MDD) is one of the most common neurological diseases in the world affecting 7-12% of men and 20-25% of women (Kessler et al., 2005) and 350 million people worldwide. The aetiology of this psychiatric disorder is unknown but key elements include dysfunctional neurotransmitter systems, neurotrophic factor levels, certain genetic mutations, as well as a miss-adaptation to stress. The network-model is the currently accepted explanation of the pathology stating that aspects of the syndrome can arise from dysregulation of neuronal activity at numerous loci on the limbic-cortical circuitry. Deep Brain Stimulation (DBS) for treatment-resistant MDD has been tested clinically over the last decade and can offer some patients symptomatic relief. Recently, bilateral DBS of the supero-lateral medial forebrain bundle (MFB) reduced depressive symptoms in a clinical trial (Schlaepfer et al, 2013). The MFB is considered to be the neural substrate for the so called SEEKING system, one of several hard-wired primary affective systems, and is thought to play an important role in depression (Panksepp, 2010). The objectives of the study were 1.) to evaluate the dynamics and robustness of depressive-like symptoms in the Flinders Sensitive Line (FSL) rodent model of depression; 2.) to identify potential physiological and metabolic deficits; and 3.) to determine whether the FSL rat model of depression is an appropriate model to investigate the mechanisms of action of MFB DBS. Eleven Flinders Sensitive Line and 11 Sprague-Dawley rats (5 M, 6 F) were tested on a battery of tests at 3-4 (Early) and at 6-7 months (Late) of age. The tests were broad spectrum covering mood, cognitive, and motor behaviours. Additional assessments included looking at corticosterone (ELISA) levels and using 18F FDG micro PET to non-invasively and longitudinally study glucose metabolism in the brain, and histological stainings. Table 1 summarizes all the behaviour tests and read-outs used in the study. Most important results are shown in Figure 1. During this study three major results were achieved. Firstly, most FSL deficits were age-dependent and transient. Some gender-dependent phenotypes such as increased anxiety and stress reaction in the male FSL rats were observed. Secondly, more stable deficits in the FSL rats were seen in the Forced Swim Test (FST) and the Double H Test (DH). Again gender differences were seen in the FST as the female FSL rats had a stronger phenotype compared to the males. The DH showed an impaired and robust learning deficit in both the male and the female FSL rats. Thirdly, micro PET brought to light a persistent bilateral hypometabolism of the entorhinal cortex (ENT) in the FSL rats at both time points, which could also be reason for the poor learning and memory performance of the FSL animals. The robust hypometabolism in the ENT in the FSL rats provides two possible mechanisms that underlie the animals' phenotype: On one hand, a dysfunctional ENT could have negative impact on modulation of VTA activity, including DA transmission via the MFB; on the other hand, a deficient DA transmission from the VTA to the ENT could also cause the hypometabolism. Overall, the results show that the FSL rat model of depression is an appropriate model to investigate the mechanisms of action of MFB DBS as the animals exhibit both long-term physiological and behavioural deficits.

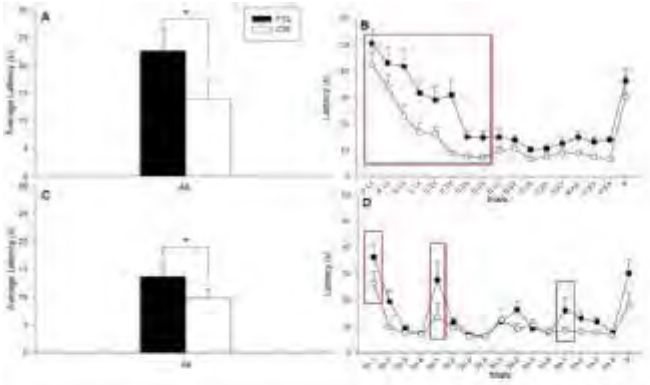

Figure 1 A-E: Behavioural Results of the Double H (A-D) and the FST (E). The FSL rats showed a increased latency until they reached the escape platform. This deficit is consistent over time. Additional a changed behaviour can be seen in the latency per trial. During the early test (B) the difference resulted from the first two days. During the late test (D) the difference resulted from the first trial of every day. In the FST only the female FSL rats showed a stable trend towards an increased immobility. This effect is also consistent over time.

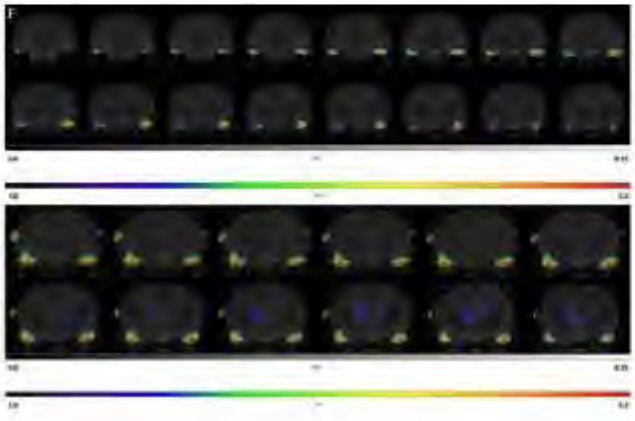

Figure F and F: Physiological results of the PET Scan (F) and the ELISA (G). The picture shows the hypometabolism of the aithorinal cortex on both sides comparing the FSL rats to the SD rats. The difference is bigger in the left cortex. 3 months later the effect amplified. In the ELISA an example for a gender-dependent deficit is shown. The male FSL rats showed an increasing corticosterone concentration in the measurements. At the same time the concentration in the SD males stayed constant. The FSL males are more stressed than the SD males.

Co-microinjection of ghrelin and D1 dopamine receptor agonist in the nucleus accumbens core enhances locomotor activity in amphetamine pre-exposed rat

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Ghrelin, a peptide hormone, is well known for its role in increasing food appetite. Ghrelin receptors exist in several brain areas including the nucleus accumbens (NAcc), a region important for the incentive motivational and locomotor activating properties of psychostimulant drugs. We investigated what roles ghrelin may play in mediating locomotor activating effects of psychomotor stimulants in this site. First, we pre-exposed rats to either saline or amphetamine (1 mg/kg, IP) every 2 to 3 days for a total of 4 times. After a week of drug-free withdrawal period, we examined the effect of either saline, ghrelin (0.5 µg/side), D1 dopamine receptor agonist, SKF 81297 (0.5 µg/side), or ghrelin (0.5 µg/side) + SKF 81297 (0.5 µg/side) directly microinjected into the NAcc core on locomotor activity. When we measured rats' locomotor activity for 1 hour immediately following microinjections, only ghrelin + SKF 81297 enhances locomotor activity, while all others have no effects. These results suggest that ghrelin may have a distinct role in the NAcc core to regulate psychomotor stimulants-induced locomotor activity, and further it may produce these effects by interaction with D1 dopamine receptors.

Crucial function of FMRP in the development of the hippocampal mossy fiber pathway

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The dentate gyrus (DG) is the main input region into the hippocampus and therefore crucial for hippocampal function in general and is of particular importance for processes of memory formation and consolidation. Granule cells of the DG form projections into the *stratum lucidum* within the *cornu ammonis* (CA), which are composed of so called large mossy fiber terminals (LMT). LMTs connect in turn with specialized postsynaptic protrusions – thorny excrescences (TE) - on CA3 pyramidal neurons. Each of these prominent large pre- and postsynaptic structures comprises several synapses and their localization close to the neuronal soma therefore renders them especially strong synaptic connections. The RNA-binding protein fragile X mental retardation protein (FMRP) is enriched in the *stratum lucidum* in so called FXS granules, indicating the special importance of protein synthesis for these structures. Hence, the question arises, whether some impairment in FXS patients might be in parts caused by defects in the development and function of the mossy fiber pathway.

In this study, the influence of FMRP on the development of both pre- and postsynapses in the mossy fiber pathway of the hippocampus was investigated by taking advantage of a *fmr1* knockout mouse model. For this purpose, organotypic slice cultures were prepared at distinct developmental stages (DIV7-28) and neurons were either electroporated with Alexa dye or transfected with farnesylated eGFP using the Helios gene gun. Moreover, spatial memory formation in the Morris Water Maze (MWM) was assessed at the respective ages (p19-p30). Mossy fiber projections of trained and untrained animals were analyzed with respect to TE number and morphology as well as the size of the LMT projections in the CA3 region. In order to shed light on possible alterations in hippocampal function and a correlation to the behavioral phenotypes of FXS, the marble burying task as a species typical behavior, which is strongly depending on proper formation of hippocampal networks, was performed as well at different ages.

Our results indicate that indeed, along with an increased activity in the marble burying task both the density and morphology of TEs was altered during development in *fmr1* knockout animals compared to WT littermates, thereby pointing towards a critical period of thorny excrescence morphogenesis that is affected in the course of FXS. Additionally, *fmr1* KO animals at this age displayed alterations in spatial learning in the MWM.

Taken together our results suggest a crucial role of FMRP for the development of tightly balanced neuronal connections and a misregulation during the development of synaptic connections in the DG-CA3 mossy fiber pathway could indeed represent a key feature responsible for prominent symptoms of FXS as compromised hippocampal networks and behavioral and cognitive deficits.

Deep brain stimulation of the centromedian-parafascicular complex attenuates deficient sensorimotor gating in a rat model for Tourette syndrome

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Certain neuropsychiatric disorders, like Tourette syndrome (TS) or schizophrenia, are accompanied by dysfunction of sensorimotor gating processes, which can be operationalized by prepulse inhibition (PPI) of the acoustic startle reaction, i.e., the reduction of the startle response to an intense acoustic stimulus when this stimulus is shortly preceded by a weaker not-startling stimulus. Experimentally induced PPI-deficits are used to investigate the pathophysiological mechanisms of neuro-modulative treatment in these disorders.

Currently deep brain stimulation (DBS) is under investigation for the treatment of pharmacoresistant TS. Targets include the centromedian-parafascicular complex (CM-Pf), the globus pallidus internus and the ventral striatum.

We recently showed that DBS of the rat's entopeduncular nucleus (EPN), the equivalent to the human globus pallidus internus, prevents an apomorphine induced deficient PPI and that a dilzocilpine induced PPI-deficit is further deteriorated by EPN lesions. We also showed that DBS of the CM-Pf alleviates PPI in rats with a breeding induced PPI-deficit.

Here we investigated whether DBS of the rat CM-Pf would affect deficient sensorimotor gating induced by either the dopamine-receptor agonist apomorphine, or the NMDA-receptor antagonist dizocilpine. For this propose electrodes were stereotactically implanted bilaterally in the CM-Pf of Sprague Dawley rats. After recovery from surgery, the rats were stimulated with 150 µA (130 Hz and 160 µs square wave pulse) or sham stimulated (without any current) for epochs of five days via a cable connected to the stimulator device. The effects of DBS on apomorphine- (1.0 mg/kg and vehicle; n = 12) or dizocilpine- (0.15 mg/kg and vehicle; n = 11) induced deficient PPI were tested on the last day of stimulation. Finally, the location of electrodes was histologically verified.

This study revealed that CM-Pf DBS alleviated an apomorphine-induced PPI-deficit, while dizocilpine-induced reduced PPI was not affected. DBS itself had no effect on PPI.

This work indicates an important role of the CM-Pf in the modulation of sensorimotor gating by the dopaminergic system, while the glutamatergic system seems to be less involved. Targeting the CM-Pf in apomorphine-induced deficient PPI may therefore be valuable to study the pathophysiology and the treatment of TS. This model may also be used to further investigate the mechanisms of action of DBS in this disorders.

Differential regulation of cocaine-induced locomotor activity by leptin in the nucleus accumbens

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Leptin, a peptide hormone secreted from adipose tissue, is known to regulate energy homeostasis by acting on the hypothalamus. Leptin receptors are expressed in several brain areas including the nucleus accumbens (NAcc), a site important for mediating the motivated behaviors and locomotor activating properties of psychostimulant drugs. The existence of leptin receptors in the NAcc suggests that leptin, besides controlling feeding behavior, may have unidentified roles in modulating rewarding effects of psychomotor stimulants. In this study, we examined the effect of leptin in the NAcc on acute or chronic cocaine-induced locomotor activity in the rat. First, leptin (0.1, 0.5 or 2.5 μg/side) was bilaterally microinjected into the NAcc, immediately followed by saline or acute cocaine (15mg/kg, IP) injection, and rat¡¦s locomotor activity was measured for 2 hours. In a dose-dependent manner, leptin inhibits the increase of acute cocaine-induced locomotor activity, while leptin alone produces no significant change in basal locomotor activity. Next, we further examined the effect of leptin microinjection on the expression of cocaine-induced behavioral sensitization. Rats were either saline or cocaine (15mg/kg, IP) pre-exposed (once daily for 7days), and after 2weeks of withdrawal, their locomotor activity were measured with a systemic cocaine challenge following microinjection into the NAcc of either saline or leptin (0.1 or 2.5 μg/side). Interestingly, microinjection into the NAcc of leptin dose-dependently enhances the increase of locomotor activity produced by cocaine challenge in cocaine pre-exposed group. These findings indicate that leptin, an appetite-suppressing hormone, may have a bidirectional role in the NAcc to regulate locomotor activity following acute or chronic cocaine injections and further suggest that leptin may have a functional role in mediating pyschostimulant-induced motivated behaviors.

Dissection and optogenetic manipulation of Habenula-IPN cell-specific neuronal networks in the control of nicotine addiction and withdrawal

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The discovery of genetic variants in the CHRNA5-CHRNA3-CHRNB4 gene cluster associated with heavy smoking and higher relapse risk has led to the identification of the midbrain medial habenula-interpeduncular (MHb-IPN) axis as a critical relay circuit in the control of nicotine dependence. Although clear roles for a3, ß4 and a5 nicotinic acetylcholine receptors (nAChR) in nicotine aversion and withdrawal have been established, the cellular and molecular mechanisms that participate in signaling nicotine usage and contribute to relapse have not been identified.

Here we present our recent findings on the circuitry, cell types and electrophysiological properties of genetically targeted MHb-IPN subpopulations. We employed recently developed transgenic reporter mouse lines for intersectional studies used for delivery of optogenetic and silencing vectors, electrophysiological recordings and neuroimaging. We discovered two novel non-overlapping IPN cell populations with distinct electrophysiological properties, cellular morphologies and segregated connectivities. We further dissected the afferents and efferents of these two IPN cell populations by Cre-dependent viral-mediated expression of channelrhodopsin (ChR2) and optogenetic stimulation in MHb-IPN brain slices. We next silenced neurotransmission in vivo from these two complementary cell populations using tethered toxins, and observed specific alterations of nicotine-dependent behaviors.

Altogether these studies identify two novel populations in the IPN, which differently contribute to nicotine withdrawal and dependence, clearly implicating the IPN as a critical relay station from the habenula. These discoveries shed light to our understanding of the brain areas and circuits involved in nicotine reward and withdrawal, furthering our knowledge on the different addictive behaviors assigned to distinct, yet overlapping, neural circuits.

DYSREGULATION OF BDNF SIGNALING DURING ABSTINENCE FOLLOWING DEVELOPMENTAL EXPOSURE TO COCAINE

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Introduction: Illicit use of drugs begins during adolescence, a period of vulnerability for drug addiction. In this stage of life, the brain is in a unique state of transition as it undergoes profound structural and synaptic changes and, therefore, interfering with brain development during this delicate period may cause adverse consequences. Such effects may be the result of long-term neuroadaptations that involve, among the others, neuroplastic molecules. It is known that cocaine exposure at adulthood causes long-lasting functional and structural changes in the rat brain that could contribute to the development of addiction.. Among the others, emerging data have indicated as key molecules of cocaine-induced alterations changes in the levels of activity-regulated cytoskeleton-associated protein (Arc) and brain derived neurotrophic factor (BDNF). However, whether and how adolescent cocaine exposure modulates markers of neuroplasticity is still unknown.

Materials and methods: Male adolescent rats were exposed to cocaine during adolescence (20 mg/kg), from postnatal day (PND) 28 to PND 42 and sacrificed at PND 90. The molecular analyses on BDNF and its associated network were carried out using Real-Time-PCR technique. Protein levels were analyzed using the western blot technique. Our analyses were performed in the medial prefrontal cortex (mPFC) that is still developing during adolescence and may be a sensitive target of the repeated administration of cocaine.

Results: We found that developmental exposure to cocaine altered transcriptional and translational mechanisms governing neurotrophin expression. Total BDNF mRNA levels were enhanced in the mPFC of PND 90 rats exposed to cocaine during adolescence, an effect sustained by increased BDNF exon IV levels through the transcription factors CaRF and NF-kB. Enhanced BDNF mRNA levels resulted in an increase of the precursor and mature forms of BDNF protein, paralleld by a reduction of several miRNAs governing BDNF translation. Such effect results in the activation of the trkB/Akt pathway which, through increased S6 kinase phosphorylation, increases Arc protein levels in the nucleus and the crude synaptosomal fraction. We also analyzed the inhibitory and degradative pathways regulating Arc expression and found reduced FMR1 and Ube3a mRNA levels as well as increased GRM5 mRNA levels. The up-regulation of Arc protein led, in turn, to reduced AMPA GluA1 mRNA and protein levels, indicating that long-term abstinence alters markers of synaptic plasticity thorugh different, but converging, mechanisms.

Conclusion: These findings indicate that interfering with the correct development of the mPFC by repeated exposure to cocaine dysregulates the BDNF system and its associated network and reveal novel mechanisms that underlie the prolonged abstinence from early in life exposure to cocaine. Moroever abstinence from developmental exposure to cocaine is associated with an increase of Arc/Arg3.1 levels which is regulated through previously unappreciated and finely tuned mechanisms.

Effect of Cadherin-13 inactivation on the GABAergic system in the mouse hippocampus

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Background

Cadherin-13 (Cdh13) is an atypical member of cadherins, a superfamily of type-1 transmembrane proteins mediating calcium-dependent cellular adhesion. Although Cdh13 shows the classical extracellular Cadherin structure, there is no expression of the typical transmembrane and cytoplasmatic domains. Instead Cdh13 is attached to the cell membrane via the glycosylphosphatidylinositol (GPI) anchor. These findings and many studies from different fields suggest that Cdh13 rather plays a role as a cellular receptor than a cell adhesion molecule. Interestingly many genome-wide association studies (GWAS) have found *CDH13* as a risk gene for attention deficit hyperactivity disorder (ADHD). The aim of this study is to analyze the effects of Cdh13 deficiency on the behavior and the hippocampal GABAergic interneuron system of the mouse.

Methods

As the group of hippocampal interneurons is very heterogeneous, double immunofluorescence studies were used to evaluate the coexpression of Cdh13 with 7 markers of GABAergic interneuron subtypes. For this purpose murine brains were double stained against Cdh13 and the respective marker and the degree of colocalization in the stratum oriens of the hippocampus was assessed. Based on the result of the immunofluorescence study, quantitative differences in interneuron subtypes in the stratum oriens of *Cdh13*-knock-out (ko) mice were investigated and compared to heterozygote (het) and wildtype (wt) animals using the Stereo Investigator system (mbf Bioscience). Additionally *Cdh13* ko and wt mice were selectively tested for hippocampus-related learning using the Barnes maze. Quantitative PCR (qPCR) was performed to analyze differences among genotypes in the GABAergic and glutamatergic neurotransmission. Primers targeting different GABA receptor subunits, vesicular GABA transporter and GABA synthesizing enzymes were used.

Results

Double immunofluorescence revealed a high degree of colocalization of Cdh13 with Parvalbumin (PV) and Somatostatin (SOM). The lowest degree of colocalization was observed with neuronal nitric oxide synthase (nNOS). After stereological quantification of these markers, no significant differences in cell number, cell density or volume between the *Cdh13* ko, het and wt mice were found. Behavioral testing showed no differences in primary latency, escape latency or distance moved but ko animals made

significantly more primary errors in the reversal phase.
Preliminary qPCR results indicate significant differences in genes of GABAergic neurotransmission and no differences in those genes involved in glutamatergic neurotransmission.

Conclusion

Cdh13 is mainly colocalized with PV- and SOM positive GABAergic interneurons while it shows little colocalization with nNOS positive cells. However, stereological investigation revealed no differences regarding the density of these cells between the different genotypes.
By contrast qPCR analysis showed changes in the expression of genes responsible for GABAergic neurotransmission. The same animals had no changes in the genes involved in glutamatergic neurotransmission. As no alterations in cell numbers have been found, we hypothesize a specific involvement of Cdh13 in the GABAergic system on a functional level, in synaptic connectivity or in development. This is supported by behavioral testing which indicates hippocampus dependent learning deficits of *Cdh13* ko mice in the Barnes maze which could be an indicator for reduced cognitive flexibility in these mice.

Effects of different pharmacological manipulations on cognitive judgment bias of rats in the ambiguous-cue interpretation paradigm

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In the present study, we investigated the effects of different pharmacological manipulations on the valence of cognitive judgement bias of rats in the ambiguous-cue interpretation (ACI) test. In this paradigm the rats must press one lever in response to one tone to receive a reward and to press another lever in answer to a different tone to avoid punishment. Cognitive judgement bias is than assessed by measuring the pattern of animals‘ responses to a tone of intermediate frequency (ambiguous-cue). In our studies, after initial behavioural training, separate groups of rats received single or chronic injections of different classes of drugs (serotonin (5-HT), noradrenaline (NA) and dopamine (DA) mimetics as well as mood stabilizers such as lithium or valproate, and were tested on the ACI test before, during and after the treatment. Results of our study clearly show that cognitive judgment bias in rats can be influenced by pharmacological intervention. The results are discussed in terms of neurochemical and neurobiological action of tested compounds.

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Effects of *Nymphaea lotus* Linn. aqueous extract on chronic mild stress-induced depression in rats

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Common stressful situations influence susceptibility to disease like depression. The present study investigates the antidepressant and antioxidant effects of extracts of Nymphaea lotus (*N. lotus*) flowers on a rat model of chronic mild stress (CMS)- induced depression. Wistar rats were separated into five groups. The first, control group was not stressed and the others groups were confronted to a 2-week CMS paradigm and tested for their responses in different behavioral situations (sucrose intake, physical state, locomotor activities and brain homogenate for biochemical status). The CMS groups received p.o. once daily for 14 days: distilled water (10 mL/Kg), Yohimbine (2 mg/kg) as standard drug, and aqueous extract of *N. lotus* at the doses of 75 and 200 mg/Kg respectively. Histopathological analyses of the neocortex and hippocampus were performed. Our findings indicate that 14 day-administration of aqueous extract of *N. lotus*, as well as Yohimbine administration, produced antidepressant-like effects in the forced swim and sucrose preference tests. Treatment with the plant extract also prevented the anxiogenic effect induced by CMS in the open field test. In the biochemical analyses, CMS increased lipid peroxidation, decreased protein levels and reduced-glutathione in the brain. The data indicate that *N. lotus* aqueous extract administration during CMS was able to prevent oxidative damage induced by stress. However, no changes were observed in the activity of the antioxidant enzymes superoxide dismutase and catalase in the brain. Our results indicate that *N. lotus* can exert anti-depressant-like behavioural effects and prevent oxidative damage induced by chronic stress in rodents.

Electroconvulsive stimulation alleviates a breeding-induced prepulse inhibition deficit in rats

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In major pharmacoresistant depression or certain forms of schizophrenia electroconvulsive therapy (ECT), i.e., the induction of therapeutic seizures via cortical surface electrodes, is an effective treatment option. Although ECT is applied since almost 80 years, the mechanisms underlying its therapeutic effect are not yet understood. Experimentally induced reduced prepulse inhibition (PPI) of the acoustic startle response (ASR), i.e., the reduction of the startle response to an intense acoustic stimulus when this stimulus is shortly preceded by a weaker not-startling stimulus, is used as an endophenotype or animal model for disturbed sensorimotor gating in schizophrenia. Here we used rats selectively bred for high and low PPI to evaluate whether electroconvulsive stimulation (ECS) applied via frontal cortical screw electrodes would alleviate PPI.

Cortical screw electrodes were stereotactically implanted above the frontal cortex in PPI low (n=13) and PPI high rats (n=14). After a recovery period of two weeks all rats were daily stimulated for five days with electrical stimuli of 1 ms pulse-width, 100 pulses/s, 1 s duration; the current was adapted to each animal reaching from 5.5 mA to 10 mA to induce a generalized seizure that lasts for = 15 s. PPI was measured one day before ECS, and on the first, seventh and 14th day after the last ECS.

In PPI low rats cortical ECS alleviated PPI deficit at the seventh day after stimulation, while in PPI high rats this measure was disturbed on the first day after stimulation ($p < 0.05$). After ECS the startle reaction was reduced in PPI high and low rats.

In summary this study provides evidence that rats with a breeding-induced PPI deficit could be used to further investigate the underlying mechanisms of ECT in neuropsychiatric disorders with disturbed sensorimotor gating like schizophrenia.

Enhanced excitability of granule cells after electroconvulsive seizures might be linked to concomitant up-regulation of activin signaling

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Activin A is a member of the transforming growth factor-beta (TGF-beta) family. In the nervous system, it was originally identified as a neurotrophic and neuroprotective factor, but more recent evidence from our and other laboratories showed that activin also tunes excitatory and inhibitory neurotransmission in the brain in a fashion that impacts on cognitive functions and affective behavior (Mol Psychiatry 14:332-346, 2009; TiNS 34:421-429, 2011). Canonical activin signaling involves activin binding to heteromeric receptor complexes consisting of type I and type II serine/threonine receptors, which then phosphorylate the recruited receptor SMADs 2 and 3. The latter multimerize with SMAD4 in the cytosol, translocate to the nucleus and modulate transcription of activin target genes. In addition to this canonical pathway, activin receptors might also act on other signaling systems, in particular mitogen-activated protein kinase (MAPK) signaling. Activin signaling is responsive to both physiological and pathological stimuli. For example, activin signaling is significantly up-regulated by novel environmental stimuli as well as by brief trains of action potentials which induce long-term potentiation at glutamatergic synapses. A more pronounced, large-scale version of this physiological responsiveness is observed after epileptic activity or acute injury. In neuropsychiatry, activin A has been implicated in the regulation of mood disorders and has been suggested to serve as an endogenous antidepressant. In fact, activin signaling is targeted by the two prevailing treatment strategies in major depression, antidepressant drugs and electroconvulsive therapy (ECT), but it is still unknown how activin might produce its putative therapeutic benefits. Here we report that electroconvulsive seizures (ECS, 25 mA for 0.5 s at 50 Hz), the mouse analog of ECT in humans, strongly up-regulate activin signaling in the granule cell layer of the dentate gyrus of mouse hippocampus. In ex vivo recordings from brain slices prepared 12 h post ECS, we observed that granule cell excitability was greatly enhanced, manifested both in field potential recordings and in whole-cell current-clamp recordings. Linking the electrophysiological effects of ECS on granule cell excitability to the accompanying rise in activin in the same hippocampal region, we found that incubation of control slices with activin (50 ng/ml) for 3 - 5 h closely mimicked the features of the ECS-mediated increase in granule cell firing. The activin-induced enhancement of intrinsic excitability was abolished by the activin-binding protein follistatin 288 (160 ng/ml), but not by SB 421532 (10 µM), which abrogates canonical activin signaling by inhibiting SMAD phosphorylation. Because follistatin causes a complete suppression of activin signaling, whereas SB 421532 only interferes with the SMAD-dependent pathway, it seems reasonable to assume that the activin-induced increase in granule cell firing is mediated by a non-canonical pathway. Our results suggest that the up-regulation of activin signaling might be responsible for a functionally significant effect of ECS in dentate granule cells and that the underlying mechanism involves a SMAD-independent pathway.

Expression inflammasome in neuronal and astrocytic cells in neurodegeneration

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Neuroinflammation is a characteristic feature of the Alzheimer's disease (AD). Neuroinflammation has a significant influence on the structural and functional plasticity of the brain (Dupret D., 2008). Significant inflammatory markers such as activated microglia and cytokines can be found surrounding the extracellular senile plaques predominantly composed of amyloid-beta protein (A). Several innate immune pathways, including Toll-like receptors (TLRs) and the NLRP3 inflammasome, have been implicated in AD inflammation. Abeta(1-42) protofibrils were able to prime and activate the NLRP3 inflammasome. Therefore there are a lot of study was devoted to study expression inflammasome in microglia (Terrill-Usery SE, 2014).

However less attention has been paid to the study of NLRP3 and NLRP1 expression in neuronal and astrocytic cells. We studied the expression inflammasome in healthy brain and neurodegeneration in neurons and astrocytes at different stages of neurogenesis.

We used Wistar male rats aged 9 months. The animal model of AD was induced by injections of beta-amyloid into CA1 area. (Li X. et al., 2011). At two week postinjection brains were removed. Immunohistochemistry staining was used to determine NLRP1, NLRP3 expression in the neuronal and glial cells. We have evaluated expression of NLRP1 and NLRP3 in the cells expressing CD133 (stem cells), PSA-NCAM (neuronal progenitor), NeuN (mature neurons), GFAP and S100beta positive cells.

The changes in the functional activity of astrocytes in the in chronic neurodegeneration were associated with the production of inflammasomes and production of proinflammatory cytokines. It determines the nature of the participation of astroglial cells in the process of neurogenesis.

Also we have investigated proliferation, migration activity of neuronal precursors and the efficiency of the integration of post-mitotic neurons in the synaptic chains depending on the nature of the expression different types of inflammasomes in experimental Alzheimer's disease.

In Alzheimer's disease we have demonstrated decline of inflammasome expression in the basal progenitor cells of the subgranular zone. The modeling of neurodegeneration is associated with reduced NLRP1 expression in the neuroblasts of the neurogenic niches.

It was not recorded significant differences in inflammasome expression in GFAP-positive cells in healthy and damaged brain in the subgranular zone, subventricular zone, olfactory bulb.

The study of the molecular mechanisms of the effect of astrocytes and humoral inflammation mediators on neurogenesis may allow the identification of new target molecules for pharmacological correction of chronic neurodegeneration.

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Entorhinal Input Contributes to an Aberrant Hippocampal Circuitry in Mesial Temporal Lobe Epilepsy

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Dentate granule cells receive their major excitatory input from the entorhinal cortex through the perforant path. In this study, we focused on the synaptic alterations of hippocampal input from the medial entorhinal cortex (MEC) via the medial perforant path (MPP) in chronic stages of medial temporal lobe epilepsy (MTLE), as Froriep et al. (2012, Epilepsia) showed changes in the activity coupling between the MEC and the hippocampus. Although histopathological reorganization associated with MTLE (i.e. hippocampal neuronal cell loss, granule cell dispersion and mossy fiber sprouting) has been studied extensively, it remains uncertain whether the entorhinal input is altered under epileptic conditions.

To address this question adult C57Bl/6 or transgenic Thy1-EGFP mice received a unilateral, intrahippocampal kainate or saline injection, followed by stereotaxic infusion of biotinylated dextran amine (BDA), an anterograde neuronal tracer, into the MEC days later. After a survival period of 21 days, brain sections were immunohistochemically stained for BDA and vGLUT-1, labeling the traced fibers and their presynaptic terminals. Quantitative morphometry and evaluation of MPP synaptic input on granule cell dendrites was carried out in confocal z-stacks by using Imaris software. Ultrastructure of MPP-synapses was further investigated using transmission electron microscopy (TEM).

We show that, following kainate treatment, the width of the MPP as well as its laminar organization remained unchanged, although dentate granule cells dispersed broadly. In turn, the density of traced fibers was reduced and high-power confocal imaging revealed axonal varicosities within the MPP. Assessment of synaptic densities pointed to a decline of MPP terminals, while postsynaptic spines are increased following KA injection. Correspondingly, PSD-95 present at the post synaptic density of mature spines was upregulated. In addition, analysis of MPP-synapses with TEM pointed to morphologic alterations on an ultrastructural level.

In conclusion, our findings suggest that under epileptic conditions the MPP is preserved on a mesoscopic scale, but it appears altered on the level of individual synapses.

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Ghrelin diminishes the neuronal output of the dorsal raphe nucleus and its responses to food-predicting cues in freely behaving rats

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Ghrelin is a peptide hormone produced by the stomach that affects reward-related brain centres and augments consumption. Modulations of the central ghrelin system are considered for treatment of eating- and psychiatric disorders that accompany alterations in reward-related behaviour. Dorsal raphe nucleus (DRN) neurons contain ghrelin receptors and have widespread serotonergic projections that target the reward system. We recorded DRN cells of male Wistar rats performing a conditioned-approach food reward task, in which pseudorandom presentation of differently pitched tone cues predicted reward delivery or omission of reward. Of 176 cells recorded to date, 103 showed short latency responses to cues. Separate 2-way RM ANOVA for each group, revealed that i.c.v. administration of ghrelin (1 µg in 1 µL ddH2O) significantly reduced the baseline activity of the DRN ($P < 0.001$). Furthermore, ghrelin significantly reduced the cue responses in the reward trials ($P < 0.001$) and showed a trend for a reduction in the responses in non-rewarded trials ($P = 0.061$). After ghrelin application, rats conducted significantly less premature head entries in the food trough ($P < 0.001$). The DRN is known to have prominent inhibitory projections to dopamine neurons of the reward system. In hungry states, where ghrelin levels are high, reduced DRN activity may enable enhanced dopamine transmission and invigorate responses to reward-predicting signals. Ghrelin induced changes in food-seeking behaviour could be responsible for reduced food trough approaches.

Inter-individual differences in behavioral inhibition and behavioral activation are reflected in human brain structure

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Individual differences in Behavioral Inhibition and Behavioral Activation Systems (BIS/BAS) may place certain people at greater risk for neuropsychiatric disorders and engagement in risky behaviors. Despite the importance of anatomical bases underlying behavioral inhibition and behavioral activation, such bases are not fully clear. Using voxel-based morphometry (VBM) to measure regional gray matter volume (rGMV), we identified the anatomical correlates of behavioral inhibition and behavioral activation using the behavioral inhibition system/behavioral activation system (BIS/BAS) scales in 382 young and healthy subjects (166 males and 216 females). Results showed that higher BAS score was associated with larger rGMV in the left parahippocampal gyrus and fusiform gyrus and smaller rGMV in the right precentral gyrus and inferior frontal gyrus. Furthermore, higher BIS score was associated with larger rGMV in the left middle frontal gyrus. Our results and previous findings suggest that structural variations in regions associated with emotion-driven learning, reward anticipation and reward selection as well as those associated with modulation of negative affect and anxiety are associated with behavioral inhibition and behavioral activation.

Modeling the dynamics of disease states in depression

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Major depressive disorder (MDD) is a common and costly disorder associated with considerable morbidity, disability, and risk for suicide. The disorder is clinically and etiologically heterogeneous. Despite intense research efforts, the response rates of antidepressant treatments are relatively low and the etiology and progression of MDD remain poorly understood. Here we use computational modeling to advance our understanding of MDD. First, we propose a systematic and comprehensive definition of disease states, which is based on a type of mathematical model called a finite-state machine. Second, we propose a dynamical systems model for the progression, or dynamics, of MDD. The model is abstract and combines several major factors (mechanisms) that influence the dynamics of MDD. We study under what conditions the model can account for the occurrence and recurrence of depressive episodes and how we can model the effects of antidepressant treatments and cognitive behavioral therapy within the same dynamical systems model through changing a small subset of parameters. Our computational modeling suggests several predictions about MDD. Patients who suffer from depression can be divided into two sub-populations: a high-risk sub-population that has a high risk of developing chronic depression and a low-risk sub-population, in which patients develop depression stochastically with low probability. The success of antidepressant treatment is stochastic, leading to widely different times-to-remission in otherwise identical patients. While the specific details of our model might be subjected to criticism and revisions, our approach shows the potential power of computationally modeling depression and the need for different type of quantitative data for understanding depression.

Keywords: *Major depressive disorder, disease states, finite-state machine, dynamical systems model*

Neuronal correlates of sustained fear in the anterolateral part of the bed nucleus of the stria terminalis

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Much of our understanding for mechanisms of fear and anxiety is based on animal studies in which Pavlovian paradigms have been used to evaluate short-term fear responses to a conditioned threat ("phasic fear"). Recently, experimental studies have begun to focus on more long-lasting states of fear that are elicited by less predictable threats ("sustained fear"). The bed nucleus of the stria terminalis (BNST), a region within the extended amygdala, has been identified as a critical element for phasic as well as sustained fear responses, but its neuronal activity pattern in relation to these different fear states is unclear so far. Therefore, we developed a fear conditioning paradigm, which allows us to differentiate between phasic and sustained fear states and combined it with extracellular local field potential and single unit recordings in freely behaving mice. Cluster analysis of neuronal activity throughout the phasic/sustained fear protocol revealed 3 distinct types of neuronal subpopulations in the anterolateral part of the BNST. (i) High phasic fear off neurons (approximately 30% of identified units), decreasing their activity at initially high phasic fear states, (ii) low sustained fear off neurons (app. 15%), reducing their activity at low sustained fear states and (iii) low sustained fear on neurons (app. 25%), showing a significantly increased activity upon the sustained fear state. Taken together, our preliminary results show that activity patterns of neuronal subpopulations of the BNST indeed correlate with the behavioral expression of phasic and sustained fear states.

Phasic dopamine activity in the dorsal striatum during variable interval responding to alcohol and sucrose

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While habitual alcohol-seeking and drinking is thought to be regulated by dorsal striatum, it is unknown whether changes in phasic dopamine accompany this behavior. In this study we measured phasic dopamine release in the dorsomedial and dorsolateral striatum of rats self-administering either alcoholic or nonalcoholic sweet solutions on a habit-promoting variable interval (VI) reinforcement schedule. We hypothesized that phasic dopamine release would occur immediately before or after lever-press action in both dorsomedial and dorsolateral striatum, and that dopamine release would be amplified in rats drinking alcohol.

Rats were trained to press a lever on a VI30 schedule for either 10% sucrose (sucrose group) or mixed 10% sucrose/10% ethanol (alcohol group). Phasic dopamine activity was evaluated ±1-sec around reinforced and unreinforced lever presses by using fast-scan cyclic voltammetry in the dorsomedial and dorsolateral striatum.

When assessing dorsomedial dopamine release at reinforced lever presses, small concentrations of DA release before and after the lever press was found. In sucrose rats, [DA]max was 7±1 and 8±3nM pre- and post-press, respectively. In alcohol rats, dopamine release was higher but not significantly different from the sucrose group: [DA]max was 15.8±5 and 16.2±2nM pre- and post-press, respectively. In the dorsolateral striatum, phasic dopamine release occurred after reinforced presses in both groups: [DA]max was 17.8±8.4 nM in the sucrose group and 25±10nM in alcohol group, and these dopamine concentrations were significantly higher than pre-press (main effect of time, $p<0.05$). Next, to better determine which dopamine signals were associated with action and which were associated with reward, we evaluated dopamine release associated with unreinforced lever presses. Similar to the rewarded lever-press activity, dorsomedial striatum dopamine around the unrewarded lever presses was again small in sucrose rats: [DA]max was 5.7±1.7 and 5±3.3nM pre- and post-press, respectively. In alcohol rats, dopamine release was higher but not significantly different than the sucrose group: [DA]max was 12±6 and 12±6nM pre- and post-press, respectively. Interestingly, dorsolateral striatum dopamine release in the alcohol group was significantly higher after unrewarded lever presses compared to pre-press dopamine (main effect of time, $p<0.01$; time X group interaction, $p<0.05$), despite the small concentration ([DA]max =9±1nM). Thus, as predicted dopamine in both dorsomedial and dorsolateral striatum accompanies VI responding in rats. Dorsolateral striatum dopamine tended to be higher, which might be related with greater involvement of the dorsolateral striatum to the habit-like behavior. Moreover, in alcohol rats, dorsolateral striatum dopamine was reward-independent, that might demonstrate involvement of phasic dopamine in the dorsolateral striatum in outcome-resistant habit-like alcohol seeking.

Positive allosteric modulation of the a7 nicotinic acetylcholine receptors enhances recognition memory in rats

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Alpha 7 nicotinic acetylcholine receptors (a7-nAChRs) are involved in the regulation of cognitive processes. Furthermore, it has been suggested that a7-nAChRs might be implicated in the pathophysiology of schizophrenia. Hence, selective activation of a7-nAChRs is considered to be a potential therapeutic strategy aimed at ameliorating cognitive and mnemonic dysfunctions associated with schizophrenia. Much of preclinical data indicated that orthosteric ligands, like the partial a7-nAChR agonist,GTS-21, produced procognitive effect, but their clinical results are equivocal.

Positive allosteric modulation (PAM) is an alternative way to activate the a7-nAChRs. Based on the functional properties of modulation, a7 PAMs are divided into two groups, type I and type II. Both appear to increase receptor sensitivity to endogenous agonist. However, type I PAMs have little or no effect on desensitization processes, while the action of type II PAMs is accompanied by a retardation of the kinetics of desensitization. CCMI is one of the recently synthesized PAMs type I and its behavioural activity has not been yet extensively characterised. Thus, little is known about the potential efficacy of this compound on cognitive processes.

Converging lines of evidence indicate impaired recognition memory in schizophrenic patients. This kind of memory can be assessed in rodent models by using the novel object recognition task (NORT). This task is based on a natural tendency of rodents to explore novel objects more than the familiar one. If the animal remembers a previously presented object, it spends more time exploring the novel one during the discrimination phase. Introduction of the long inter-trial interval (24 h) results in natural forgetting.

The aim of present study was to evaluate the effects of CCMI, a type I a7-nAChR PAM, and of GTS-21, an orthosteric agonist of a7-nAChR in the NORT in rats. Additionally, to elucidate whether the demonstrated improvement of recognition memory was due to the compounds' action at the a7-nAChRs, the ability of an a7-nAChRs antagonist, methyllycaconitine (MLA), to block the effects of active doses of CCMI and GTS-21, was assessed.

Both GTS-21 (1 mg/kg) and CCMI (3 mg/kg), administered IP 30 minutes before the acquisition trial, caused an improvement of the recognition memory when tested 24h following their administration. These procognitive effects were fully blocked by MLA (3 mg/kg).

The present study demonstrates the beneficial effects of both an orthosteric agonist of a7-nAChRs and type I a7-nAChRs PAMs on recognition memory in rats. Therefore, our results support the notion that a7-nAChRs allosteric modulation may constitute a potential procognitive therapy.

Social behavior at the molecular level

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Providing the understanding of the neural, humoral and genetic factors that control social behavior is a key for human well-being. The formation and maintenance of social relationships in young and middle age are essential components of human mental health. A deficit in the healthy behavioral formation (autism, schizophrenia, social phobia) leads to social isolation. Thus, it is of particular importance to understand the molecular mechanisms that sustain the establishment and modulation of relationships between individuals, especially in the context of treatment and drug therapy for patients. The richness of developmental processes in behavior, including multiple sources and the consequences of experience are significant in understanding processes of human development. A responsibility for different behavioral pathology is laid down at the neurochemical level. However, yet little is known about the brain molecular mechanisms of oxytocin secretion and its release in the implementation of social behavior. The behavioral study of humans would be much diminished today without the influence of animal research. Mice are social animals and ‘knockout’ mice are powerful models for investigating the neurobiological mechanisms of the cognitive process control leading to the development of social relationships, skills and for potentially extending our understanding of the human condition, and have been of major importance to theories of child development and to psychiatry. Understanding the neurobiological bases of social recognition, social leaning, emotions and the use of social information transmission in mice provides new perspectives by which to view and regulate social behavior among human beings.

Programming of promoter DNA methylation in heterozygous serotonin transporter deficient mice by prenatal stress

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Stress exposure, especially in early life, can have severe effects on emotional health in later life. The serotonin transporter gene-linked polymorphic region (5-HTTLPR) in humans has been suggested to play a modulatory role in mediating effects of early-life stress exposure on psychopathology, rendering carriers of the short (s)-allele more vulnerable to adversity in later life. However, the underlying molecular mechanisms are not well understood. Epigenetic mechanisms such as DNA methylation could represent mediators of such gene-by-environment interactions. To elucidate mechanisms involved in the programming of later life vulnerability, we used a maternal restraint stress paradigm of prenatal stress in heterozygous (+/-) 5 HTT deficient mice and found that the effects on behaviour and gene expression were particularly marked in the hippocampus of female *5-Htt+/-* offspring. Following these examinations, we performed a genome-wide hippocampal DNA methylation screening using methylated-DNA immunoprecipitation (MeDIP) on Affymetrix GeneChip® Mouse Promoter 1.0R arrays, to learn to which extend these effects might be mediated by differential DNA methylation. Subsequent analysis of this data focussing on female offspring showed that *5-Htt* genotype, PS and their interaction differentially affected the DNA methylation signature of numerous genes, a subset of which showed an overlap with the expression profiles of the corresponding transcripts. One especially interesting candidate, coming up in our analysis was the myelin basic protein (Mbp). DNA methylation, at specific CpG sites located at the promoter of this gene, was associated with gene expression and also anxiety-related behaviour in these animals. In more detail, we observed a decreased DNA methylation and an up-regulation of gene expression in the hippocampus of wild type animals induced by prenatal stress, while no change in DNA methylation and gene expression was found in the hippocampus of *5-Htt +/-* mice following stress exposure. Most remarkably we found the same expression pattern displayed in several other myelin related genes, as well. In conclusion, our behavioural data, in combination with gene expression and DNA methylation results, hint towards DNA methylation to be one distinct molecular mechanism contributing to the behavioural consequences of heterozygous 5-HTT deficiency, prenatal stress and their interaction.

Responsiveness of activin A and its novel target gene *Pmepa1* to environmental stimulation and antidepressant treatment

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The transforming growth factor-ß (TGF-ß) family member activin A exerts multiple neurotrophic and -protective effects in the brain. More recently, we and others identified activin A as a modulator of both glutamatergic and GABAergic neurotransmission, influencing synaptic plasticity, cognitive functions and affective behavior (Zheng et al., Mol Psychiatry 14:332-346, 2009; Krieglstein et al., TiNS 34:421-429, 2011). With respect to the latter, forebrain-specific disruption of activin receptor signaling induces a low-anxiety phenotype in mice, whereas infusion of activin into the hippocampus exerts antidepressant-like effects. Activin A signaling is furthermore targeted by antidepressant therapies including electroconvulsive seizures (ECS) and pharmacological drugs. However, the functional implications of the dramatic increase of activin signaling after ECS and mechanisms to counteract activin effects in the brain are still largely unknown. Another pending question is if activin is responsive to physiological stimuli known to ameliorate depression-like behavior in rodents such as environmental enrichment (EE). To explore these issues, we assessed the impact of EE and ECS on activin signaling and its functional inhibitors in C57BL/6 mice. We found that EE produced a rapid and significant up-regulation of the activin ßA subunit (*Inhba*) that resembled the massive increase in *Inhba* mRNA after ECS in its time course, but was more modest. Both EE and ECS selectively up-regulated *Inhba* expression and did not appreciably affect the mRNA levels of other TGF-ß family members such as *Tgfb1*, *Tgfb2* and *Tgfb3*. The elevated *Inhba* expression was accompanied by enhanced phosphorylation of SMAD2/3, the intracellular effectors of activin signaling, indicating functionality of the signaling pathway. Several activin signaling inhibitors including follistatin and *Pmepa1* (=prostate transmembrane protein, androgen induced 1) were increased in response to ECS and EE with an appropriate time delay to the maximal up-regulation of *Inhba* mRNA, suggesting a self-limiting feedback loop induced by activin. Using chromatin immunoprecipitation (ChIP) with anti-SMAD2/3 antibodies, *Pmepa1* was identified as the first *in vivo* target gene of activin signaling in the hippocampus following ECS. Underlining the functional importance of this finding, up-regulation of *PMEPA1* mRNA by activin was conserved in human neurons generated from embryonic stem cells. Taken together, our findings substantiate the notion that activin is a promising candidate for endogenous anti-depressant action and identifies PMEPA1 as a novel player in the self-limiting activin response.

Study hydrolysis myelin basic protein by IgG of schizophrenic patient according to the extension of the disease

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Schizophrenia remains one of the leading causes of disability. Schizophrenia is mental illness with polymorphic symptomatology leading to cognitive and related skills deficits marred by reduced levels of independence. The end result is considerably reducing lifespan. Involvement of the immune system in the pathogenesis of schizophrenia was shown in many articles. There is the observed dysregulation between the nervous and immune systems, the causes of which may be changes in brain structure and dysfunction of immune cells. Furthermore the serum of patient with schizophrenia contains autoantibodies to human myelin basic protein (MBP). Although the blood level of IgG in group of patient significant increase as compared to concentration of IgG in blood of healthy person. Research of catalytic activities antibodies of the sera of patient with schizophrenia has not been carried out previously.

IgG fractions were purified individually from the sera of 10 healthy donors and 30 schizophrenic patient. Electrophoretically homogeneous IgG were obtained by affinity chromatography of serum proteins on protein G-Sepharose under conditions that remove nonspecifically bound proteins. The homogeneity of Abs was confirmed by SDS-PAGE with silver staining. It showed single bend which had molecular weight equal 150-kDa. It has been shown that catalytic activity is an own property of antibodies. Number of strict criteria was testing to assign the detected catalytic activity to the antibodies: electrophoretic homogeneity of Abs, gel exclusion chromatography of Abs at conditions of dissociation of immune complexes (pH shock analysis).

In this work we investigate proteolytic hydrolysis MBP by Abs of schizophrenic patients according to the extension of the disease. Subjects were grouping according to duration of disease: group A – 2-5 years duration of disease, group B – 6-12 years, group C – more than 13 years disease duration. It was shown that Abs of schizophrenic patients specifically hydrolyzed MBP. Proteolitic activity belongs to purified Abs. Increase of proteolytic activity IgG towards MBP associated with extension of the disease (p-level 0,5): level of MBP hydrolysis in group A (2-5 years) is 6,0%, Abs from group B (6-12 years) demonstrate higher proteolytic activity - 54%, Abs from group C (more than 13 years) showed the most higher level of MBP hydrolysis – 97%. Based on these results we can suggest that IgG may play a role in the pathogenesis of schizophrenia.

The blockade of NMDA receptors reduced the extinction period on morphine-conditioned place preference in the rat

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Introduction: Several studies showed that brain reward system including the nucleus accumbens (NAc) and ventral tegmental area (VTA) play an important role in morphine-induced reward and reinstatement. Activation of NMDA glutamatergic receptors in the NAc and VTA may be a component of the mechanism of the drug induced reward. In addition, the NAc also receives a major dopaminergic projection from VTA. Intra-accumbal glutamate and dopamine are affected the reward system through actions on the NMDA receptors. So, in this study, by using a NMDA receptor antagonist (AP5), the role of NMDA receptor in the brain reward system during extinction period was investigated in maintenance of the morphine rewarding properties by conditioned place preference (CPP) paradigm in the rats.
Materials and methods: Forty adult male albino Wistar rats were used in these experiments. After administration of effective dose of morphine (5 mg/kg; sc) during the CPP paradigm, animals received three doses of AP5 (1, 5 and 25 mM/5 µl in saline) or saline intracerebroventricularly in extinction period (free morphine stage). The conditioning score and motor activity were recorded during extinction period by Ethovision software.
Results: Our findings showed that ICV injections of different doses of AP5 (1, 5, 25 mM/5 µl) significantly reduce the extinction phase of morphine-induced CPP in a dose-dependent manner.
Discussion: It seems that blockade of NMDA receptors maybe act through memory pathways associated with reward circuitry in the rat.

The effects of the positive allosteric modulator of a7-nAChRs in cognitive tasks in rats.

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Nicotinic acetylcholine receptor (nAChR) systems are widely recognised as playing an important role in the regulation of cognitive processes. Recently, studies investigating the utility of nAChRs as a possible target for the therapy of cognitive decline in Alzheimer's disease and schizophrenia have focused primarily on the a7-nAChRs subtype. A promising approach is based on the use of positive allosteric modulators (PAMs) of a7-nAChRs due to their several advantages over the direct agonists. Nevertheless, the behavioural effects of this class of compounds, particularly with regard to higher-order cognitive functions, have not been broadly characterised.

In the present study, the potential procognitive efficacy of a7-nAChRs PAM, CCMI (N-(4-chlorophenyl)-[[(4-chlorophenyl)amino]methylene]-3-methyl-5-isoxazoleacet-amide, also known as compound 6, AVL-3288 or XY4083) was assessed in the attentional set-shifting task (ASST), odour span task (OST) and five-choice serial-reaction time task (5-CSRTT) in rats.

In the ASST, rats must select a bowl containing a food reward based on the ability to discriminate the odours or the media covering the bait. The ASST requires rats to initially learn a rule and form an attentional "set" within the same stimulus dimensions. At the extra-dimensional (ED) shift stage, the essential phase of the task, animals must switch their attention to a new, previously irrelevant stimulus dimension. The animal's performance at the ED stage is considered an index of cognitive flexibility.

The OST assesses working memory (WM) capacity, i.e., the amount of information kept concurrently in the WM. Rats are trained to dig in scented bowls for food rewards. Upon retrieval of the reward, another bowl, marked with different scent, is added. Only the novel bowl is baited and must be selected over the previously sampled bowls. The number of odours remembered provides a measure of working memory span.

In the 5-CSRTT, used to assess sustained attention, rats are required to detect and respond to brief light stimuli that are presented randomly in one of the five holes. The task allows for the simultaneous examination of multiple aspects of performance. The ratio of correct responses to total responses offers a measure of the accuracy of attentional processes. Additionally, impulsivity is measured by premature responses and the latency to the correct response reflects the speed of processing.

The acute administration of CCMI (1 mg/kg) facilitated cognitive flexibility, as indicated by a reduced number of trials to criterion during the ED phase of the ASST. CCMI also decreased the number of errors made in the OST. However, the compound's effect on the span length was not statistically significant. CCMI did not affect any aspect of rats' attentional performance on the 5-CSRTT.

The present study demonstrates the beneficial effects of CCMI on some aspects of cognition, i.e., set-shifting ability. Further studies are necessary to evaluate the efficacy of this compound in models of cognitive impairment.

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The endocannabinoid system in the extended amygdala network modulates mechanisms of sustained fear.

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The extended amygdala, in which neurons of the central (CeA) and medial (MeA) amygdala are connected to neurons of the bed nucleus of the stria terminalis (BNST), has received a lot of attention as a possible mediator of sustained fear. In line with this, recent studies show that the endocannabinoid system is involved in the modulation of the synaptic transmission and plasticity in the BNST.

Therefore, we used a combination of in vivo and in vitro approaches to analyse whether the cannabinoid type 1 (CB1) receptors are directly involved in the modulation of amygdala-BNST synapses. By adeno-associated virus-mediated expression of channelrhodopsin in specific amygdala nuclei we studied their connectivity with BNST upon optogenetic activation. In vitro patch-clamp recordings combined with light stimulation showed that the monosynaptic amygdalar input from CeA and MeA are GABAergic while basal amygdala (BA) input is glutamatergic. Futhermore, CB1 receptors in the BNST mediated short term plasticity in terminals originating from the CeA and BA, but not from MeA. Local pharmacological intervention in behaving animals showed that blocking CB1 receptors in BNST with AM251 before fear retrieval reduced expression of sustained fear whereas a phasic component was unaffected. These results were replicated in experiments using CB1 receptor-deficient mice. Therefore, we suggest that CB1 receptor activation in the BNST shifts animal behavior from phasic to sustained fear state. Together these results indicate the endocannabinoid system to play a critical modulatory role in mechanisms of fear sustainment within the extended amygdala network

THE TPH2 KNOCKOUT RAT: PHYSIOLOGICAL AND BEHAVIORAL ANALYSIS OF A SEROTONIN DEFICIENT RAT MODEL

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Serotonin (5-HT) is a neurotransmitter in the central nervous system, which plays a role in the etiology of depression, phobias, and other psychiatric dysfunctions. It is also involved into central regulation of respiration, circadian rhythmicity, immune and cardiovascular systems. There are two independent 5-HT synthesizing enzymes in mammals and knockout animal models for each pathway represent indispensable tools to understand the various functions of 5-HT. The recently generated knockout rat for the brain-specific enzyme Tryptophan hydroxylase 2 (TPH2) is a unique model which lacks 5-HT exclusively in the brain. In this study we firstly established the protocol for the genotyping of *Tph2*-deficient rats and then evaluated cardiovascular and behavioral phenotypes in these animals. Results of different physiological and behavioral experiments indicated that the lack of central 5-HT causes alterations in social, aggression-like, and stress coping behaviors and blood pressure regulation. Furthermore, male *Tph2*$^{-/-}$ rats exhibit significant growth retardation during adolescence. Investigation of behavior in adolescent rats revealed alterations in tickling-induced ultrasonic vocalization and adult hippocampal cell proliferation. This study gives a first neuromolecular and behavioral characterization of the *Tph2*$^{-/-}$ rat. Since the rat 5-HT system is highly similar to human, these results highlight the opportunities for new translational research from rat to man.

Variability of Cerebral Lateralization for Perception of Speech Emotional Prosody in the Course of Perceptual Learning (in different acoustic environment)

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The theoretical and research contributions of neuroscience have underlined the importance of speech emotional prosody in human communication and have thoroughly examined the mechanisms of its processing. Sufficiently large number of studies have considered and discussed cerebral lateralization features for speech emotional intonation perception. However, there are still no unequivocal data on this question. We also know that perceptual learning plays an important role in the processing of emotional prosody of speech. However we found no papers that studied cerebral asymmetry for perception of speech emotional prosody that develops in the process of perceptual learning. It is known that external noise influences the process of perceptual learning and, as well, may cause variation of the interhemispheric relationships. In this study we investigate features of cerebral lateralization of speech emotional intonation perception in the process of perceptual learning in the changing acoustic environment (at white noise background and without it). The created corpus of speech signals of happy, angry, neutral emotional intonations contained two dissyllable pseudo words. Such test stimuli were selected to avoid lexical-semantic component of the signal. The stimuli were presented through the headphones at random to the right or left ear, without noise or at ipsilateral white noise background. Simultaneously, white noise of the same intensity as the valid signal was fed to the contralateral ear through the other channel. Adult listeners had to recognize the valence of emotional intonations of test stimuli. The sample consisted of 38 adults (23 females and 15 males) of mean age 21.1 ± 0.4. They were students of St. Petersburg universities, had normal hearing and were right handers. Reaction time (RT) and accuracy of recognition (AR) were recorded in two consequent sessions of trials and generalizing index considering the both of them was calculated (relative recognition efficiency – RRE=AR/RT). Analysis of variance showed the factor of session's sequence to be highly significant for RRE ($p < 0.00014$). In the absence of noise perceptual learning is observed for negative emotions that are recognized through the right ear, and through the left ear for neutral and positive emotional intonations. At white noise background we have found the changing of perception lateralization with training, that is with transition from the first to the second series of presentation. For emotional intonation "anger" significant improvement of the RRE, unlike noiseless experimental conditions occurs when a signal is applied to the left ear ($\Delta = 21.34\%$ / s, $p = 0.045$). Improvements of RRE for "joy" is not significant both for recognition through right and left ears. It can be assumed that structures of the right hemisphere are more susceptible to training than the structures of the left hemisphere under noiseless conditions for positive emotional intonation and at noise background – for negative ones. Thus, lateralization revealed for speech emotional tone recognition in the course of perceptual learning depends on acoustical environment and the emotional intonation valence that, in its turn, confirms variability of functional cerebral asymmetry.

Impaired fast-spiking interneuron function in a genetic mouse model of depression.

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Rhythmic neuronal activity provides a frame for information coding by co-active cell assemblies. Abnormal brain rhythms are considered a pathophysiological mechanism causing mental disease, but the underlying network defects are unknown. We find that mice expressing truncated Disrupted-in-Schizophrenia 1 (Disc1), which mirror a high-prevalence genotype for human psychiatric illness, show depression-related behavioural despair. Theta and low-gamma synchrony in the prelimbic cortex (PrlC) is impaired in Disc1 mice and inversely correlated with the extent of behavioural despair. While weak theta activity is driven by the hippocampus, disturbance of low-gamma oscillations is caused by local defects of parvalbumin (PV)-expressing fast-spiking interneurons (FS-INs). The number of FS-INs is reduced, they receive fewer excitatory inputs, and form fewer release sites on their targets. Computational analysis indicates that weak excitatory input and inhibitory output of FS-INs leads to impaired gamma oscillations. Our data therefore provide a link of network defects with a risk gene mutation underlying depression in humans.

Poster Topic

T14: Vision: Invertebrates

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T14-4D Ultrastructure and Anatomy of Microglomerular Synaptic Complexes in the Polarization Vision Pathway of the Honeybee
Martina Held, Uwe Homberg, Keram Pfeiffer

A ciliary protein in motion-vision gain control

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During locomotion, motion-sensitive interneurons react with higher gain to stimuli[1-3]. Even though octopamine seems to mediate this gain-control[4], it is unknown if a defined cell population is necessary to control the motion vision gain. We tested the optomotor reflexes of over 20000 flies and found that the knock-out of a single ciliary protein in a single neuron population of the medulla decreased the gain in the optomotor reflex. This observation holds true for the ON and OFF channel of the motion vision pathway. Furthermore, when we genetically clamped the membrane potential of these cells to resting potential, the flies did not show any optomotor reflex. An increase of the membrane potential via thermogenetics tricked the animal into over-estimating the stimulus. Thus we have shown that the interneuron is nescessary for motion vision and sufficient to alter its gain.

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Anatomical organization of tangential neurons of the central complex in the brain of the desert locust

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Insects show exceptional abilities for spatial orientation that in many respects parallel those of vertebrates. The diversity of navigational strategies like landmark-aided orientation, path integration and sophisticated sky compass orientation reflects adaptations to different living conditions of insects. In many navigational tasks, an assembly of midline-spanning neuropils in the insect brain, the central complex (CX), is involved. The CX constitutes a 3D matrix composed of rows of 16 vertical slices within its subdivisions, the protocerebral bridge, the upper and the lower division of the central body, and a pair of noduli composed of stacks. Following the analysis of columnar neurons that innervate and interconnect single slices of CX subunits (Heinze and Homberg 2008, J Comp Neurol 511:454), we studied the organization of tangential neurons as a basis for further functional analyses of the CX.

Data are based on reconstructions of single dye injected neurons and mass injection of dye for mapping cell clusters of tangential neurons. With few exceptions, tangential neurons have fine, putatively dendritic ramifications outside the CX and beaded or varicose ramifications likely to be axonal within the CX. Ramifications of tangential neurons outside the CX were largely subdivision-specific. Tangential neurons of the protocerebral bridge (TB neurons) arborized in the posterior optic tubercles or wide areas of the posterior slope of the brain. Tangential neurons of the noduli (TN) ramified in parts of lateral accessory lobes. Tangential neurons (TL) of the lower division of the central body had dendritic processes in the medial and lateral bulbs of the lateral complex and surrounding areas. Tangential neurons of the upper division of the central body (TU) showed the largest variety of cell types. Their ramifications outside the CX were concentrated in subfields of the lateral accessory lobes or the superior medial protocerebrum and the lateral protocerebrum. The data suggest relative specific input to the protocerebral bridge, the noduli, and the lower division of the central body and, in contrast, a large variety of brain areas directly connected to the upper division of the central body. No direct connections were found with the mushroom bodies.

Circadian clock resetting in *Drosophila* by non-canonical Rhodopsin signalling and the zona pellucida protein Quasimodo

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Circadian clocks regulate multiple aspects of animal behaviour and physiology to occur at optimal times within the 24-h day. In order to do this, they must be accurately synchronized to rhythmic environmental cues such as light and temperature. In mammals and invertebrates synchronisation to environmental light:dark cycles occurs via visual and non-visual pathways. In Drosophila, synchronization is mediated both by the compound eyes, and by the blue-light photoreceptor Cryptochrome (Cry), present in many but not all of the clock neurons. Cry undergoes a light-induced conformational change that allows it to bind to the clock protein Timeless (Tim) and the F-box protein Jetlag (Jet), which results in both the degradation of Tim and Cry and the re-setting of the molecular clock. However, in the absence of Cry and retinal photoreception, some pacemaker neurons still retain the ability to regulate Tim levels in response to light and to reset their clock.

The clock controlled gene quasimodo (qsm) encodes a GPI-anchored zona pellucida protein that acutely responds to light via a Cry-independent mechanism. Qsm mutants interfere with the light-dependent degradation of the clock proteins Tim and Period (Per), which results in abnormal behavioural rhythmicity in constant dim light (LL), a condition in which wild type flies exhibit arrhythmic behaviour due to the constitutive degradation of Tim.

Wild type flies synchronize to a 12h:12h light:dark (LD) cycle and following exposure to a 6 hour phase delay of this cycle (similar to rapidly crossing 6 time-zones westward) they re-entrain to the new regime after only one day. In contrast to this, mutants lacking both Cry and norpA (a gene encoding Phospholipase C-ß, an essential enzyme in the fly visual photo-transduction pathway) need 5 to 7 days to resynchronize their clocks. Although this is much longer than normal flies, it does show that some some residual sensitivity of their circadian clock to light.

However, the additional removal of Rh5 and Rh6 abolishes this residual light sensitivity of the norpAP41;; cryb double mutants, suggesting the existence of a PLC and Cry-independent mechanism clock entrainment. Our preliminary results show that when qsm mutants are also combined with norpAP41 and cryb, the same inability to re-synchronise is observed. We therefore propose that Qsm interacts with Rh5 and/or Rh6 via a non-canonical photo-transduction pathway and mediates light based entrainment of the clock. To test study this hypothesis we have studied the electro-retinogram (ERG) response of these flies to light. The remaining ERG response found in norpAP41 mutants is absent or altered when the mutation is combined with the removal of Rh5 and Rh6, therefore implying that Rh5 and Rh6 have a norpA independent function.

The anchoring of Qsm in the cell membrane and the lack of a direct physical interaction with Tim implies

that it exerts its effect on the clock via another route. We predict that Qsm interacts with ion channels and/or transporter proteins that influence membrane properties and intracellular and/or intercellular signalling mechanisms. It has been shown that Qsm interacts genetically and physically with the Na+-K+-Cl- cotransporter (NKCC). Our preliminary results show that in constant dim light (LL), the same conditions in which qsm mutants display abnormal rhythmicity, overexpression and knockdown (via RNAi) of NKCC produces similar high rhythmicity. Strikingly, under the same LL conditions NKCC overexpressing flies also exhibit robust Tim oscillations similarly to what was observed for qsm mutants.

Based on our findings, we propose a possible light entrainment pathway involving non-canonical photoreception. After activation by light of the Drosophila Rhodopsins 5 & 6 we believe that this may promote the cleavage of Qsm from the membrane which leads to the activation of the Na-K-Cl contransporter (NKCC), which affects neuronal polarisation and ultimately the degradation of Tim and the re-setting of the molecular clock.

Circadian rhythmicity in behavioural and neuronal sensitivity in locusts

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Locusts demonstrate phenotypic plasticity in behaviour, morphology and physiology, driven by changes in population density. At low densities, locusts become solitarious and are cryptic in behaviour and appearance. At high densities, locusts become gregarious, are conspicuously coloured and primarily day-active active. Previous work has shown a variation in the circadian rhythm of a looming-sensitive interneuron (Gaten *et al*., 2012), while a circadian aspect to hatching has also been shown (Padgham, 1981). Here we carry out electroretinogram (ERG) and behavioural analyses in response to visual stimuli, and hatching assays, all of which show a circadian pattern of differing robustness in the two phenotypes. Solitarious animals show a more robust clock than gregarious animals which do not show a sustained ERG rhythm into constant darkness. The type of behavioural response to a looming stimulus differed between the phenotypes, with solitarious animals less likely to hide than gregarious animals and more likely to freeze. The amplitude of hiding response was significantly diurnal in solitarious animals but was not in gregarious animals. The temporal characteristics of hatching for gregarious and solitarious animals differed significantly with solitarious animals emerging 20 minutes earlier than gregarious hatchlings. Intra-pod variation in hatching time was significantly lower in solitarious egg pods than gregarious egg pods. These experiments combined suggest a reduced robustness in the gregarious circadian clock relative to that of solitarious individuals.

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Direct visual input to motoneurons controlling wing steering muscles in *Drosophila*

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Locomotor control in animals requires the integration of multiple sensory modalities. The impressive aerial performance of flies particularly depends on the continuous fusion of visual information from the compound eyes with stabilizing mechanosensory feedback from campaniform sensilla on both the wings and the halteres. Halteres are gyroscopic, club-shaped organs that beat in anti-phase with the wings and finely control phasing of flight muscles spikes. Electrophysiological studies in resting flies previously suggested that during maneuvering flight, visual input is rerouted through the haltere system rather than directly activating wing steering muscles. Owing to this bypass, visual steering commands were thus considered to manipulate the halteres' precisely-timed feedback signals within each wing-stroke cycle. Hence, the halteres would be required for both body stabilization and vision-guided flight control.

Here we investigated the alternative hypothesis that visual information and mechanosensory feedback are integrated at the level of the steering muscle motoneurons. To this end, we investigated fruit flies *Drosophila* in which we abolished sensory feedback on both body sides by mechanically disabling haltere movement and ablating the wing nerve with an infrared laser. We scored wingbeat kinematics and behavioral performance of the tethered flying animals under vision-guided feedback conditions in a flight simulator. Compared to a control group, we found significant differences in the ability of the tested flies to modulate bilateral wing stroke amplitude in response to a single, horizontally moving visual object under closed-loop control. Wingbeat amplitude response of haltere-immobilized flies was significantly reduced, while the wing nerve-treated group showed a strong increase in response amplitude. Similar changes in wingbeat amplitude modulation were also obtained from flies responding to a vertically oscillating pattern under open-loop feedback conditions. By contrast, we found only subtle behavioral differences in both groups in their ability to actively fixate a visual object in the frontal field of view and no significant changes in visio-motor response delays during vision-induced escape maneuvers, compared to unrestrained animals.

Our results provide behavioral evidence for the existence of a direct and strong neural pathway from the visual system to wing steering muscles, bypassing the mechanosensory feedback from the halteres. The characteristic changes in wingbeat amplitude modulation in the manipulated flies, moreover, suggest antagonistic impact of the two mechanosensory inputs on the efficiency with which steering muscles control wing kinematics. Consistent with electrophysiological studies on sensory integration processes in motoneurons of the fly's neck muscles, our findings support the idea of sensory integration of vision and mechanosensory feedback by motoneurons of wing steering muscles. In this scenario, motoneurons appear as efficient and fast, low-level information processing units, which might give rise to a new conceptual framework for locomotor control in flies.

Expression Plasticity of Opsin Genes in *Camponotus rufipes* workers

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Camponotus rufipes workers start to forage outside the nest after a period of about 4 to 6 weeks. Outside the nest, vision plays a more important role since foragers need to orient and navigate in a light environment. In the present study, we investigate if the transition from indoor to outdoor activity is accompanied by changes in opsin expression and therefore with spectral sensitivity. Using qRT-PCR method, we aimed to answer: Which opsin genes do *C. rufipes* workers express? What is the expression plasticity of opsin genes in relation to age and light exposure?
Our results showed that *C. rufipes* workers expressed long-wavelength (LW1), ultraviolet (UV) and blue (BL) opsin mRNAs at varying levels, respectively, from most to least expressed levels. Phylogenetic analyses revealed that the inferred amino acid sequences were similar to visual opsin sequences of other insects such as *A. mellifera* and *C. floridanus*.
Considering the plasticity in opsin gene expression in relation to age, Post hoc analysis showed that expression levels of all three opsins increased up to 14 days of age in workers kept under constant darkness (DD). Although all three opsin levels started to decrease after 28 days, it was only significant for the UV opsin mRNA and continued until the age of 42 days.
To test for light effects, 28 days old workers were exposed to a 12:12 hours light/dark regime for 1 day, 4 days and 14 days (LD) and compared to workers of the same age which were kept under constant darkness. Our results showed that expression levels of all opsins were significantly higher in the light/dark group except for the 4 days LD group where only the increase of the BL opsin level was significant.

We conclude that *C. rufipes* workers, like many other hymenopterans, express three opsins (LW1, UV, and BL) in their compound eye and show expression plasticity in relation to age and light exposure. Opsin expression is regulated by multiple factors at the same time (e.g. light, age and circadian clock) as shown in honey bees, and therefore expression plasticity of opsins is strongly related to environmental factors and physiological adaptations.
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Head-body-coordination in walking *Drosophila melanogaster*

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Due to the small stereobasis and interocular overlap, most insects lack stereoscopic vision. Therefore, other cues for distance estimation become prevalent as for example the retinal image shift by self-motion. During translational movements, close objects will travel faster across the retina than distant ones, whereas during rotational movement all objects move with the same speed. Therefore, only translational movements provide distance information[1]. Insects overcome this problem by using a saccadic strategy, which consists of very short and fast rotations, called saccades[2,3], that disrupt long translational movements. This strategy has been found in different insects[4–6].
Here, we show that walking *Drosophila melanogaster* perform body saccades, without the typical head saccades described for other insects[4]. This was also paired with the absence of haltere movement during walking, which seems to coordinate head movement in other insects. Modeling of the visual field of *Drosophila* revealed that head movements affect the retinal input only marginally.

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How much *Drosophila* visual behavior is predicted by models with asymmetric motion responses?

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Insects show very robust visual behaviors that require sophisticated computation. We use *Drosophila* as our model system to analyze the underlying principles of the visual information processing because of the neuro-crystalline structure of the optic system, genetic tools, and stereotypical and stably elicited behaviors, such as object fixation and figure ground discrimination. In particular, these responses to small-field objects do not necessarily require small-field tuned neurons, but can also theoretically arise from asymmetric motion processing. While recent experiments in flies with blocked T4-T5 cells revealed object responses independent of this T4-T5 circuitry [1], our behavioral experiments show that a T4-T5 independent system cannot account for object fixation under strong coupling conditions or in a low-contrast figure-ground scenario. Under these conditions, intact T4-T5 cells are required for object fixation. We created two dynamical models based on measured properties of well-studied Horizontal System (HS) cells [2], downstream of T4-T5. The main feature of these models is asymmetric motion processing, and these models predict object fixation in the named conditions, therefore suggesting a key role of asymmetric motion processing for fly visual behavior. One of our models is a simple phenomenological model allowing mathematical evaluation to predict experimental outcomes. The second model is more physiologically realistic computational model. Very few assumptions go into the models, and those that do are mostly taken from the literature. Here we examine several published results to determine whether these outcomes are also predicted by our models. First, we examined an open-loop random noise figure-ground discrimination experiment used to create spatio-temporal action fields and argued to require two parallel streams dedicated to figure and ground responses [3]. Next, we examined closed loop experiments on one-eyed flies that required application of constant torque to enable object fixation [4]. In both cases, we show that asymmetric motion processing in wide-field integrating neurons is theoretically sufficient to predict these and other measured experimental outcomes. Our models are incapable of predicting other visual behaviors, such as operant conditioning or place learning, but overall suggest that asymmetric motion responses in neurons such as the well-studied HS cells should be considered as a potential basis for a range of visual behaviors.

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Investigating the neuronal substrate mediating 3D vision in the praying mantis

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The praying mantis is a predatory insect that catches edible animals with a rapid strike of its two front legs. It relies on vision to determine the location of its prey in space. Mantises are the only invertebrates known to possess 3D vision - the ability to combine images from the two eyes to compute depth. This enables them to accurately judge the range of a target in space and to decide whether it is in reach or not.

We study the mechanisms underlying 3D vision in mantises by means of behavioural and electrophysiological experiments. We provide visual stimuli by using anaglyph 3D technology. This means that the images intended for left and right eyes are presented in different colours. Spectral filters in front of the animal's eyes ensure that each eye sees only its intended image. In this way, we are able to present stimuli with binocular disparity, in which each eye views a slightly shifted image of the same object. To find out which neurons are involved in computing depth from this disparity and which kind of computations are taking place, we are carrying out intracellular recordings with tracer injections in the brain of the animals during visual stimulation. We will present preliminary results from this project.

Missing links and unexpected properties of motion-detecting circuits

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Motion vision has been studied in many systems and well-known computational models describe how motion cues can be extracted from the environment. It is thus considered a model for understanding paradigmatic neural computations. How these computations are implemented at the network level is still not understood. Recent progress has provided insights into the circuits that perform these computations in *Drosophila*: Immediately post-synaptic to photoreceptors, the lamina neurons L1, L2 and L3 act as three input channels to motion-detecting circuits. Whereas L1 provides the sole input to a pathway that is required for the detection of moving bright edges, a combination of L1, L2 and L3 tunes dark edge detecting pathways. While these cells respond to light signals regardless of their motion, the T4 and T5 neurons of the lobula complex are direction-selective, preferring a particular direction of motion. A key challenge is to identify the cells that connect the lamina inputs to the T4 and T5 outputs and to uncover the mechanisms by which they extract directional information, a hallmark of motion computation. Here, we identify and characterize such a functionally critical link.

Recent anatomical evidence and physiological characterization has implicated two medulla neuron types, Tm1 and Tm2, as the interneurons of the dark edge detecting pathway that connect L2 to T5. However, lacking functional evidence that these neurons are required for motion-evoked behaviors, we took a forward genetics behavioral approach using the InSITE system. We identified another medulla neuron, Tm9, as a behaviorally relevant component of circuits that detect moving dark edges. By genetically manipulating Tm9 alone, as well as in combination with the three inputs channels, we have assigned Tm9 function to a pathway in motion-detecting circuits, that is specialized for the detection of moving dark edges. We have then used *in vivo* calcium imaging to describe the physiological properties of Tm9. Finally, combinatorial genetic approaches in which one cell type is silenced and another is imaged have allowed us to determine the functional connectivity of L3, Tm9 and T5. Our data demonstrate that this cell type represents a missing link in motion computation, significantly informing our understanding how critical computations are performed in this neural network.

Modulation on the fly: The role of neuromodulation in visually guided flight behavior

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Neuromodulation of neuronal circuits is a crucial mechanism underlying neuronal plasticity and behavioral flexibility. However, the precise mechanisms are only partially understood. Technical advances in the field of proteomics and genetics have identified a variety of molecules involved in neuromodulatory signaling such as neuropeptides and their corresponding receptors. First attempts have been made to unravel the functional role of these neuropeptides in well-defined neuronal networks for example underlying circadian rhythm, olfaction or memory by using the genetic model organism Drosophila (Reviews in: Nässel & Winther, 2010, Prog Neurobiol.; Taghert & Nitabach, 2012, Neuron). Although the neuronal networks underlying insect vision and motion detection have been well characterized (Egelhaaf, 2006,Invertebrate Vision; Borst et al 2010, Annu. Rev. Neurosci.), studies of neuropeptides in visual neuronal circuits and their modulatory role in visual-motor reflexes have only rarely been addressed. Using a virtual reality flight environment, we combine molecular genetics and high-throughput behavioral assays to study the role of precise molecules and specific neurons in the neuronal networks underlying visual-motor control in freely flying Drosophila (Stowers et al., 2014, IEEE Computer).

We have built a flight arena, called the FlyCube, which consists of five displays projecting onto the four vertical sides and floor of a glass cube. To monitor flies' flight trajectories, we use multi-camera real-time tracking software with high-speed cameras (Straw et al., 2011, J R Soc Interface). This behavioral setup allows us to study the flight behavior of flies in natural flight whilst interfering with normal sensory feedback. We designed a stimulus capable of actively directing flies to fly along arbitrary trajectories using the natural fly optomotor responses. We use this stimulus to test the flies' ability to perform optomotor following. In initial experiments we studied the role of one already well-described neuropeptide in the optic lobes, pigment-dispersing factor (PDF). Interestingly, in those experiments (for each condition 35 flies, 5 experiments) we found a strong increase in the total amount of flight trajectories in flies with chemically silenced (n=1379) as well as in flies with completely ablated PDF-neurons (n=2468) compared to control flies (n=1037). Furthermore, those flies show a significant increase in the trajectory length and we found a small increase in the correlation between the flies turn rate and the stimulus rotation rate. In general, PDF-impaired flies show an increase in flight activity and a slightly better performance in optomotor following. With this work we aim to provide an unprecedented understanding of single molecules, such as neuromodulators, with naturalistic and potentially complex behavior in freely moving animals.

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Neuropeptides in the regulation of worker ontogeny in the ant *Cataglyphis fortis*

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The desert ant *Cataglyphis fortis* is well-known for its sophisticated navigation capabilities. Solitary foragers leave the nest for up to several hundred meters, searching for food in a zig-zagging manner, and return to the nest in a straight line. This navigational task is predominantly based on a path integrator as well as visual and olfactory landmarks. *C.fortis* workers undergo an age-related polyethism from newly hatched callows (1-2 days), interior I and II ants (~28 days), to foragers (~7+ days). Interior I workers serve as repletes and are mostly inactive, while interior II workers are actively moving inside the nest, care the brood and queen to later be involved in nest digging and garbage disposal. This last phase of interior II workers is assumed to be the first time the ants get exposed to visual input. After the indoor phases, workers leave the nest for the first time and start to perform orientation runs for about three days before they finally start foraging. Our studies showed that this process is accompanied by significant synaptic reorganization of microglomeruli in the mushroom bodies, learning and memory centers (Stieb et al. 2012 *Dev Neurobiol*), and in visual brain centers involved in processing of the polarized skylight patterns (giant synapses in the lateral accessory lobe). These studies suggest that synaptic reorganization is not strictly age dependent, but rather triggered by first exposure to visual input during the orientation runs close to the nest entrance. However, the age-related mechanisms that drive the ants to start their first orientation runs still remain enigmatic.

We hypothesize that neuropeptides and –hormones are key regulators for the initiation of the behavioral changes underlying division of labor between indoor and outdoor workers. We propose mechanisms involved in general activity levels and phototaxis. Peptides of the tachykinin-related peptide (TK) family are promising candidates. Downregulation of TK in the *Drosophila* central complex (CX) led to altered locomotor behavior, hyperactivity and centrophobia (Kahsai et al. 2010 *J Exp Biol*). Thus, age-related timing of changes in the expression of TK in the *C.fortis* CX may as well control activity levels inside the nest or change the probability for the ants to approach the nest entrance and light stimuli. We further assume that positive phototaxis changes with age in a way that young ants avoid light, whereas older ants get attracted by light.

As a first step to investigate neuropeptidergic regulation of division of labor in *C.fortis* we used mass spectrometry to detect and de novo sequence neuropeptides and analyze their distribution. Among all peptides characterized, those of the TK peptide family were of particular interest. We performed TK-immunostainings with a focus on the CX in ants of different ages and revealed a differential distribution of TK in the fan shaped body (FB). In behavior assays, we investigated general activity levels and light affinity. While young ants mostly remained inactive in the dark, older ants actively moved and did not show a specific preference between light and dark. This change in behavior can be triggered experimentally by light exposure and is in line with low TK levels in the brain of young ants. Supported by DFG, SFB 1047 “Insect Timing” (B6 and B2) to WR, CW.

Orientation runs of the desert ant *Cataglyphis fortis*

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The desert ant *Cataglyphis fortis* is a famous model organism for orientation. The ants use path integration to navigate successfully through their hostile habitat, i. e. sabkhas and chotts in Northern Africa. Throughout their lifespan, the ants perform different tasks. Leaving the nest for the first time poses a challenge for the unexperienced newcomers: They have to find their way back to the entrance that is an inconspicuous, little hole in the flat saltpan. The transition phase between indoor workers and foragers lasts up to three days. During this time, the ants perform so-called orientation runs during which the ants do not move far away from their nest and they do not bring any food item back. They walk slowly and circuitously. Furthermore, they frequently turn around themselves (so-called pirouettes). To analyze the orientation runs in detail we recorded the ants leaving the nest entrance with a high-speed camera in three different settings (environment with no, one, and three artificial landmarks). Furthermore, we performed a displacement experiment with three landmarks to investigate the learning progress of the ants. The more experienced an ant is, the more it searches at the correct position for the nest entrance on the test field.

System Identification of Drosophila Flight Behaviour

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In a freely flying subject exploring its environment, many visuomotor programs may be operating concurrently. To what extent can the gross flight behaviour be considered consequences of an active, perhaps higher order decision process, or merely an emergent property of the interaction of these lower level programs?

In order to understand the interaction between visuomotor programs, one must first understand the temporal dynamics of single behaviours in isolation.

Much of the present understanding of insect flight is based on steady-state measurements performed on tethered individuals. While such experiments can highlight sensory cues relevant for a particular task; they are unable to address the dynamics of a control process. A model based on steady-state measurements might not be able to predict the time course of the response, amplifying or attenuating the dynamic features of the underlying system, or failing to recognize differences in transient behaviour between genotypes.

Using our novel virtual reality assay for freely flying drosophila, and a unique type of open-loop perturbation experiment, we are studying the wide-field motion response of Drosophila in free flight. Using control theoretic methods taken from the engineering fields we perturb freely moving animals, and through careful design of stimulus, generating behavioural models for individual flies.

By applying physical constraints (laws) in the model estimation process, components of the model relating to the plant (the mass and aerodynamics of the fly) can be separated from the behavioural program (the dynamics of the optomotor response for example).

By comparing these models one may identify behavioural differences between populations. By understanding the temporal characteristics of these models one may see how behaviours, which may appear to the observer as a ‘decisions’, may appear as direct consequence of the stimulus alone.

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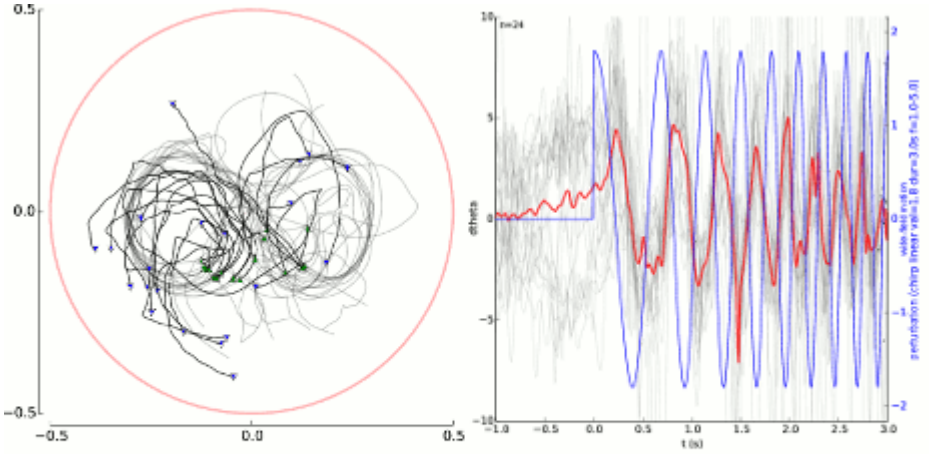

Flies are directed along a "figure 8" path (left, light grey) until they reach a fixed point in the arena (green triangles). Immediately a perturbation experiment begins (left, dark grey), where they are shown a predefined wide-field stimulus (right, blue trace).

Ultrastructure and Anatomy of Microglomerular Synaptic Complexes in the Polarization Vision Pathway of the Honeybee

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Honeybees, among many other insects, use celestial cues for spatial orientation and navigation. The main source of sky-compass information is the sun but, in addition, the color gradient and the polarization pattern of the sky can be exploited as well. While sky compass orientation of the honeybee has been investigated in a large number of behavioral studies, the underlying neuronal systems and mechanisms are still largely unknown.

The neuronal basis of the polarization vision system is best-known in desert locusts (Schistocerca gregaria). The polarization vision pathway receives input via specialized photoreceptors in the dorsal rim area of the compound eye. Signals are relayed through the optic lobe to the anterior optic tubercle. Neurons of the anterior optic tubercle connect to GABA-immunoreactive tangential neurons of the central body via conspicuous microglomerular synaptic complexes.

In the present study, we used dye injections combined with anti-GABA immunocytochemical staining to investigate whether these microglomerular synaptic complexes also exist in the polarization vision pathway of the honeybee (Apis mellifera). To this end we labeled projection neurons of the anterior optic tubercle (TuLAL1 neurons) by injection of Dextran-Texas Red and tangential (TL) neurons of the central body with antibodies against GABA. In addition we investigated the ultrastructure of the synaptic connections using transmission electron microscopy (TEM).

We found microglomerular synaptic complexes each formed by one large cup-shaped terminal of a TuLAL1 neuron that enclosed many small processes from tangential neurons of the central body. TEM images showed that the vesicle stock in the large terminals of the TuLAL1 neurons consists of small clear and larger dense core vesicles. This gives evidence that these neurons are presynaptic whereas the small profiles of TL neurons represent postsynaptic partners in these complexes. Furthermore we could identify two types of synapses: divergent dyads and synapses formed with at least three postsynaptic partners.

Comparing the results of honeybees with locusts we assume that these synaptic complexes are highly conserved characteristics within the polarization vision pathway of insects. However, we could distinguish some differences between both species regarding the shape of the complexes as well as the diversity of synapses. In locusts the shape and the distribution of the synapses is very consistent with a chain-like alignment of dyadic synapses. In contrast, the complexes in honeybees appear less uniform including at least one additional type of synapse.

Poster Topic

T15: Vision: Retina and Subcortical Pathways

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Daniel von Poschinger-Camphausen, Cornelius Weber, Stefan Wermter

T15-2A Centrosomal protein Pericentrin interacts with Nesprin protein Syne-2 in the retina
Nathalie Falk, Kristin Kessler, Johannes Glöckner, Karsten Boldt, Marius Ueffing, Ronald Roepmen, Christian Thiel, Johann Helmut Brandstätter, Andreas Gießl

T15-3A Classifying retinal ganglion cells in the salamander retina
Fernando Rozenblit, Tim Gollisch

T15-4A Depolarization- and light-evoked calcium action potentials in horizontal cells of the mouse retina
Andreas Feigenspan

T15-5A Diverse Features of Spatial Contrast Adaptation in the Mouse Retina
Mohammad Hossein Khani, Tim Gollisch

T15-6A Effects of locomotion throughout the mouse early visual system
Sinem Erisken, Agne Vaiceliunaite, Ovidiu Jurjut, Matilde Fiorini, Steffen Katzner, Laura Busse

T15-1B Encoding of Natural Images by Retinal Ganglion Cells
Jian Liu, Tim Gollisch

T15-2B Following the visual signal across the entire mouse retina: From cone calcium to ganglion cell spikes
Tom Baden, Katrin Franke, Sinziana Pop, Miroslav Roman Roson, Robin Kemmler, Philipp Berens, Matthias Bethge, Timm Schubert, Thomas Euler

T15-3B Functional characterization of the signal processing chain in the mouse early visual system
Miroslav Roman Roson, Thomas Euler, Philipp Berens, Laura Busse

T15-4B Influence of cortico-thalamic feedback on temporal and spatial response properties in the mouse dorsolateral geniculate nucleus
Agne Vaiceliunaite, Sinem Erisken, Ovidiu Jurjut, Steffen Katzner, Laura Busse

T15-5B Mechanisms underlying the modulation of horizontal cell gap junctions by all-trans retinoic acid
Jasmin Segelken, Sebastian Hermann, Reto Weiler, Ulrike Janssen-Bienhold

T15-6B What the mouse eye tells the mouse brain: Fingerprinting the retinal ganglion cell types of the

mouse retina
Katrin Franke, Thomas Baden, Philipp Berens, Miroslav Rezac, Matthias Bethge, Thomas Euler

T15-1C Motion Encoding in the Salamander Retina
Norma Krystyna Kühn, Tim Gollisch

T15-2C Optical imaging of neurons responsible for transformation of sensory information into motor commands
Gytis Baranauskas, Gytis Svirskis, Natasa Svirskiene, Tatiana Tkatch

T15-3C Order in the chaos - unscrambling the interlaced fine structures of anchovy cone pedicles
Maximilian Scheungrab, Katja Schulze, Gerhard Wanner, Martin Heß

T15-4C Power spectra changes induced by repetitive transorbital alternating current stimulation: a longitudinal approach to study after effects
Ting LI, Carolin Gall, Doreen Brösel, Ying Gao, Michal Bola, Bernhard A. Sabel

T15-5C Processing of motion stimuli by cells in the optic tectum
Josine Verhaal, Harald Luksch

T15-6C Receptive field mapping in blind retinae using localized electrical stimulation
Henrike Stutzki, Florian Helmhold, Günther Zeck

T15-1D Response characteristics of ON stellate-varicose amacrine cells and their interactions with ON midget ganglion cells in primate retina
Christian Puller, Michael B. Manookin, Maureen Neitz, Fred Rieke, Jay Neitz

T15-2D Similarity across saccades is encoded in the retina
Vidhyasankar Krishnamoorthy, Michael Weick, Tim Gollisch

T15-3D Spatial integration properties of mice retinal ganglion cells: a closed-loop approach
Luis Giordano Ramos Traslosheros López, Michael Weick, Tim Gollisch

T15-4D Spike generator desensitization in retinal ganglion cells underlies transient light response and sensitivity to high contrasts
Thomas Euler, Timm Schubert, Olivia Auferkorte, Elisabeth Butz, Laura Hüser, Silke Haverkamp, Le Chang

T15-5D The NIMA-related serine/threonine kinase -Nek1- at cilia of sensory cells
Andreas Gießl, Kristin Kessler, Johann Helmut Brandstätter, Christian Thiel

T15-6D Type 2 wide-field amacrine cells in tyrosine hydroxylase (TH)::GFP mice show a homogenous synapse distribution and contact small ganglion cells
Bianca Brüggen, Arndt Meyer, Franziska Boven, Reto Weiler, Karin Dedek

A Generative Model of Decorrelating Color Sensitive Retinal Ganglion Cells

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Neuroanatomical studies have shown that the mammalian retina consists of many parallel, equally potent microcircuits [3] which in turn drive numerous different retinal ganglion cell (RGC) types, indicating the retina's functional role of pre-processing visual-information before reaching the visual cortex. Theoretical models suggest that the retina encodes information efficiently, using minimal resources whilst transmitting a maximum of information [1].

Generative Model

The effect of minimizing metabolic costs had been explored by [4] utilizing a generative model with one hidden layer. In this simple model connection weights are equated to synaptic strengths and the network's output to neural firing rates. By reconstructing the input from hidden layer activity and by comparing reconstruction and original input an error signal is generated, which in turn is used to update the networks weights. Also, a weight constraint is applied. Constraining the connection strengths in this model is crucial for the emergence of localized, difference of Gaussian (DOG) shaped receptive fields, resembling those of retinal ganglion cells (RGC).

Extension to Color

We extended the model, which processes gray scale images [4], to color images. Additionally the hidden layer neural activations were only allowed to be positive and the imposed weight constraint was simplified by removing one threshold parameter. After the training process converged, the receptive fields were first fitted with a spatio-chromatic elliptical DOG parametric model and then clustered via k-means into 7 clusters.

Decorrelation of Input

The resulting receptive fields decorrelate the input into several distinct channels each spanning the entire simulated visual field, resembling parallel pathways [2] found in the primate retina. As a consequence of applying the weight constraint continuously, receptive fields not contributing to reconstructing the presented images slowly die off (their weights decay to zero). An efficient code emerges that uses a small number of RGC neurons and small weights to encode the presented input. However, slightly different model parameters, or training images with different overall statistics, tend to result in vastly different distributions of RGCs.

The Figure shows parametric fits of the resulting receptive fields for 6 of the 7 clusters. Ellipses are drawn in the color of the strongest weight for each RGC. As the model has been trained from rgb images, the color of these center weights is mostly close to the rgb color space cube's edges. (Top row: red, green, blue. Bottom row: cyan (red-off), magenta (green-off), yellow (blue-off).) The 7th cluster contains non-local, non-DOG shaped receptive fields. The numbers of RGCs in each channel are: red (47), green (38), blue (60), cyan (48), magenta (38), yellow (63), non-DOG (26), zero (256), total (576).

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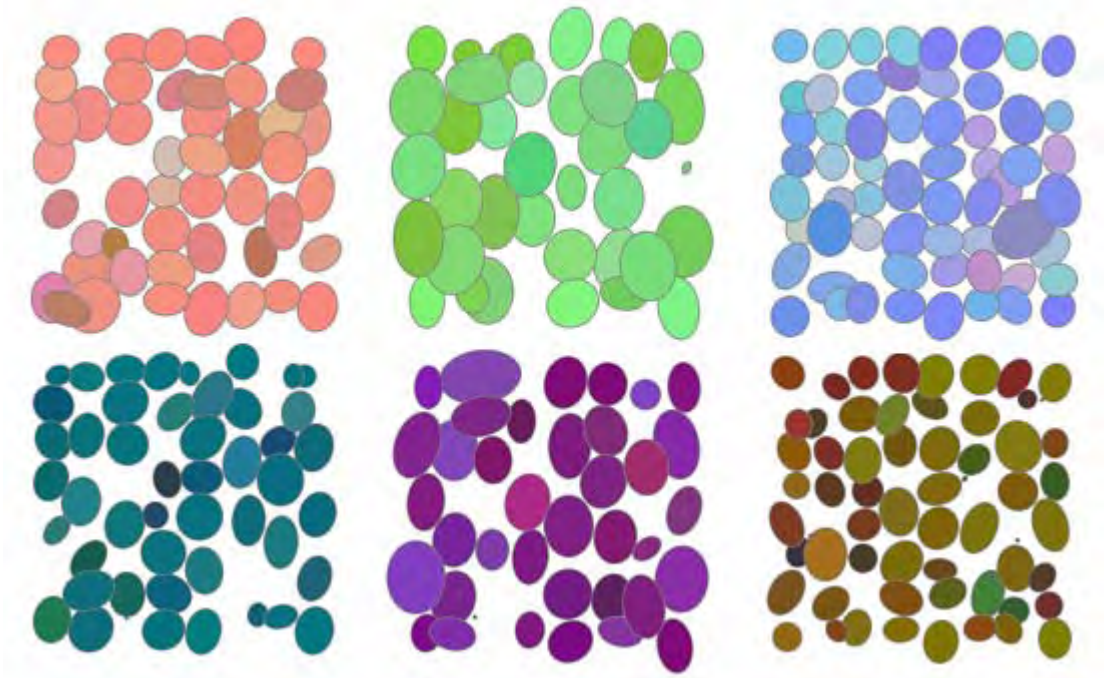

Centrosomal protein Pericentrin interacts with Nesprin protein Syne-2 in the retina

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Pericentrin (Pcnt), a conserved protein of the pericentriolar material (PCM), serves as a multifunctional scaffold for numerous proteins and plays an important role in microtubule organization. To date, three Pcnt splice variants from orthologous genes in mice and humans are known. Structurally, Pcnt is characterized by coiled-coil domains throughout most of the protein, which mediate interactions between resident structural lattice proteins of the PCM and a number of regulatory centrosomal proteins. Furthermore, Pcnt contains a pericentrosomal matrix targeting motif, called the PACT domain, which is responsible for the targeting of Pcnt and other PCM proteins to the centrosome. Through its many interactions, Pcnt contributes to a diversity of fundamental cellular processes.

Mutations in the human PCNT gene are associated with a range of diseases including primordial dwarfism and ciliopathies. As diseases associated with Pcnt mutations display heterogeneous clinical manifestations, it is difficult to pinpoint the functional role of Pcnt.

In the mouse retina, Pcnt is localized at the basal body complex of the connecting cilium between the two photoreceptor compartments. Here Pcnt colocalizes with the protein machinery that regulates the ciliary transport of proteins from the inner to the outer segment. In order to get more insights into the function of Pcnt in the retina, we try to identify new ciliary as well as centrosomal interaction partners.

With several independent methods, e.g. tandem affinity purification, yeast two-hybrid using a self-constructed cDNA library from mouse retina, and GST pull-down, we were able to show that Pcnt interacts with Klarsicht/ANC-1/Syne-homologue (KASH) domain-containing protein Syne-2. Syne-2 seems to be distributed in the whole inner segment of photoreceptors, and it partially colocalizes in the ciliary region with Pcnt.

Syne-2 is a large multifunctional protein that belongs to the nuclear envelope spectrin repeat containing proteins (Nesprin). Structurally, Nesprin proteins are characterized by an actin-binding domain linked by a variable number of spectrin-repeats to a C-terminal membrane-spanning region, called the KASH domain.

Yu *et al.* (2010) investigated the role of Syne-2 during mouse retinal development and demonstrated that a deletion of the KASH domain leads to thickness reduction of the outer nuclear layer and nuclear mislocalization. Based on further experiments they suggested an interaction between Syne-2 and dynein/dynactin as well as kinesin complexes as the molecular motor of nuclear migration in mouse retina.

The interaction between Pcnt and Syne-2 could be the missing link to understand more precisely how interkinetic nuclear migration and photoreceptor cell migration works in general, something we aim to show in future experiments.

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Classifying retinal ganglion cells in the salamander retina

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The retina is a complex neural network which extracts visual features from the environment. Retinal ganglion cells (RGCs) form the output layer of this network. More than 15 different RGC types have been identified in the vertebrate retina using a variety of anatomical and physiological properties. Each RGC type is believed to relay specific features of the visual scene. It is not fully known, however, which features are being encoded. Thus, considerable effort is put into classifying RGCs based on their response properties. We identified RGCs in the axolotl retina, employing a spectral clustering algorithm. Spiking activity of RGCs was recorded extracellularly, using a 252-channel multielectrode array with offline spike-sorting. With this setup, typically more than 200 RGCs could be recovered from a single retina. The retina was stimulated with spatiotemporal white noise, and the receptive field dynamics for each RGC were obtained by reverse correlation (spike-triggered average). We classified the cells by the clustering algorithm, based on the similarity of the cells' receptive field sizes and their temporal filtering characteristics. We estimated the number of clusters using Akaike and Bayesian information criteria to be between 15 and 20. We observed groups with similar temporal properties (e.g., fast OFF cells), which could be separated based on their receptive field sizes. Moreover, a mosaic pattern of the receptive fields (a tell-tale sign of a single RGC type) was seen in more than a single cluster, in contrast to what had been previously reported for salamanders.

Depolarization- and light-evoked calcium action potentials in horizontal cells of the mouse retina

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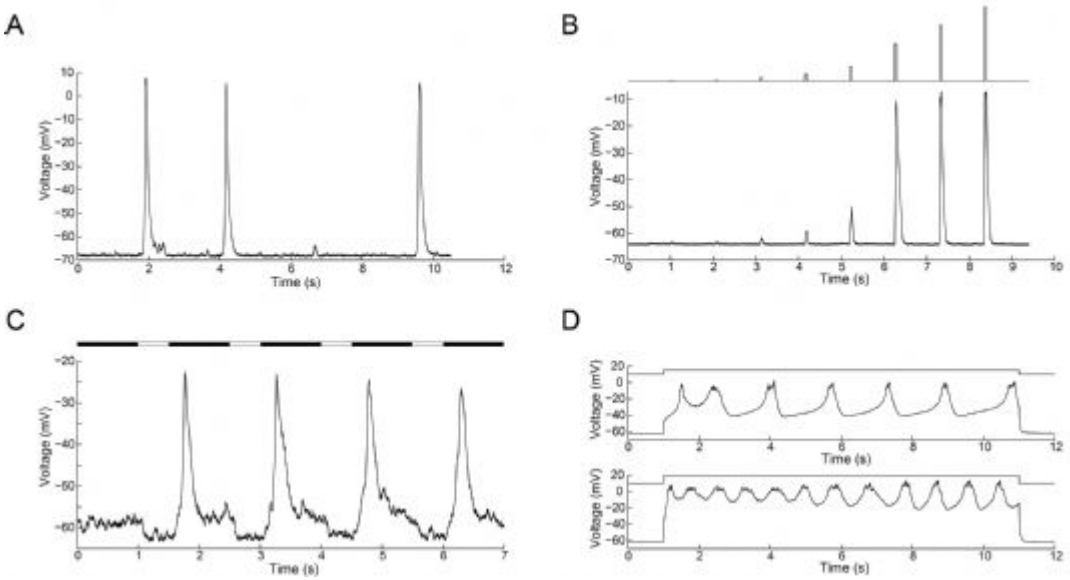
Horizontal cells are interneurons at the first synapse of the visual system which are thought to provide negative feedback to photoreceptors and to contribute to the center-surround organization underlying the receptive field properties of many retinal neurons. In vertebrates, horizontal cells receive major synaptic input from photoreceptors, which release glutamate at highly specified ribbon synapses. The mouse retina contains only axon bearing horizontal cells with dendrites contacted exclusively by cones and an elaborate axon terminal system postsynaptic to rods. Soma and axon terminal system are connected by a long and thin axon which is likely to electrically isolate the two compartments.

Since horizontal cell bodies lack voltage-gated sodium channels, they have so far been classified as nonspiking interneurons. However, expression of L-and N-type voltage-gated calcium channels suggests a functional role of these channel types in mediating voltage responses triggered by glutamatergic photoreceptor input. Therefore, I studied the properties of horizontal cell calcium signaling using (1) patch-clamp recordings of horizontal cell bodies in vibratome sections of the mouse retina, and (2) calcium imaging in enzymatically and mechanically isolated horizontal cells.

Calcium-mediated action potentials occurred either spontaneously (A) or following depolarization (B). Their duration at threshold (–56 ± 6 mV) was 152.2 ± 10.5 ms, and they displayed an amplitude of 54.6 ± 3.3 mV. Interestingly, calcium spikes could be induced at light offset, when horizontal cells responded with a large transient inward current to glutamate released from photoreceptors (C). Injection of longer depolarizing currents caused repetitive calcium spikes, with frequencies depending on current amplitudes (D). In addition, calcium spikes encode incoming periodic signals with a resolution determined by the differential dV/dt of the depolarizing input.

These results suggest that calcium-mediated action potentials contribute significantly to the encoding and processing of synaptic signals in horizontal cells of the mammalian retina.



Diverse Features of Spatial Contrast Adaptation in the Mouse Retina

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Retinal ganglion cells adjust their responses when the contrast of the visual scene changes. These adjustments or adaptations manifest themselves as changes in the kinetics of response and sensitivity of the ganglion cells. The temporal scale of these adaptation processes have been studied extensively. However, the spatial scale of contrast adaptation, that is how mammalian ganglion cells process contrast changes within the local sub-fields of their receptive fields, is not completely understood. Here, we investigated the spatial extent of contrast adaptation in mouse retina. To do so, we stimulated some regions of the receptive field alternately with high and low contrast while keeping constant low contrast at other regions with a grey background covering the area between these regions. We were able to categorize the response characteristics of retinal ganglion cells to contrast alternations into four categories of fixed filter, globally adaptive, locally adaptive and action-at-distance classes. Fixed filter cells do not show substantial changes in their response characteristics following the contrast change. In globally adaptive cells, contrast alternations in local sub-fields lead to global adaptation over the entirety of the receptive field. In locally adaptive cells, each local sub-field adapts independently to contrast. Action-at-distance cells adapt not at the local subunits that were stimulated with alternating contrast, but they adapt at the other subunits that were stimulated with constant low contrast. This diversity of contrast-adaptation features is important for supporting a diverse set of visual functions performed by different classes of retinal ganglion cells.

Effects of locomotion throughout the mouse early visual system

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Neural responses in primary visual cortex (V1) depend not only on sensory input but are also profoundly modulated by behavioral context. One such behavior is locomotion, shown in mice to enhance the gain and reduce the variability of cortical neurons. Beyond global state-dependent changes, V1 single neurons integrate locomotor and visual signals in complex ways and can exhibit tuning for running speed. Response modulation by locomotion along the visual pathway is currently thought to be restricted to cortical neurons, however, little is known about how locomotion affects cortical populations and pre-cortical processing stages.

Using extracellular recordings from multiple neurons in head-fixed mice running on a spherical treadmill, we investigated the influence of locomotion on neural populations in area V1 and in the dorsal lateral geniculate nucleus (dLGN). We measured pupil position and size using camera-based eye tracking under infrared illumination.

We assessed how locomotion shapes population responses in V1 upper layers by computing pairwise spike count correlations between simultaneously recorded neurons. During spontaneous activity, locomotion de-correlated population activity, reducing both variability of individual cells and shared variability between pairs. Pairwise correlations, during both locomotion and stationary periods, varied as a function of orientation and run-speed tuning similarity, with highest correlations for pairs preferring similar orientations and running speeds. Remarkably, despite variable speed preferences within the population, locomotion globally reduced pairwise correlations across all degrees of run-speed tuning similarity.

Given the prominent effects of locomotion on cortical populations, we next re-assessed if these effects are indeed restricted to cortex. Contrary to current understanding, we found that locomotion can have powerful non-multiplicative influences on response magnitude and tuning already at the level of the dLGN. First, locomotion modulated responses as early as the dLGN: individual cells increased not only their activity around locomotion onset, but also their average responses to grating stimuli. Second, locomotion profoundly influenced dLGN selectivity for stimulus size. Similar to V1, neurons in dLGN had larger RF center sizes during locomotion, and were less suppressed by stimuli extending to the RF surround. Third, responses in dLGN exhibited a smooth and diverse dependency on run speed, irrespective of the presence or absence of visual input. Even in complete darkness, dLGN neurons were diversely influenced by run speed: with increasing run speed, responses of individual neurons showed monotonic increases, monotonic decreases or band-pass tuning properties.

Finally, we found that pupil size closely followed running speed. Importantly, pupil size could not account for locomotion-based response enhancements, as blocking pupil constriction by topical application of atropine did not alter run speed tuning of neurons in neither dLGN nor V1. Instead, changes in pupil size seemed to serve as a reliable marker for behavioral state.

These findings document previously unknown and far-reaching effects of locomotion throughout the early visual system and further demonstrate that state-dependent changes can exert powerful non-multiplicative influences beyond response gain modulations.

This work was supported by funds awarded to the Centre for Integrative Neuroscience (DFG Exec 307); and by a Starting Independent Researcher grant from the European Research Council (project acronym: PERCEPT) awarded to S.K.

Encoding of Natural Images by Retinal Ganglion Cells

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How natural scenes are encoded by retinal ganglion cells (RGCs) is still an unsolved problem. Here, we study how natural images are represented in the RGC responses by recording spikes from isolated axolotl retinas, using a multielectrode array, stimulated with a set of natural images. For each image of the stimulus set, we obtained a linearly filtered image by weighting the image with the receptive field profile. We then obtained a prediction for the spike count response through a linear-nonlinear (LN) model.
To go beyond the LN model, we also analyzed the standard deviation of the linearly filtered images as a measure of local spatial contrast (LSC). We found that LSC is positively correlated to the spike count response, and incorporating LCS in the LN model could improve spike count predictions. Finally, we used closed-loop experiments to compare responses to natural images with different degrees of spatial blurring while keeping the mean contrast over the receptive field of a given ganglion cell constant. These experiments showed that the spike count responses decay substantially when LCS is decreased. Together, these results suggest that, besides mean light intensity, spatial contrast exerts a strong effect on RGC responses. This implies that spatial nonlinearities are important for predicting responses to natural images and that some of these effects can be captured by looking at the spatial contrast inside the receptive field.

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Following the visual signal across the entire mouse retina: From cone calcium to ganglion cell spikes

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The vertebrate retina, with its exquisitely regular organisation and its planar, near transparent structure offers a powerful playground for the detailed exploration of principles in sensory information processing in general. Moreover, detailed knowledge the anatomy of the mouse retina is paralleled by few other model systems in neuroscience today. Here, we present a systematic approach to add a physiological dimension to this description, by optically imaging light-evoked activity to visual stimuli that systematically survey key transformations during retinal signal decomposition, including contrast and frequency response functions and responses to Gaussian noise. By following such ”elementary visual responses” at key sites within the retinal circuitry, including a clear link to anatomy, that we believe will prove instrumental in exploring a computational description of retinal signal decomposition as whole.

We recorded from synapses, dendrites and somata of all excitatory neurons of the mouse cone-pathway. In addition, we recorded from a subset of inhibitory neurons. In the outer retina, we imaged (i) calcium responses from S- and M-cone photoreceptor pedicles in retinal slice of the HR2.1:TN-XL mouse line (Wei et al. 2012, Baden, Schubert et al. 2013). In addition, we monitored (ii) calcium responses in both somata and individual varicosities of horizontal cells using GCaMP3 and GCaMP6 expressed in the Cx57cre/+ line (Ströh et al., 2013) using cross-breeding and AAV, respectively. In the inner retina, we recorded (iii) calcium responses in individual presynaptic terminals of bipolar cells (Baden et al. 2013) and (iv) dendritic tips of retinal ganglion cells labelled with the synthetic calcium indicator OGB-1 or GCaMP6 introduced using AAV. We also surveyed (v) glutamate release based on iGluSnFR responses (Marvin et al., 2013, Borghuis et al., 2013), expressed either ubiquitously or in specific Cre lines (PV:Cre, Feng et al. 2000; Farrow et al. 2013; Pcp2:Cre, Lewis et al. 2004; Ivanova et al., 2013; ChAT:Cre, Lowell et al., 2006). We also recorded calcium responses in the somata of (vi) all RGCs and (vii) displaced amacrine cells in the ganglion cell layer after electroporation with OGB-1 (Briggmann and Euler 2011). These recordings were complemented with (viii) single-unit spike recordings and subsequent intracellular fillings, as well as the use of reporter lines PV:Ai9tdTomato, PcP2:Ai9tdTomato or subsequent immunohistochemistry (GAD67, ChAT) to aid genetic/anatomical classification.

Reference to this database will benefit the development of computational models aiming to describe retinal function. In addition, it will form the foundation for a more systematic approach towards understanding the changes in processing during degeneration.

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Functional characterization of the signal processing chain in the mouse early visual system

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More than 20 types of retinal ganglion cell (RGC) represent parallel channels transmitting different aspects of visual information from the retina to various parts in the brain. Retinal output is most directly conveyed to the cortex via the retino-geniculo-cortical pathway, comprised of RGCs, relay cells in the dorsolateral geniculate nucleus (dLGN) and the primary visual cortex (V1). While it has long been known that this pathway is not homogenous but consists of parallel channels each carrying specific information, it is still debated which RGC types project to the dLGN and how their output is transformed at the level of the dLGN. Here, we started to characterize, in the mouse model, the functional properties of dLGN-projecting RGCs and to compare responses of RGCs and dLGN neurons to the same set of visual stimuli.

We explored two approaches for selective labeling and physiological characterization of dLGN-projecting RGCs. First, we injected a retrograde tracer (“mini-Ruby”, Molecular Probes) into the mouse dLGN. After 7 days, the retina was removed and electroporated with a synthetic calcium indicator (Briggman & Euler, J Neurophysiol 2011). Using two-photon in-vitro imaging, we recorded light-evoked calcium activity from the population of RGCs that had been labelled by the retrograde tracer. Visual stimuli included frequency/contrast modulated full-field flicker, dense noise, moving bar, and chromatic stimuli. Second, we injected an adeno-associated virus (AAV) encoding the calcium biosensor GCaMP6 into the dLGN. Through transfection of RGC terminals this leads to retinal biosensor expression, which enabled us to selectively record light-evoked calcium responses in dLGN-projecting RGCs. In a separate set of experiments, we characterized the responses of dLGN neurons to the same visual stimuli using in-vivo extracellular multi-electrode recordings in the dLGN of awake, head-fixed mice.

Combining the data sets from the retina and the dLGN, we seek to build computational models that will test if and how dLGN responses can be described as specific combinations of RGC output channels and the influence of local inhibitory circuits. Specifically, we will ask if response features are simply inherited from the RGC input or if they are modified within the LGN.

In conclusion, this study promises to yield a functional characterization of the population of dLGN-projecting RGCs, and to provide fundamental insights into how the representation of visual information changes along the first stages of the retino-geniculo-cortical pathway.

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Influence of cortico-thalamic feedback on temporal and spatial response properties in the mouse dorsolateral geniculate nucleus

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Visual information processing is mediated not only by feedforward inputs but also profoundly modulated by feedback. Whether cortico-thalamic feedback substantially changes the representation of visual information in the dorsolateral geniculate nucleus (dLGN) remains an open question, as previous investigations have yielded inconsistent results. Here, we used the mouse model to test how cortico-thalamic feedback affects temporal and spatial processing of dLGN neurons.

To study the role of cortico-thalamic feedback we performed extracellular recordings with multisite linear silicon probes in dLGN of awake head-fixed mice.
To interfere with cortico-thalamic feedback, we used two optogenetic approaches. In the first approach, we used PV-Cre mice to transiently silence visual cortex by optogenetically stimulating parvalbumin–positive (PV+) inhibitory interneurons. In the second approach, we used Ntsr1-Cre mice to perform direct manipulations of cortico-thalamic feedback by selective activation and inhibition of L6 cortico-thalamic neurons.

To assess feedback-related changes of temporal response properties, we first examined spontaneous activity. We found that during global inactivation of V1, spontaneous firing rates decreased abruptly. After ~200 ms of cortical inactivation, dLGN neurons transitioned into burst mode firing, leading to a 3-fold increase in the burst-tonic firing ratio.
To assess feedback-related changes in spatial response properties, we measured size tuning in the dLGN by presenting gratings of different diameter. We found that numerous neurons in mouse dLGN exhibit surround suppression. Importantly, in dLGN neurons, transient global inactivation of visual cortex led to an expansion of receptive field center size (22.3%) and a reduction of surround suppression (21.8%). We observed many dLGN neurons in which the decrease in surround suppression during cortical inactivation seems driven by two effects: a reduction of responses to stimuli up to the optimal size and an increase of responses to stimuli exceeding the optimal size. We obtained broadly complementary results when we directly interfered with activity of L6 cortico-thalamic neurons.

We conclude that, similar to what has been reported for higher-order mammals, both temporal response properties and spatial integration in mouse dLGN are at least partly shaped by cortico-thalamic feedback circuits.
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Mechanisms underlying the modulation of horizontal cell gap junctions by all-trans retinoic acid

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Horizontal cells (HC) are extensively coupled via gap junctions (GJ) composed of connexins. Recently different connexin isoforms have been identified in carp HC. One isoform is cpCx53.8 which is expressed in all four HC types. There is evidence that GJ function is highly modulated by the phosphorylation of the involved connexins.

Here we investigated whether cpCx53.8 phosphorylation is modulated by all-trans retinoic acid (at-RA), a byproduct of the phototransduction cascade. Therefore we used an antibody detecting the phosphorylated isoforms of cpCx53.8 in western blot and immunhistological experiments. To identify the underlying signalling cascade, we additionally tested if at-RA effects could be inhibited by different protein kinase blockers. As a control light adapted and dopamine (DA)-treated retinas were used, as it is known from several previous studies that DA induces the cAMP-triggered phosphorylation of cpCx53.8 via PKA.

Western blot analyses revealed increased cpCx53.8 phosphorylation following light adaptation. Treatment of dark-adapted retinas with at-RA resulted in a comparable intense phosphorylation of cpCx53.8. Pre-incubation of retinas with specific PKA and PKC activators (cAMP and PDBu, respectively) and blockers (H89 and stau, respectively) showed that DA and at-RA mediate their effects by the activation of two different intracellular signalling cascades, whereby at-RA acts via PKC. Immunhistological studies on retinas treated with at-RA and PDBu alone and in combination with stau showed changes in cpCx53.8 immunoreactivity (IR) pattern, indicating that changes in the distribution of the connexin are related to its phosphorylation.

To unravel mechanisms on the cellular bases, N2A cells transiently transfected with PCS2+/cpCx53.8 were used. Transfected cells were treated with at-RA and PDBu, whereas cAMP was used as a positive control. In this case activation of PKA and PKC resulted in an increase in cpCx53.8 IR associated with the plasma membrane. These effects were blocked by pre-incubation with specific PKA and PKC inhibitors (H89 and stau, respectively), indicating that phosphorylation of cpCx53.8 is important for its incorporation into the plasma membrane. Western blot analyses confirmed the observed increase in cpCx53.8 IR in membrane fractions of the cells after treatment with cAMP and PDBu. In contrast, treatment with at-RA had no effect on the distribution of cpCx53.8 in N2A cells. These results suggest that at-RA does not interact directly with PKC to support incorporation of cpCx53.8 into the plasma membrane as shown for PDBu. Furthermore, this result is in contrast to retinal studies, which showed an at-RA induced increased cpCx53.8 IR in the outer plexiform layer.

In summary these findings suggest that at-RA exerts its effect on cpCx53.8 phosphorylation via PKC, whereupon *in vivo* an additional protein, like a receptor, seems to be involved in the signalling cascade, as at-RA treatments in the artificial system had no effect. Therefore future experiments need to clarify whether an at-RA receptor is expressed in HC and which PKC isoform (Ca^{2+}-dependent or -independent) is involved in the signalling cascade.

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What the mouse eye tells the mouse brain: Fingerprinting the retinal ganglion cell types of the mouse retina

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The retinal ganglion cells (RGCs) relay the output of each parallel feature detecting channel – established through complex interactions in the retina's two synaptic layers – to higher visual centres. Understanding how the visual scenery is encoded by the outputs of the ~20 RGC types will thus yield a complete picture of the representation of the visual scene available to the brain.

To reliably record from each RGC type in the mouse retina, we bulk-electroporated the tissue with a synthetic calcium indicator (OGB-1) and used two-photon calcium imaging to record light stimulus-evoked activity at the level of the ganglion cell layer (GCL) (Briggman & Euler, J Neurophysiol 2011). So far, our database contains recordings of >10,000 cells from the GCL. In addition, we obtained recordings from transgenic PV and PCP2 mice, in which 13 morphologically distinct RGC types are fluorescently labelled and can be identified based on their anatomy (Farrow et al., Neuron 2013; Ivanova et al., J Comp Neuol 2013). Moreover, we performed electrical single-cell recordings from RGCs to relate their spiking responses to the somatic Ca^{2+} signals and to compare their morphologies with published RGC catalogues (e.g., Völgyi et al., JCN 2009).

We implemented a probabilistic clustering framework for separating RGCs into functional types based on features extracted from their responses to the different visual stimuli using PCA. We employed an automated mixture of Gaussians Clustering algorithm to cluster the cells based on their physiological properties. The total number of clusters is chosen by minimizing the Bayesian Information Criterion, which balances the likelihood of the data under the model with the model complexity. Subsequently, clusters were grouped according to genetic labels and morphological criteria (e.g. soma size).

For our data, we obtain ~35 functional groups, which separate into ~25 RGC groups and ~10 displaced amacrine cell (dAC) groups, as verified using glutamate-decarboxylase (GAD) immunostaining. These numbers match well the number of RGC and dAC types expected in mouse retina. The RGC types include many known cell types (OFF and ON alpha, W3, ON-OFF direction-selective), as verified using our genetic label and single cell data (e.g. alpha RGCs) and additional information available (e.g. soma size/shape and retinal tiling). In addition, they include new functional RGC types, such as (1) an OFF orientation selective RGC, (2) an ON transient DS RGC with single cardinal direction and, (3) a contrast-suppressed type.

Our results suggest that a functional fingerprint for each RGC in the mouse retina is within reach.

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Motion Encoding in the Salamander Retina

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Salamander is a common model to study visual encoding in the retina, in particular the encoding of motion. We investigate the response of retinal ganglion cells (RGCs) to different motion schemes like global and local drifts of object and background and tremor-like motion.
We found three functional types of RGCs. First, object-motion-sensitive (OMS) cells preferably respond to local motion of objects, but not when the whole scene is moving coherently (Ölveczky et al., Nature 2003). Second, we found direction-selective (DS) cells, which are known from mouse and rabbit, but not well studied in salamander. Third, we found a ganglion cell type that combines both functions. These OMS-DS cells respond selectively to a certain direction of motion and prefer small moving objects instead of global background motion.
The two kinds of DS cells do not only differ in their preferences for global and local stimuli, but also in the organization of preferred motion directions. For standard DS cells the preferred directions occur in three clusters, separated by 120 degrees angular difference. The OMS-DS cells have two or more primary directions of 90 degrees angular difference. This suggests an interesting analogy to the ON and ON-OFF DS cells observed in the mammalian retina. Furthermore, a pharmacological study revealed GABAergic inhibition to be involved into the computation of direction selectivity in both cell types.

Optical imaging of neurons responsible for transformation of sensory information into motor commands

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Optical imaging methods such as monitoring of internal Ca2+ concentration in multiple neurons or in multiple sites of dendritic branches of neurons provide a wealth of information because of ability to simultaneously record from multiple sites of information transfer such as neurons or synapses. However, most such imaging studies in vertebrates are limited either to in vitro experiments such as slices or neuronal cultures or to superficial layers of cortex imaged in vivo. These types of experiments do not permit to study in full the transformation of sensory information into motor commands because in slices no realistic sensory input can be presented while superficial cortical neurons neither receive a direct sensory input nor send their output to neurons controlling muscles. Therefore it is desirable to have a preparation, suitable for optical imaging of neurons, which transform unprocessed or minimally processed sensory input into a motor command.

Recently we have developed such a preparation, namely an integrated frog eye-optic tectum preparation, in which tectal neurons can be recorded by patch-clamp technique. In amphibians the optic tectum, a homolog of superior colliculus in mammals, receives sensory inputs directly from retina and the principal tectal neurons send their axons to motor centers, neurons of which make synapses on muscles. Thus, a single tectal neuron is largely responsible for transformation of sensory information into a motor command.

Such an information transfer can happen when a collision stimulus triggers an escape behavior. Collision detection is conserved across species and is performed by specialized collision-sensitive neurons in the optic tectum of frogs and the superior colliculus of mammals, including humans. The collision sensitive neurons not only detect objects approaching on a collision course but also generate an escape from a potential collision response. We have previously reported that a large number of tectal neurons in layer 6 are indeed collision sensitive neurons capable of generating the escape command from a potential collision. We recorded these neurons by employing the patch-clamp technique while visual stimuli, generated by the PsychoPy software package, were presented to the frog eye on a computer screen.

These patch-clamped tectal neurons have extensive dendritic branches, which are the sites of synapses formed by the incoming retinal axons. Patch recordings do not permit to understand how multiple retinal inputs sum up to generate the collision response. To investigate how these retinal signals are integrated on tectal neuron dendritic branches we plan to perform Ca2+i imaging by employing a calcium sensitive dye OGB-1 and a high resolution, high speed and low noise sCMOS camera Andor Neo. Preliminary results show that patch-clamped neurons can be visualized from cell body up to fine branches in the upper layers of the optic tectum, where most synapses from the retinal axons are formed.

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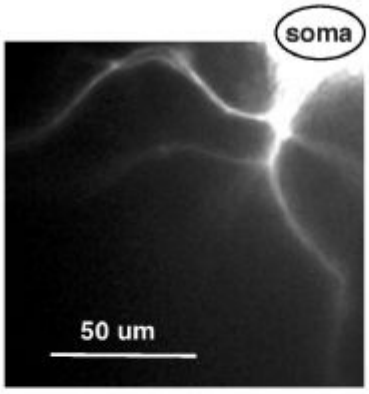
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Order in the chaos - unscrambling the interlaced fine structures of anchovy cone pedicles

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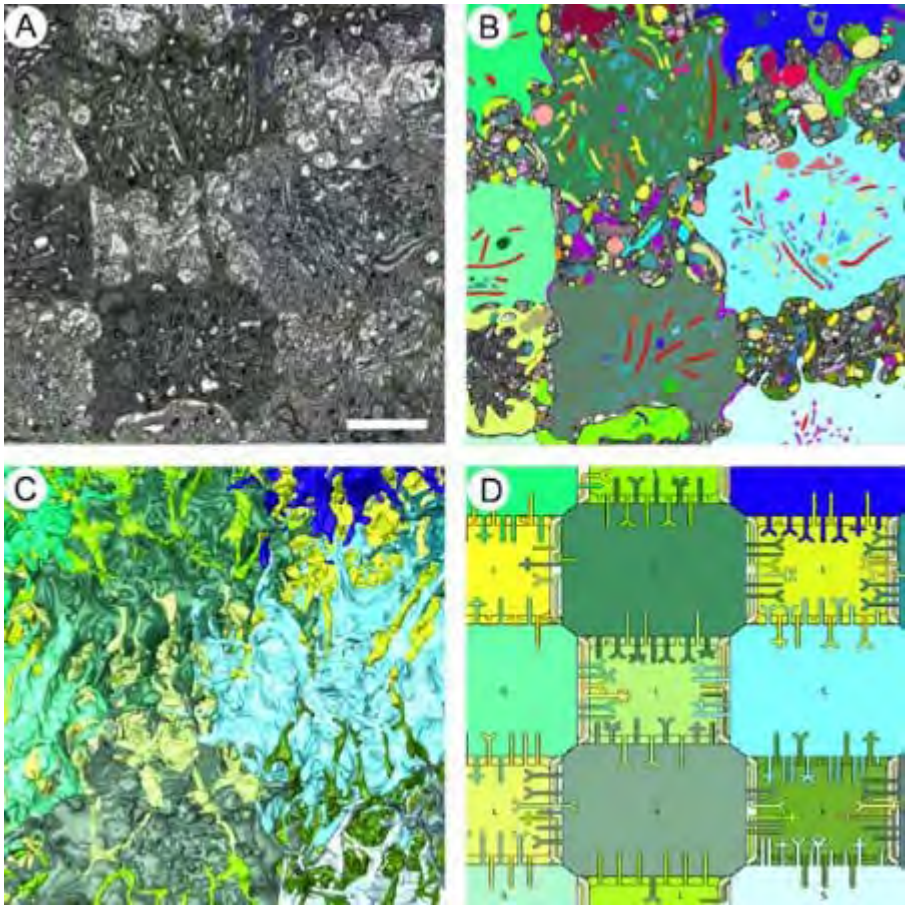
In the teleost retina cone photoreceptors generally are arranged in geometrically regular patterns. Readily visible at the level of cone ellipsoids these patterns are maintained throughout the outer nuclear layer and re-emerge in the outer plexiform layer (OPL) as corresponding pedicle patterns. The striking regularity in the arrangement and the fixed proximity relationships of these photoreceptor terminals likewise suggest both, a cell-type specific and a spatially well-ordered wiring scheme between cones and secondary order neurons. In addition also cone-cone contacts, e.g. via telodendrites, are established.
In most parts of the anchovy retina two cones types with orthogonal e-vector sensibility are found (long and short cones), most likely subserving polarization contrast vision. These cones are arranged alternatingly in parallel rows (“polycones”), in a body forming a tessellate pedicle pattern in the OPL. In contrast, confined parts of the retinal periphery are dominated by linear triple cones (with two large lateral and one small central component), most likely subserving bichromatic color vision, that again can be recognized in the OPL.
In order to disentangle the OPL connectomics of the anchov retina we record, visualize and analyse the 3D fine structure of the cone pedicles with their horizontal pattern, cell-type specific radial stratification, number and arrangement of presynaptic ribbons, synaptic triads and not least with their inter-pedicular contacts. Retinal fragments of the European anchovy (Engraulis encrasicolus) were processed for TEM and embedded in epoxy resin. The samples were sliced and imaged with a FIB/FESEM crossbeam workstation (resulting voxel size 9 nm in x-, y- and z-direction: Fig A, bar 2 µm). The resulting image stacks (ca. 1000 slices each) were reconstructed with Amira® performing manual segmentation (Fig B) and digital surface rendering (Fig C).
We found that the prevailing polycone pattern as well as the triple cone pattern of some retinal regions is retained at the pedicle level. A distinctive radial displacement of long/lateral and short/central cone pedicles and their ribbons can be revealed (ca. 1.5 µm). By digitally crystallizing individual pedicles and combining them with their neighbors successively, an unexpectedly dense interconnecting network of fine pediclular telodendrites with cone type specific 3D geometry was uncovered. On the one hand the corners of neighboring long cones (i.e. between but not within polycones) and also the corners of neighboring short cones are connected via gap junctions. On the other hand many fine protrusions are leaving each pedicle basally and invade the synaptic cavities of neighboring pedicles (Fig D) with no synapses in evidence (at least at 9 nm isotropic resolution). This is less pronounced in triple cone pedicles due to their long proximity distances.
Masked by the 3D entanglement and complete space filling of the pedicles with their protrusions and a multitude of invaginating or attaching secondary order neuron dendrites a well-ordered and cell-type specific wiring scheme emerges in the anchovy’s OPL. The telodendritic network indicates a strong lateral and directional modulation of downward signals already among the photoreceptors. To complete the OPL wiring scheme the dendritic trees of horizontal and bipolar cells have to be included in further analyses.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Power spectra changes induced by repetitive transorbital alternating current stimulation: a longitudinal approach to study after effects

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Introduction: Non-invasive brain stimulation using alternating current stimulation (ACS) has recently been shown to improve vision after optic nerve damage. It is assumed that transorbital ACS induces neuronal networks to propagate synchronous firing probably activating partially damaged areas surviving the injury.
Objectives: The goal of this study is to investigate if rtACS induces EEG power spectra changes that are related to the stimulation.
Methods: One subject with hemianopic visual field loss due to stroke first received 10 days sham stimulation (daily duration: between 25min on day1 to 50min at the end of the treatment period). After a four-month treatment free interval, rtACS was given on another 10 consecutive days. EEGs were recorded before and after each stimulation session. EEG spectral power changes and visual field alterations were investigated.
Results: After rtACS contrast sensitivity in perimetry was enhanced in the central visual field, but the extent of the visual field defect remained unchanged in the more peripheral visual field. rtACS induces an increase in EEG power in classic bandwidths with an alpha-peak shift. During sham-stimulation it was observed that there were also an increase in EEG power in classic bandwidths with an alpha-peak shift, but the change in amplitude is smaller than that by rtACS.

Conclusion: rtACS induces changes in EEG power spectra in a regular pattern as increase in EEG power and a spectral shift in alpha frequency. sham-stimulation induces smaller change in in EEG power spectra and a similar spectral shift in alpha frequency.

Processing of motion stimuli by cells in the optic tectum

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The ability to detect motion across the visual scene is crucial for detection of potential mates or predators and is a basic capability in the animal kingdom. In the earliest station of the visual pathway of birds, the optic tectum, cells have been found which seem to be perfectly suited to encode motion. These cells are located in the deep output layer of the optic tectum, the Stratum Griseum Centrale, have large dendritic fields have and make a direct synaptic contact with the retinal ganglion cells. It has been hypothesized that the sequential activation of the synaptic endings is the basis of the encoding of motion by these cells. However a direct confirmation of this hypothesis by electrophysiological data is still missing.
We measured the response of neurons in the intermediate and deep tectal layers to stimuli moving at different speeds. We recorded cells that responded with a regular (‘non-bursting’) and a bursting firing pattern. The bursting cells could be further divided into cells, which showed an extremely high firing rate within bursts (‘fast-busting’ ~500 Hz), and cells with lower firing rates (‘slow-busting’ ~150Hz). For the ‘fast-bursting cells’ the number of bursts evoked by visual stimulation was independent of the speed of the stimulus while this was not the case for ‘slow-bursting’ cells.
These results indicate that the number of burst evoked by visual stimulation might indeed be related to the sequential activation of the bottlebrush endings by visual stimuli. Therefore, these cells might be involved in the encoding of movement across the visual scene.

Receptive field mapping in blind retinae using localized electrical stimulation

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The hypotheses of preservation of the inner retinal circuitry in the incurable disease of retinitis pigmentosa encourages attempts of a partial restoration of visual function using electrical or optogenetic stimulation. This hypothesis can be tested by evaluating the receptive fields of the retinal projection neurons, the retinal ganglion cells (RGCs).
Here we experimentally mapped receptive fields of RGCs in blind retinas using electrical stimuli delivered by an implantable subretinal microchip. Whole-mount retinas from adult, blind mouse retinas (rd10 and rd1) and from wild type (C57Bl6) were interfaced to the microchip, which contains an array of 1600 stimulation electrodes (electrode spacing: 70 µm). Individual photodiodes on the microchip were illuminated by an oLED monitor. The change in photocurrent was transferred to constant-current stimulation pulses (duration: 2.1 ms).
The induced spiking of RGCs was recorded using a flexible, transparent and perforated microelectrode array (16 electrodes, 10 µm electrode diameter, 60 µm spacing). Perforation of the array allowed for appropriate oxygenation of the retina and therefore for recording times exceeding more than one hour. Electrically evoked receptive field sizes were mapped using narrow stripe-like stimuli (70 x 3000 μm^2 and 140 x 3000 μm^2 respectively). At each stimulus position (separation: 70 µm) the number of induced RGC spikes was calculated as an average over multiple stimulus presentations and a post-stimulus time histogram was calculated. A Gaussian fit to the post-stimulus time histogram revealed the receptive field size.
The mean receptive field size of RGCs in rd10 retinas mapped using electrical stimuli was 260 µm (n = 34 RGCs). This value is similar to the mean RF size obtained under identical experimental conditions for RGCs in healthy, control retinas (310 µm; n = 30). Moreover the spatio-temporal response patterns in retinas of both mouse strains shared similar features. We note that although most response patterns were ON-type responses (of transient and sustained type), some RGCs are inhibited by electrical stimuli. In addition, preliminary experiments using rd1 retinas, a mouse strain where photoreceptor degeneration starts earlier compared to rd10, reveals oscillatory spiking patterns upon brief electrical pulses.

In summary, we prove that RGCs in blind rd10 retinas are sensitive to spatially localized stimuli. Evaluation of the receptive field sizes suggests that the inner retinal circuitry remains intact to a large degree. However, we also detect a large percentage of RGCs which display oscillatory spiking in both rd10 and rd1 retinas. For these RGCs elaborate electrical stimuli will need to be developed.

Response characteristics of ON stellate-varicose amacrine cells and their interactions with ON midget ganglion cells in primate retina

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Morphological studies have distinguished more than twenty different types of amacrine cells in primate retina. However, their functional roles and the neuronal circuitries of most cells remain unknown. Among the group of wide-field amacrine cells, OFF stellate-varicose cells have been previously identified in Golgi-impregnated retinas of macaque monkey and human (Mariani, 1990, JCN 301:382-400; Kolb et al., 1992, JCN 318:147-187). Here, we describe the light response properties of ON stellate-varicose amacrine cells and identify ON midget ganglion cells as one of their putative postsynaptic partners.

Electrophysiological recordings of light responses from displaced ON stellate-varicose cells were performed in the in vitro flat mount preparation of macaque retinas. These experiments were combined with dye-injections into overlapping ON midget ganglion cells and subsequent immunostainings for confocal imaging. Lucifer yellow or neurobiotin was added to the pipette solution to recover the morphology of the cells after whole-cell patch-clamp recordings. Light responses were measured in response to several stimuli at photopic or mesopic light levels. Specimens were then counterstained with antibodies against choline acetyltransferase (ChAT), the vesicular GABA transporter vGAT, gephyrin, or the alpha1 subunit of $GABA_A$ receptors.

The overall morphology of ON stellate-varicose cells resembled that of the OFF type. Three to four primary dendrites emanated from small somata (~ 14 µm) located in the ganglion cell layer. They branched multiple times in a radiate fashion and stratified slightly below the level of the inner ChAT band. Characteristically large varicosities were found along the full extent of the otherwise very fine dendrites and were positive for vGAT. Dendrites of co-injected ON midget ganglion cells and the corresponding postsynaptic markers were found in close apposition to these sites. The dendritic fields of stellate-varicose cells were large (~1 mm) and showed strong overlap in neighboring cells. The pattern of neurobiotin spread to nearby cells suggested homologous and heterologous coupling via gap junctions. Axons were not observed, and neither current injections nor light stimulation elicited spikes. At photopic light levels, 500 ms light pulses from a dark background elicited a strong depolarization and a sustained response modulation lasting for more than 3 sec after light offset. The sustained response component was strongly suppressed by large stimuli while the early, transient component was barely affected by stimulus size. The membrane voltage was constantly shifted to more positive potentials at a grey background and the cells responded with more transient depolarizations or hyperpolarizations to light or dark stimuli, respectively. Stimulation at mesopic light levels revealed a putative rod input to the cells.

We report the existence of ON stellate-varicose amacrine cells in primate retina and ON midget ganglion cells as their putative postsynaptic partners. Our data suggests that stellate-varicose cells pool input from rods and cones over large areas and may continuously release GABA onto postsynaptic cells depending on background light levels.

Similarity across saccades is encoded in the retina

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Saccadic eye movements form an essential feature of visual behaviour. During saccadic vision, the visual signals on the retina are segmented into brief image fixations separated by global motion signals. It has been shown that the activity of retinal ganglion cells (RGCs) is strongly affected during saccade-like image shifts, either by way of short bursts of spikes or by suppression of spiking activity. This suggests that there may be a specific representation of saccades by different types of ganglion cells. Also, saccades are known to modulate RGC response to subsequent fixations. However, it is not known how the stimulus history (i.e., the saccade and an earlier fixation) shapes the response to the current fixation. Here we address this problem by studying the retinal coding under simulated saccadic vision. We recorded spiking activity from retinal ganglion cells of isolated mouse retina to a stimulus with saccade-like shifts of either a grating pattern or a natural image. We analysed the RGC responses to both the saccade-like motion and the fixation after a saccade. We found a variety of response types to this stimulus. Surprisingly, we observed a group of cells that selectively responded to scenarios when the newly fixated image is similar to the image before the saccade. We identified these cells as transient OFF cells with large receptive fields. Furthermore, we show that the similarity response is brought about by a disinhibition circuit involving GABAergic interneurons. Our results demonstrate that this ganglion cell type shows novel dynamics under saccadic stimulus presentations, allowing it to detect image similarity across saccades.

Spatial integration properties of mice retinal ganglion cells: a closed-loop approach

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How neurons integrate the incoming streams of information largely constraints their role within the computations performed by the system. The final integration process in the retina is carried out by ganglion cells, which often have been described by a center-surround organization of their receptive field. We here study how different ganglion cells in the mouse retina integrate visual contrast over space. We do so by performing cell-attached patch-clamp recordings of individual ganglion cells in the isolated retina and use closed-loop control of visual stimulation in the receptive field center to identify sets of stimuli that evoked the same neuronal response ("iso-response stimuli"). With this method of iso-response measurements we can evaluate the integration nonlinearities preceding cell-intrinsic nonlinearities, such as spike generation. For example, in the salamander retina, this method already unraveled different types of integration nonlinearities in both center and surround of the receptive field [1, 2]. We also go beyond the comparison of the different nonlinearities showed by different ganglion cell types, and use computational modeling to fit them to models of signal processing. Finally, we use the models to infer mechanisms underlying the different nonlinearities.

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Spike generator desensitization in retinal ganglion cells underlies transient light response and sensitivity to high contrasts

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Mammalian retinas contain about 20 types of retinal ganglion cell (RGC), each with their own distinct set of morphological and physiological features. Many of them still lack comprehensive functional characterization. Here, we analysed a transgenic mouse line (Igfbp5) with GFP expression in two mirror symmetric strata in sublamina 2 and 3 of the inner plexiform layer and the corresponding cell bodies in the inner nuclear layer and ganglion cell layer. Anatomical examination revealed that several cell classes and types are labelled in the retina of this mouse. These include bipolar cells, at least one type of displaced wide-field amacrine cell, and two populations of RGCs. Here, we studied the Igfbp5 ON RGC type, which co-stratifies with the GFP-labelled wide-field amacrine cells in sublamina 3 and morphologically resembles the previously documented PV-2/3 cells in the transgenic Pvalb^{Cre} mouse line (Farrow et al., Neuron 2013).

By visualizing the GFP-labelled cells under the two-photon microscope, we were able to selectively target Igfbp5 ON RGCs and measure spiking responses to visual stimulation using loose-patch juxtacellular recordings. The voltage-gated sodium channels of the cells were characterized in a separate set of experiments using voltage-step protocols and whole-cell patch-clamp recordings. For comparison, we also recorded “alpha-like” ON RGCs, which we identified by their large soma size and their dendritic morphology recovered via post-recording dye fills.

Despite some similarities with the direction-selective ON RGCs, visual stimulation of the Igfbp5 ON RGCs revealed no directional sensitivity. Instead, the cells displayed transient ON-responses with a striking preference for high-contrast stimuli. In comparison, alpha-like ON RGCs responded already at much lower contrast. Current injection and voltage-step experiments, as well as computational modelling suggest that spike generation desensitization observed in Igfbp5 ON RGCs (but not in alpha-like ON RGCs) is based on a strong desensitization of the voltage-gated sodium channels themselves and shapes the coding of the Igfbp5 cells in two ways: they become more transient and less sensitive for low-contrast stimuli. In line with earlier studies on RGC contrast adaptation (e.g. Kim & Rieke, J Neurosci 2003; Weick & Demb, Neuron 2011), our finding suggest that desensitization of the spike generator plays a fundamental role in at least three different aspects of retinal coding at the RGC level: kinetics (transient vs. sustained), contrast adaptation and dynamic range of contrast encoding.

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The NIMA-related serine/threonine kinase -Nek1- at cilia of sensory cells

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We identified mutations in a gene called NEK1 (NIMA-related serine/threonine kinase) causes the human short-rib polydactyly syndrome type Majewski, a lethal osteochondrodysplasia. NEK1 encodes a serine/threonine kinase with proposed functions in DNA double-strand repair, neuronal development, and coordination of cell-cycle-associated ciliogenesis. We showed that loss-of-function-mutations in the NEK1 gene significantly reduce cilia number and alter ciliary morphology in a patient-derived fibroblast cell line. We found only centrioles at stage 1 of ciliogenesis with an abnormal microtubule growth but no enveloping ciliary pocket, confirming a ciliogenesis defect in NEK1 mutated cells.

Many genes of syndromic ciliopathies are coding for components of the cilium or the basal body complex, the ciliary protein organization center, and play a role in protein selection and transport in or along the cilium. Hence, sensory systems like the retina or the olfactory epithelium with sensory neurons containing a cilium can also be affected by these mutations. Mutations in genes encoding ciliary proteins mostly result in a degeneration of the whole ciliary complex, leading in patients to blindness or the loss of olfaction. In certain cases, e.g. the Bardet-Biedl syndrome (ciliopathic human genetic disorder), both sensory organs are affected.

Recently, we identified the protein Nek1 at the basal body complex of the sensory neurons in the retina and the olfactory epithelium of the mouse. In the other cell types Nek1 is localized at the centrosomes. The aim of this project is to analyze whether Nek1 plays a role in ciliary transport and/or ciliogenesis in sensory cells. Deciphering the function of Nek1 in the retina and olfactory epithelium will hopefully advance our understanding of ciliopathic human genetic disorders in sensory cells.

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Type 2 wide-field amacrine cells in tyrosine hydroxylase (TH)::GFP mice show a homogenous synapse distribution and contact small ganglion cells

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In vertebrate retinas, wide-field amacrine cells represent a diverse class of interneurons, important for the extraction of selective features, like motion or objects, from the visual scene. Most types of wide-field amacrine cells are axonless and collect inputs and provide outputs within the same process. However, some types spatially segregate outputs from inputs: A subset of amacrine cells - the polyaxonal amacrine cells - receive inputs via their short dendrites and deliver spikes along their long axonal processes (Greschner et al., 2014, J. Neurosci., 34, 3597–3606.), thus mediating long-range inhibitions.
In the TH::GFP mouse line, two types of GFP-expressing wide-field amacrine cells have been described: Dopaminergic type 1 cells stratifying in layer 1 of the inner plexiform layer (IPL) and GABAergic type 2 cells, which were shown to contribute to the second calretinin-positive band in the middle of the IPL (Knop et al., 2011, J. Neurosci. 31, 4780-4791).
Similar to polyaxonal amacrine cells, type 2 cells possess short and long radial processes of several hundred microns in length. Via these neurites, type 2 cells collect excitatory and inhibitory inputs from ON and OFF bipolar cells and other amacrine cells, giving rise to ON-OFF light responses (Knop et al., 2011, J. Neurosci. 31, 4780-4791). To test for segregation of synaptic in- and outputs, we aimed to analyze the spatial distribution of synapses along type 2 cell processes. Also, we aimed to identify ganglion cells postsynaptic to type 2 cells to shed light on the function of type 2 wide-field amacrine cells in the mouse retina.
To this purpose, we dye-injected type 2 cells and ganglion cells in TH::GFP retinas and labeled for connexin36 (Cx36, a gap junction protein for electrical synapses), CtBP2 (a ribbon marker for excitatory synapses) and gephyrin, neuroligin-2, $GABA_A$ receptor subunits alpha3 and rho (all postsynaptic markers for inhibitory synapses).
We show that along dendrites of type 2 cells, which form a weakly coupled network, electrical synapses (made of Cx36) and chemical (excitatory and inhibitory) synapses are uniformly distributed, independent of distance from the soma and dendrite length, thus excluding a segregation of synaptic in- and outputs.
Moreover, we reveal that type 2 cells contact at least two types of small ganglion cells: W3 cells (Kim et al., 2010, J. Neurosci., 30, 1452–1462), a ganglion cell sensitive to object motion, and G5 cells (Völgyi et al., 2009, J. Comp. Neurol., 512, 664–687). Contacts were typically located at type 2 cell varicosities and often associated with markers of inhibitory synapses.
In summary, our data indicate that type 2 wide-field amacrine cells of the TH::GFP mouse line are electrically coupled to each other via Cx36 and do not spatially segregate synaptic output from input. We suggest that type 2 cells mediate presynaptic inhibition to bipolar cell terminals and provide postsynaptic inhibition to at least two ON-OFF ganglion cells with small receptive fields: W3 and G5 cells. Inhibitory contacts to W3 cells suggest that type 2 cells play a role in the suppression of moving stimuli in the surround of W3 cells and are thus important for the detection of object motion in the mouse retina.

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Poster Topic

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

T16-1A Bimodal role of matrix metalloproteinases in adult visual cortex plasticity in a healthy and lesioned brain
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T16-2A Changing saccade plans: timing of response competition in spatial decision-making
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Sophia Katharina Stodieck, Xiaojie Huang, Oliver M. Schlüter, Siegrid Löwel

T16-4B Learning to discriminate stimulus orientation shapes response properties in mouse primary visual cortex
Ovidiu Jurjut, Sinem Erisken, Agne Vaiceliunaite, Laura Busse, Steffen Katzner

T16-5B Neurons in visual cortex retain a memory of their inputs after monocular deprivation
Tobias Rose, Juliane Jäpel-Schael, Mark Hübener, Tobias Bonhoeffer

T16-1C Ocular dominance plasticity after stroke was preserved in PSD-95 knockout mice
Franziska Greifzu, Daniel Parthier, Oliver M. Schlüter, Siegrid Löwel

T16-2C Orientation selectivity in a network of cortical neurons *in-vitro*
Manuel Schottdorf, Hecke Schrobsdorff, Walter Stühmer, Fred Wolf

T16-3C Processing of contrast-modulated second-order stimuli in mouse visual cortex.
Zeinab Khastkhodaei, Ovidiu Jurjut, Steffen Katzner, Laura Busse

T16-4C Reward expectancies modulate sensory processing in mouse primary visual cortex
Alexandra Wal, Ovidiu Jurjut, Laura Busse, Steffen Katzner

T16-5C Saccade Related Layer Specific Local Field Potential Activity in Macaque V1 during Free Viewing
Richard Meyes, Junji Ito, Yukako Yamane, Ichiro Fujita, Hiroshi Tamura, Sonja Grün

T16-1D Spatial integration in mouse V1 is shaped by NMDA receptors in parvalbumin-positive interneurons
Matilde Fiorini, Agne Vaiceliunaite, Sinem Erisken, Ovidiu Jurjut, Steffen Katzner, Laura Busse

T16-2D The effects of pulvinar microstimulation on cortical BOLD activity in the behaving monkey
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T16-3D The preserved ocular dominance plasticity in the visual cortex of enriched mice is heritable
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T16-4D The retinotopic representation of the visual wulst in a laterally eyed bird, the zebra finch (*Taeniopygia guttatta*)
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T16-5D The Yin and Yang of cortical plasticity: the role of postsynaptic density proteins 93 and 95 for mouse vision and visual cortical plasticity
Leon Hosang, Sophia Stodieck, Bianka Götze, Plinio D. Favaro, Oliver M. Schlüter, Siegrid Löwel

Bimodal role of matrix metalloproteinases in adult visual cortex plasticity in a healthy and lesioned brain

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The ability of the adult brain to undergo plastic changes is of particular importance in recovery from central nervous system (CNS) injuries or for improving learning and memory during aging. Matrix metalloproteinases (MMPs) have emerged as important players in CNS plasticity in both the healthy and injured brain. A recent study showed that blockade of MMPs prevented the experience-induced potentiation of open-eye responses in juvenile rat visual cortex (V1) (Spolidoro et al, 2012); however, little is known about their function in adult experience-dependent V1 plasticity. Even though increased activity of MMPs can be beneficial for a healthy brain, in an injured brain, MMPs promoted the spread of a lesion (Agrawal et al, 2008), and may actually impair plasticity (Cybulska-Klosowicz et al, 2011). Studies from our laboratory have recently shown that a small cortical lesion in the primary somatosensory cortex (S1) prevented plastic changes in the mouse visual system (Greifzu et al., 2011). Specifically, monocular deprivation (MD) did no longer induce ocular dominance (OD) shifts in V1 towards the open eye. Similarly, neither visual acuity nor contrast sensitivity through the open eye improved (sensory learning) when assessed behaviorally by optomotry.

Here we examined: (i). the role of MMPs in adult V1 plasticity and sensory learning and (ii) their therapeutic potential in restoring plasticity after a cortical lesion in the neighboring somatosensory cortex. To induce plastic changes, adult mice (80-90 days old) were deprived of vision in one eye for 7 days during which the broad spectrum MMP-inhibitor GM6001 (50mg/kg in 2% cyclodextrin) or control solution (2% cyclodextrin) was applied via intraperitoneal injections once per day. After this treatment, we monitored V1-activities by intrinsic signal optical imaging and tested vision by optomotry. In control mice, MD induced OD-shifts towards the open eye and increase in visual acuity. In contrast, mice treated with GM6001 did neither show an OD-shift after MD nor improve their visual capabilities, suggesting that MMPs are crucial for both experience-dependent visual cortex plasticity and sensory learning in the adult brain. To additionally probe the therapeutic potential of MMPs in rescuing impaired plasticity after a cortical lesion, we performed experiments in mice with a photothrombotic (PT) stroke lesion in S1. Specifically, animals were treated with either GM6001 or control solution one hour after PT and were subsequently subjected to MD or no MD for 7 days. As expected, in PT-lesioned mice treated with control solution, MD did not cause an OD-shift nor induce any improvements in visual capabilities of the optomotor reflex. In contrast, a single injection of GM6001 1 hour post PT preserved both an OD-shift and increased visual capabilities of the open eye after 7 days of MD. Altogether, our data suggest a bimodal role of MMPs in visual cortex plasticity and sensory learning: while a 7-day treatment with MMP-inhibitor during MD prevented OD-plasticity and sensory learning in adult mice, a single treatment after PT preserved both OD-plasticity and sensory learning in adult animals. It remains to be determined which types of MMPs account for the present findings.

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Changing saccade plans: timing of response competition in spatial decision-making

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Animals and humans change goals and actions according to updated demands of the environment. This flexible action selection is thought to be mediated by bihemispheric visuomotor circuitry through competition of neuronal signals representing different response options (“affordance competition” model, Cisek 2007). The aim of this study was to understand how updating the goals at different intervals during motor planning modifies the selection of spatially lateralized options in instructed or free choice conditions.

Ten human subjects participated in two sessions and performed three different oculomotor tasks in separate blocks: 1) Primary saccade task, 2) Follow Second Cue (FSC) task, and 3) Follow First Cue (FFC) task. The primary task included only one central symbolic cue which also served as a “go” signal and either instructed subjects to saccade to one of the two opposite targets (instructed trials) or let them freely choose between the targets (choice trials). In the FFC and FSC tasks 67% trials were as in the primary task, but in 33% trials the cue changed following a variable delay after the first “go” cue (“change trials”, change signal delay, CSD: 17, 67, 134, 184, 251, 301 ms). In the FSC task subjects were required to change their action if the cue was updated. In change trials of FSC task, subjects also reported the direction of their initial motor plan (left or right) with a button press. This allowed us to distinguish “real” change trials from “congruent” trials in which the second cue directed the subjects in the same direction as their initial plan (e.g. as in a large proportion of choice trials). In third, FFC task subjects always had to follow the initial cue and ignore the second cue. This task served as a control to demonstrate that subjects perceived the first cue when it was followed by the second cue

We hypothesized that in the choice trials, two alternative motor plans are initially present and it takes longer to resolve the competition and arrive at decision threshold, as compared to the instructed trials. In accord with this hypothesis, in all three tasks subjects were significantly faster in instructed as compared to choice primary (one cue) trials (by 10-34 ms). In the FSC task the reaction times in both instructed and choice change trials (measured from the second cue) were faster than in corresponding primary trials (by 19 ms and 38 ms), significant for the choice trials. This suggests that it is easier to switch between actions during motor planning than to initiate the action from a fixation baseline, especially when both action plans might be engaged. Correspondingly, subjects had better performance in the choice change trials as compared to the instructed change trials, at early and middle CSDs (significant at 134 and 184 ms), but not at late CSDs. The (task-irrelevant) second cue also biased the target selection in FFC task up to CSD of 184 ms in choice trials. Together, these results support the hypothesis that the competing oculomotor plans are present in the interval up to 200 ms leading to the saccade execution.

Clusters of intralaminar synaptic inputs on the dendrites of cortical layer 5 pyramidal cells suggest recruitment of non-linear dendritic integration

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The spatial organization of synaptic inputs on the dendritic tree of cortical neurons plays a major role for dendritic integration and neural computations but remarkably little is known about it. For mapping inputs with synaptic resolution we combined optogenetics and 2-photon calcium imaging, which we refer to as Channelrhodopsin (ChR2)-assisted synapse identity mapping (CASIM). The principle is as follows: NMDA receptor mediated calcium signals identify those dendritic spines on a postsynaptic pyramidal cell, which receive input from photostimulated presynaptic neurons expressing ChR2. The main advantage of CASIM over other methods for mapping synapses by light or electron microscopy (e.g. Rah et al., 2013; Druckmann et al., 2014) is that it identifies synapses in functional rather than structural or molecular terms. We applied CASIM to map the synapses between layer 5 (L5) pyramidal neurons in the primary visual cortex of transgenic Thy1-ChR2 mice (Arenkiel et al., 2007) and found a clustered organization of the synapses on the basal dendrites of L5 cells. To analyze the spatial organization of synaptic inputs we developed a new mathematical approach based on combinatorial analysis of the likelihoods to observe specific arrangements. The identified input clusters have the size and spacing (4 to 9 synapses within 20 µm of dendrite) required for local non-linear dendritic integration (Losonczy and Magee, 2006; Branco and Hausser, 2011).

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Experimental model for testing of cortical visual prosthesis prototype.

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Brain-computer interface (BCI) development will promote progress in the field of impaired sensory functions compensation including visual function. Visual perception issue is central for visual prosthesis development. It is assumed that natural activation of primary visual cortex areas is accompanied by the appearance of elementary visual sensations which correspond to phosphenes – sensations that appear when these cortical areas are electrically stimulated directly. Analysis of phosphene vision studies conducted so far has shown that today due to many reasons we need to develop more complex experimental schemes and to switch to more simple model objects than primates and human. We suggest the use of rats with pigmented iris as such model objects. The experimental model for testing phosphene vision hypothesis and development of visual prosthesis prototype consists of instrumental differential conditioning: a rat presses on the pedals in response to light stimulus presentation with certain localization in the visual field and should not press on the pedal when localization of the same stimulus is different. If the phosphene hypothesis is true then artificial electrical stimulation of the primary visual cortex areas activated with the presentation of mentioned above light stimuli should also lead to behavioral reactions achieved earlier (direct transfer). Moreover changing of artificial stimuli meaning as a result of reconditioning should lead to corresponding changing of natural stimuli meaning for the animal (back transfer). Electrical stimulation of ventral tegmental area is used as a reward in the model. Electrodes for visual cortex stimulations are implanted previously and corresponding natural visual stimuli locations are determined using visual evoked potentials registration (amplitude maximum of the N1 wave with 35-48 ms latency). Light stimuli should be projected to the certain region of the retina in spite of eye movements presence. For fulfillment of this condition the system of painless head fixation is used together with video monitoring for eye position detection and oculography for eye movements evaluation.

Face patch resting state networks link face processing to social cognition

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Faces transmit a wealth of social information. How this information is transmitted from highly selective face-processing centers to areas supporting social cognition remains unclear. Here we noninvasively identify these routes using resting state functional magnetic resonance imaging in six macaque monkeys at high resolution and with full brain coverage. We find that face areas are connected with specific frontal, temporal, parietal, and subcortical structures supporting socially relevant cognitive functions. Furthermore, we show that there is significant overlap between the face patch rsfMRI connectivity maps and another large scale network, the default mode network. Interestingly, the face patch resting state networks and the default mode network in monkeys show a pattern of overlap akin to that between the social brain and the default mode network in humans. This overlap localizes to the posterior superior temporal sulcus and dorsomedial prefrontal cortex, areas supporting in high-level social cognition in humans. Together, these results reveal the embedding of face areas into larger brain networks, and suggest that the face patch system offers a readily accessible venue into studying the evolution of primate social cognition circuits.

Impaired binocular microsaccades in hemianopia

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Microsaccades are small, fast, and jerk-like eye movements which occur several times per sec both during voluntary fixation and free viewing. Microsaccades, especially binocular microsaccades, counteract foveal and peripheral fading and thus facilitate high-acuity perception and improve spatial resolution. Abnormal microsaccades are found in amblyopia, progressive supranuclear palsy, Parkinson's disease, Alzheimer's disease and mild cognitive impairment, but they have not been studied in hemianopia. Discovering distinctive characteristics of these pathological microsaccades is of significant importance for early diagnosis, dynamic monitoring of disease progression or treatment efficacy. We now recorded the eye movements of 14 patients with homonymous hemianopia and 14 age matched participants with normal vision. While microsaccade magnitudes, velocities, durations, and frequencies were comparable between the two groups, hemianopic patients produced significantly less conjugate binocular microsaccades. Our results indicate that binocular microsaccades are impaired in patients with homonymous hemianopia, which might contribute to their vision loss.

Interhemispheric brain processing and action selection in human and non-human primates

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Adaptive action selection often relies on interhemispheric transfer (IHT) of visuomotor information. However, it is still not known how the two hemispheres integrate such information, especially when there is a need for action selection as compared to the situation with only one response option. Furthermore, there are no related monkey-human comparative studies considering potential species differences due to brain lateralization. Here we investigate the behavioral and neural correlates of IHT with and without action selection in both species. In contralaterally-organized primate brains, when the hemisphere encoding the motor response does not receive the visual information directly ("crossed condition"), motor responses are typically slower than during "uncrossed conditions", indicating additional interhemispheric processing. IHT has been assessed by asking subjects to respond manually to lateralized visual stimuli with pre-determined hand (Poffenberger, 1912), resulting in the crossed-uncrossed difference (CUD) of ~4 ms. To test IHT during action selection, we designed an experiment in which humans and monkeys performed manual responses after presentation of lateralized stimulus which also cued the use of the right or the left hand. In this case, the CUD for 20 human subjects and one rhesus monkey were 18 ms and 20 ms. To test differences in brain activation patterns between crossed and uncrossed conditions during action selection, human subjects performed the task inside a scanner. Crossed responses elicited stronger fMRI activation in multiple regions of the visuomotor network, in both hemispheres. During IHT without action selection (hand use cued before the go signal) the CUD human subjects decreased to previously reported 4 ms. In conclusion, human and monkey brains require similar times to integrate information across hemispheres while selecting an action and, at least in humans, this time is much longer than without action selection. We hypothesize that the longer CUD during action selection reflects not only a "single-pass" transfer of visuomotor information but recurrent interhemispheric. We are testing this hypothesis and potential inter-species differences by comparing the brain activation patterns in tasks with or without action selection using fMRI.

Knock-down of the signaling scaffold postsynaptic density protein-95 (PSD-95) in the adult primary visual cortex restored a juvenile ocular dominance plasticity

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Neural circuits are highly susceptible to experience-dependent modifications during the critical period, where silent glutamatergic synapses, highly efficient plasticity substrates, are abundant and unsilencing them may mark maturational processes of neurocircuits. The postsynaptic density protein-95 (PSD-95) is a signaling scaffold present at mature excitatory synapses, but its role for synaptic maturation remains elusive. As we showed recently, experience-dependent maturation of silent synapses was absent in the primary visual cortex (V1) of PSD-95 knock-out (KO) mice and a juvenile ocular dominance (OD) plasticity, visualized by intrinsic signal optical imaging, was preserved lifelong[1].

To clarify the locus underlying the increased plasticity in PSD-95 KO mice, we tested the effect of time and region specific silencing of PSD-95 expression with viral vectors (AAV-sh95). First, we tested whether silencing PSD-95 expression in V1 of WT-mice at postnatal day (P)0 induced the same phenotype as observed in PSD-95 KOs. Since interhemispheric interactions are important for OD-plasticity[2,3], we silenced PSD-95 expression in V1 of one or both hemispheres (control: AAV-GFP). OD-plasticity was tested in P80 mice after 4 days of MD.

Indeed, a knock-down of PSD-95 in both hemispheres phenocopied our previous results in PSD-95 KOs: after 4 days of MD, the OD-index (ODI) was 0.01±0.02, whereas the ODI in controls was: 0.28±0.02 ($p<0.001$, t-test). Quantitative analyses of V1-activation revealed that the OD-shift was primarily mediated by a decrease of deprived eye responses ($p<0.05$), demonstrating a juvenile OD-shift. OD-plasticity was also preserved in mice with a knock-down exclusively in the hemisphere contralateral to the deprived eye (ODI=0.05±0.03, $p<0.001$, t-test), but not in mice with a knock-down in the hemisphere ipsilateral to the deprived eye (ODI=0.24±0.01, $p>0.05$, t-test).

Finally, we tested whether plasticity can be restored in adulthood by silencing PSD-95 after the critical period for OD-plasticity. To this end, we knocked-down PSD-95 (control: AAV-GFP) in P40 mice and tested OD-plasticity after 4 days of MD at P80. Indeed, in sh95-injected but not in GFP-injected mice, the ODI shifted towards the open eye (ODI=0.03±0.03 compared to 0.26±0.01, $p<0.001$, t-test).

Our results demonstrate for the first time that knocking down PSD-95 in V1 of mice can restore OD-plasticity even after the critical period for OD-plasticity. Furthermore, silencing PSD-95 in the hemisphere contralateral to the deprived eye is sufficient to reliably induce OD-plasticity. Since the knock-down of PSD-95 can also reduce the AMPA/NMDA ratio, and thus likely restore silent synapses also in adult V1-neurons, we envisage PSD-95 or related postsynaptic scaffolds as potential targets for the therapeutic increase of synaptic plasticity to aid neuronal regeneration in adult nerve cell circuits.

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Learning to discriminate stimulus orientation shapes response properties in mouse primary visual cortex

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In visual cortex, the response properties of neurons are thought to depend on the behavioral relevance of the sensory information. Selective attention, for instance, can shift the tuning to stimulus features (David et al., 2008) and reduce trial-to-trial variability of individual neurons, or shared fluctuations in neuronal pairs (Cohen & Maunsell, 2009; Mitchell et al., 2009). The behavioral relevance of any stimulus is typically learned over time. How does learning progress affect response properties of neurons in mouse primary visual cortex (V1)?

In head-fixed mice, we used a classical conditioning paradigm, in which the presentation of one of two orthogonal grating orientations was immediately followed by a fluid reward. We measured licks occurring before reward delivery and characterized learning progress by comparing anticipatory licks during stimulus presentation to baseline licks before stimulus onset. In individual animals (n=7), quantitative analysis revealed step-like changes in behavior. First, lick rates were unaffected by the presentation of either orientation (‘naïve stage’). Second, an unspecific conditioned response appeared abruptly, with lick rates higher than baseline for both grating orientations (‘intermediate stage’). Finally, this conditioned response became specific, such that lick rates increased strongest for the rewarded orientation (‘well-trained stage’).

To examine if these distinct learning stages are reflected in the response properties of V1 neurons, we recorded with multi-contact silicon probes from several single neurons throughout cortical layers, and measured response properties of V1 ensembles while the animals learned to discriminate stimulus orientation. First, we examined how learning affected responses of individual neurons. Consistent with previous observations (Kreile et al., 2011), in naïve mice the distribution of preferred orientations peaked at the horizontal orientation. In the intermediate stage, however, the distribution was biased towards the unrewarded orientation. Finally, in the well-trained stage, the distribution of preferred orientations shifted towards the rewarded orientation. These differences across learning stages were pronounced in superficial layers, and largely absent in deep layers. In addition to affecting orientation preferences, learning also decreased trial-to-trial variability in spiking responses (Fano factor). Second, we analyzed how learning shapes population activity by computing, in 200-ms time windows, shared variability in spike counts between pairs of neurons to repeated presentations of the same stimulus (noise correlations). We found that noise correlations were strongly reduced in well-trained compared to naïve stages. This reduction was specific to the processing of the stimulus, as noise correlations measured before stimulus onset did not differ between learning stages.

We conclude that mice learn to discriminate stimulus orientation in a distinct sequence of stages, which can shape the representation of stimulus orientation and increase the robustness of responses in area V1.

Neurons in visual cortex retain a memory of their inputs after monocular deprivation

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A classic example of experience-dependent cortical plasticity is the shift in ocular dominance (OD) after monocular deprivation (MD). Up to now, however, changes in cortical responsiveness after MD have largely been studied on the population level, and it therefore has remained open how alterations in tuning and response strength of individual neurons give rise to global OD changes. In particular, it is unclear if population OD shifts are realized by changes in the tuning of individual neurons, or by selective recruitment or silencing of distinct populations of cells. Moreover, it is unknown if and how single cells recover from an OD shift, given that MD is accompanied by the formation of stable spine synapses [1].
We perform chronic two-photon imaging of cellular structure and function after viral cotransduction with a genetically encoded Ca^{2+} indicator together with a bright structural marker (AAV1/2-mRuby2-P2A-GCaMP6s). In adult mice, the OD of the same excitatory L2/3 neurons imaged repeatedly over months is largely stable under baseline conditions (14 day session-to-session Pearson's correlation coefficient r=0.58). The change in population OD after MD (see figure) is achieved by single-cell tuning changes in 66% of the visually responsive neurons as a result of a variable combination of increased open eye and decreased deprived eye responses. Whereas 30% of the originally deprived eye responsive neurons are silenced after MD, the number of neurons responding to the non-deprived eye remains largely stable. Even though cellular OD-shifts after MD can be pronounced, the majority of cells faithfully return to their pre-deprivation OD after ~3 weeks of recovery (pre-MD to full recovery correlation r=0.49). Interestingly, when challenged with a second MD episode, predominantly the same cells undergo repeated OD plasticity (1st to 2nd MD correlation r=0.60). Therefore, individual L2/3 pyramidal neurons retain a memory of both their baseline OD and their propensity for experience-dependent plasticity, suggesting the existence of plastic subsets of neurons that dominate the adaptive capacity of visual cortex. We currently assess how these reversible cellular OD shifts relate to functional and structural synaptic changes by chronic dual color imaging of dendritic spines of sparsely transfected neurons.

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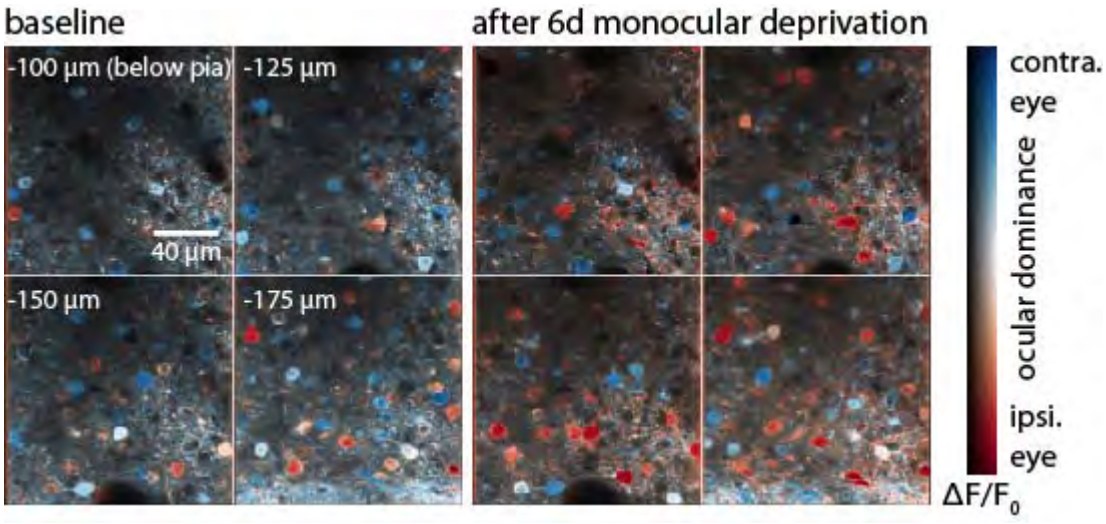

Ocular dominance plasticity after stroke was preserved in PSD-95 knockout mice

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Neuronal plasticity is essential when the brain suffers damage, such as following a stroke, to enable rehabilitation. One of the most established models to study cortical plasticity is the ocular dominance (OD) plasticity induced by monocular deprivation (MD) in the primary visual cortex (V1). Using optical imaging of intrinsic signals we have shown that this plasticity is absent after a photothrombotic stroke lesion induced in the vicinity of V1 (somatosensory cortex) in adult mice[1]. It was shown that OD-plasticity in adult standard cage reared rodents is limited by age and can be enhanced by experimental reduction of the inhibitory tone[2]. Likewise, exposing mice to conditions, which reduce inhibition in V1, such as enriched environment[3] or dark exposure[4] also preserved OD-plasticity after an S1-lesion.

Here we tested whether modification of excitatory circuits can also preserve plasticity after stroke. We could recently show that mice lacking the postsynaptic density protein 95 (PSD-95), a signaling scaffold protein present at mature excitatory synapses, have almost livelong juvenile-like OD-plasticity, an increased number of silent synapses but an unaltered inhibitory tone[5]. In fact, in adult PSD-95 KO mice (postnatal day 93-149), OD-plasticity in V1 was preserved after a S1-lesion: the OD-index of -0.01±0.02 was significantly lower compared to WT mice (0.21±0.04) after MD. Notably, plasticity was preserved although some of the mice were already beyond the sensitive phase for OD-plasticity when raised in standard cages. In contrast, experience-enabled enhancements of the optomotor reflex after MD were compromised in PSD-95 KOs, as previously observed in WT-mice[1]. V1-activation and retinotopic map quality were not different between PSD-95 KO mice and their WT-littermates.

From the preserved OD-plasticity after stroke of the PSD-95 KO mice we conclude that a certain excitatory-inhibitory balance is necessary for promoting V1-plasticity which can be established by either reduced intracortical inhibition or an increased number of silent synapses, as it is present in juvenile mice that also have preserved OD-plasticity after stroke[3].

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Orientation selectivity in a network of cortical neurons *in-vitro*

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A landmark of visual cortical architecture is orientation preference. It is organized roughly repetitively across the visual cortex with neighbouring neurons preferring similar orientations. Its spatial layout shows numerous invariants across Eutherian mammals widely separated in phylogeny [1]. Hypotheses to account for orientation selectivity on one hand and its spatial organization on the other reach from simple feed-forward ideas to cortical self-organization in randomly connected networks and are subject to a long standing debate [e.g. 1, 2], reflecting in part the limited possibilities of testing the often minimalistic theoretical models in experiments.

Here, we propose a new experimental framework to test various models for their biological plausibility. We use a cell culture of optogenetically modified rat neurons as surrogate cortex as such cultures resemble cortical tissue to some extent in structure, activity and development [3-5]. We then stimulate these cells with spatially complex light patterns as moving gratings, generated by a holographic photostimulation system [6]. The neural responses are monitored with a multielectrode array.

We show that cultured neurons react differently to various moving gratings, that cells show direction tuning and that they have a preferred direction (and orientation). This behaviour resembles to some extent cortical orientation selectivity.

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Processing of contrast-modulated second-order stimuli in mouse visual cortex.

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In primates, visual processing in the ventral stream takes place across a hierarchy of areas, starting with representations of low-level features in primary visual cortex (V1), which are gradually transformed into high-level object representations in inferotemporal cortex (IT). In mice, extrastriate visual areas seem to form anatomically and functionally distinct groups, with areas LM, LI, and PM possibly constituting a pathway analogous to the ventral stream in other mammals (Wang et al., 2011; Anderman et al., 2011; Marshel et al., 2011; Glickfield et al., 2013). Whether in the mouse these areas contain increasingly complex, cue-invariant representations of visual information remains an open question.

To test this question we compared visual processing of first-order gratings, characterized by changes in luminance, and second-order gratings, characterized by changes in contrast only. We recorded extracellular activity in areas V1 and LM of anesthetized and awake mice, and measured orientation tuning curves for first- and second-order gratings.

We found that in areas V1 and LM responses to luminance-modulated gratings were stronger and more selective than those to contrast-modulated gratings. This difference was particularly pronounced during anesthesia: while in either visual area the majority of neurons recorded lacked clear orientation selectivity for contrast-modulated gratings in anesthetized mice, we found in awake mice numerous neurons with orientation selective responses, even to contrast-modulated gratings.

We next asked whether this neural representation of second-order orientation could mediate cue-invariant judgments of orientation and tested behaviorally whether mice can transfer learned orientation-reward contingencies from luminance-modulated to contrast-modulated gratings. Using a classical conditioning paradigm, we trained mice to perform a coarse orientation discrimination task. After mice reached stable orientation discrimination for luminance-modulated gratings we replaced the visual stimuli with contrast-modulated gratings. We found a massive breakdown of performance with little generalization of orientation discrimination across the two types of stimuli. This breakdown was not due to the lower root-mean-square (RMS) contrast of the contrast-modulated gratings: when switching back to a luminance-modulated grating with matched RMS contrast, discrimination performance was again high. Hence, although mice can see contrast-modulated gratings, they show little cue-invariant generalization of orientation discrimination.

We conclude that the mouse visual system seems to be specialized for luminance-based perception.

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Reward expectancies modulate sensory processing in mouse primary visual cortex

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A large body of literature has documented effects of attention throughout the visual system, including primary visual cortex (V1). In contrast, few studies have investigated how V1 activity is shaped by other top-down influences, such as reward expectancy (e.g., Stanisor, 2013). We asked whether reward expectancy can affect spiking responses of single neurons in mouse V1.

Using head-fixed mice on a spherical treadmill, we presented stimuli that provided identical sensory stimulation, but differed in reward contingencies. We used a single drifting grating, which was presented either behind a square or diamond aperture. Only one of these two stimuli could be used to earn a fluid reward. Mice started a trial by moving forward on the treadmill, which triggered the presentation of a randomly selected stimulus. In case of the rewarded stimulus, mice could earn a fluid reward by continuing to run for an additional 3.5 s. At any point in time, mice could abort a trial by stopping and thereby immediately request a new trial. The animals (n = 5) learned this task in about 4 weeks.

After the mice had reached a stable level of performance, we used multi-contact silicon probes to record extracellular activity from multiple V1 neurons during task performance.

Analyses of running behavior showed robust effects of reward. For stimuli associated with reward, mice ran until reward delivery at relatively constant speed (15-20 cm/s) in most of the trials, yielding low abortion rates (35 %, average across 5 animals). For unrewarded stimuli, in contrast, average trial abortion rates were high (75 %).

In addition to the effects on behavior, reward expectancy modulated firing rates of individual V1 neurons. During a time window of 1.5 s after stimulus onset, firing rates were higher for rewarded than for unrewarded stimuli. Across the population of recorded neurons, firing rates increased by about 13%. These reward-related modulations of firing rates could not be caused by an unequal sensory drive provided by the different grating shapes, as sensory control measurements revealed largely similar firing rates.

We conclude that, in mice, expectation of reward can enhance firing rates already at the level of V1.

Saccade Related Layer Specific Local Field Potential Activity in Macaque V1 during Free Viewing

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Abstract:

Primates perform frequent saccadic eye movements (SEMs) to sample visual information from their environment. These SEMs are accompanied in the primary visual cortex (V1) by local field potential (LFP) modulations that precede the arrival of the visual input *[Ito et al. (2011) Cereb Cortex 21:2482-97]*.
In the present study we aim to elucidate how the SEM related LFP modulations depend on the properties of visual input during saccades. For this purpose, we presented stationary, full-field grating stimuli to an awake macaque monkey, who freely explored the stimuli with voluntary saccadic eye movements. Eye movements were recorded with a scleral eye coil and the LFP from V1 with a 24-channel linear electrode array. We identified SEM related LFP modulations by averaging the LFP signal aligned to the onsets of saccades and subsequent fixations, and reconstructed the corresponding current source density (CSD) signal (Figure a and b) from the LFP via the inverse CSD method *[Pettersen et al. (2006) J Neurosci Meth 154:116–33]*. For a comparison we also computed the average LFP and CSD signal aligned to the onset of the stimulus images preceded by fixation on a central fixation spot (Figure c).
The monkey preferred to perform saccades in the direction parallel to the stripes of the stimulus image. Those parallel saccades (PSs) had a larger median amplitude, velocity and duration than orthogonal saccades (OSs) and intermediate saccades. We found that the SEM related LFP modulation was stronger for OSs than for PSs. The CSD signal showed a current sink in the granular layer, that was observed for the saccade-onset and fixation-onset triggered averages but not for the image-onset triggered averages, confirming that this sink reflected SEM related activity (Figure a and b, red frames vs. c, red frame). The OSs evoked stronger current sinks than the PSs. We observed a positive correlation between the amplitude of saccades and the magnitude of the evoked current sink. The increase of the CSD signal for larger saccades saturated more quickly for OSs than for PSs (Figure d vs. e). This SEM related current sink probably reflects an inhibitory process because it was accompanied by a concurrent decrease in high-gamma band (~160 Hz) power, which has been shown to be strongly positively correlated to the firing rates of local neurons *[Ray et al. (2008) J Neurosci 28:11526-36]*.
In summary, saccades causing larger changes in the retinal image, such as those with larger amplitude and/or in directions more orthogonal to the stripes of the stimulus images, evoked stronger CSD modulations. This seems to suggest mere excitation of V1 by the retinal changes caused by saccades, but a comparison with the concurrent high-gamma power modulation rather suggests that the CSD modulation reflects an inhibitory process. A possible implication of these observations would be that the CSD signal might reflect an active suppression mechanism that matches the amount of inhibition to the strength of the visual input during saccades.

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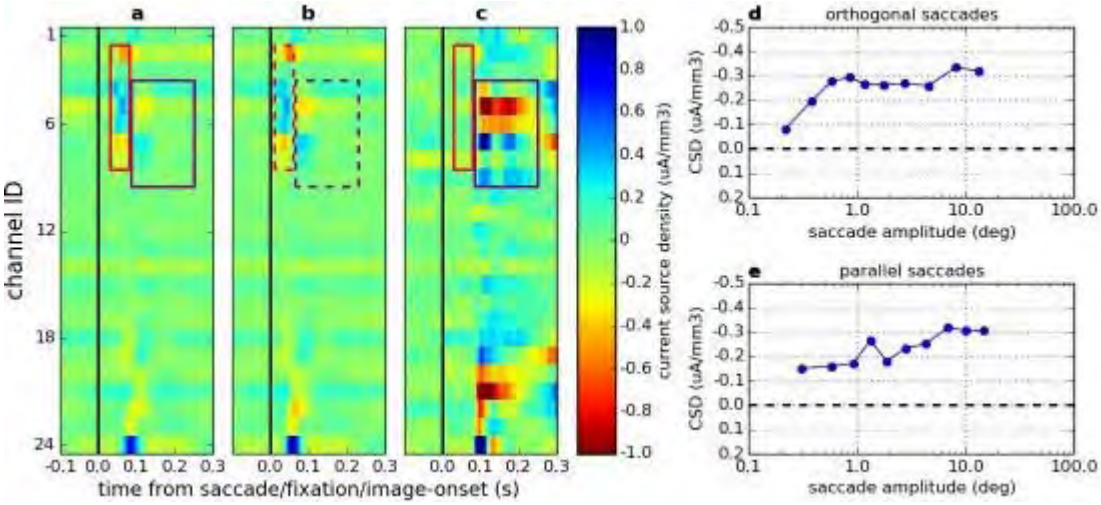

Spatial integration in mouse V1 is shaped by NMDA receptors in parvalbumin-positive interneurons

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In cortex, fast-spiking parvalbumin positive (PV+) interneurons represent the largest neuronal population mediating GABAergic inhibition. In primary visual cortex (V1), PV+ interneurons modulate the tuning properties of excitatory cells by contributing to selectivity for stimulus orientation, contrast and size. Like pyramidal cells, PV+ interneurons receive excitatory input through glutamate receptors, the disruption of which has been implicated in impairments of visual feature integration and figure-ground segregation (Self et al., 2012). Here we examined the impact of NMDA-glutamate receptor ablation in PV+ interneurons on visual information processing.

We generated mice lacking NMDA receptors in PV+ neurons by crossing PV-Cre mice with mice carrying floxed NR1 alleles (NR1-PVCre-/-; Korotkova et al., 2010; Carlen et al., 2012). We recorded V1 extracellular activity of head-fixed, transgenic and littermate control mice placed on a Styrofoam ball, where they could run or remain stationary. We assessed spatial integration by measuring size tuning curves with gratings of different diameters. We measured selectivity for orientation and contrast by presenting full-field gratings of different orientations and contrasts.

We first compared overall neural activity, and found that both baseline and peak firing rates were largely similar between genotypes. We next tested the role of NMDA receptors in PV+ interneurons for spatial integration. Compared to control mice, V1 cells in NR1-PVCre-/- mice had smaller receptive field center sizes and stronger surround suppression. This difference in spatial integration between mutant and wildtype mice persisted even after equating for differences in their locomotion behavior, such as time spent running and average running speed.

Since previous studies suggested that PV+ interneurons might shape spatial integration via contrast gain control mechanisms (Vaiceliunaite et al., 2013; Nienborg et al., 2013), we also investigated whether NMDA receptors in PV+ interneurons contributed to contrast sensitivity. Compared to control mice, V1 cells in NR1-PVCre-/- had higher sensitivity for stimulus contrast (i.e. lower semisaturation contrasts c50). We speculate that this higher sensitivity could, at least partly, mediate sharper tuning for stimulus size.

We conclude that NMDA transmission in PV+ interneurons shapes selectivity of neighboring pyramidal cells for stimulus size, possibly via mechanisms of contrast gain control.

The effects of pulvinar microstimulation on cortical BOLD activity in the behaving monkey

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The pulvinar nucleus, the largest thalamic complex in primates, is reciprocally connected with areas in frontal, parietal, and temporal cortex involved in visuomotor planning and execution of eye and hand movements. The combination of electrical microstimulation and functional magnetic resonance imaging (fMRI) has been shown to be a powerful technique to identify functional brain networks. Here we used unilateral electrical microstimulation and time-resolved event-related BOLD fMRI to map functional connections of dorsal and ventral pulvinar in a monkey performing a fixation and a memory-guided saccade task to left or right targets. The stimulation (biphasic 100-250 µA 200 ms 300 Hz trains, 1 train per s) was delivered during 10 s memory period or the corresponding fixation period and was interleaved with control trials without stimulation. Consistent with the anatomical connectivity, stimulation of both right dorsal and ventral pulvinar led to an increase in BOLD activity in a wide range of interconnected areas in the frontoparietal network (dlPFC, FEF, a45, a44, LIP, AIP) and along the superior temporal sulcus (MT, MST, FST, PGa, TPO/STP). In addition, stimulation of right ventral pulvinar strongly activated extrastriate visual cortex. The stimulation-induced enhancement was present in both the stimulated and the opposite hemisphere, suggesting a spread of activation not only via monosynaptic but also polysynaptic connections, but the effect was weaker in the opposite hemisphere. In most regions, the stimulation proportionally enhanced task-related fixation, cue and memory delay activity, such that relative amplitude differences between fixation, memory left and memory right conditions remained similar in stimulation and control trials. Interestingly, effects of dorsal pulvinar microstimulation on cortical BOLD activity were diminished in several frontal and temporal areas (FEF, FST, TPO) when the monkey was in the resting state (sleeping) compared to performing a task. In conclusion, both dorsal and ventral pulvinar seem to be functionally connected to cortical areas that are part of the visuospatial fronto-parieto-temporal bihemispheric network. These results set stage for further investigations of task and state dependency in thalamocortical communication using a combination of perturbation techniques (microstimulation, inactivation) and functional imaging.

The preserved ocular dominance plasticity in the visual cortex of enriched mice is heritable

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Ocular dominance (OD) plasticity in the mouse primary visual cortex (V1) declines with age in standard cage (SC) raised animals but can be preserved in mice raised in an enriched environment (EE)[1]. EE-raising provides the mice with more social interactions, voluntary physical exercise and cognitive stimulation compared to SC rearing. Motivated by a recent study showing that juvenile enrichment rescued a genetic defect in long-term potentiation also in the non-enriched offspring of the enriched mice[2], we here investigated whether the plasticity-promoting effect of EE could also be transferred from EE-parents to pups born and raised exclusively in SCs. To this end, mice were raised in EE. After mating, pregnant mothers were transferred to SCs few days before delivery (at postnatal day (PD) 7). Offspring were raised in SCs into adulthood (PD>120) and then OD-plasticity after monocular deprivation (MD) was visualized using intrinsic signal optical imaging. The offspring of EE-parents showed an OD-shift after 7 days of MD (ODI: 0.11±0.04) compared to mice without MD (ODI: 0.26±0.03; p=0.0026, t-test) or to mice with SC-raised parents. V1-activation after contralateral eye stimulation decreased from 2.37±0.14 for mice without MD to 1.85±0.24 for MD-mice, whereas V1-responses after ipsilateral eye stimulation did not change (noMD: 1.59±0.11, MD: 1.59±0.13 p=0.886, t-test).). Thus, the observed OD-shift was mediated by decreased deprived eye responses, a typical signature of “juvenile-like” plasticity, observed after 4 days of MD in 4-week-old SC raised mice or after 7 days of MD in adult EE mice. To summarize, the adult offspring of enriched parents still displayed a juvenile OD-plasticity in V1, even if they did not experience any EE. We hypothesize that this is due to epigenetic modifications that can be transmitted from either EE-mothers or EE-fathers or both to their non-enriched offspring.

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The retinotopic representation of the visual wulst in a laterally eyed bird, the zebra finch (*Taeniopygia guttatta*)

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The structure and function of the visual wulst, the avian homologue of the mammalian visual cortex, is not yet well investigated. In laterally eyed birds like the zebra finch, the foveae are "looking" into different directions, and the corresponding visual field of the contralateral eye has been shown to be represented in the visual wulst in one or more retinotopic maps (Keary et al 2010). There is also anatomical evidence for input from the ipsilateral eye, but its role is not clear. We here examine whether the most prominent and stable visual wulst map covers the entire visual field and whether the fovea is more strongly represented than the periphery, as was shown for the primary visual cortex (V1) of mammals. To evaluate a possible role of the ipsilateral input, we examined visual wulst activation patterns after stimulation of the ipsilateral eye and the interaction of ipsi- and contralateral stimulation.

To visualize wulst activation, we used autofluorescent flavoprotein imaging (Michael et al 2013). Stimuli were moving bars of 4° width presented on a monitor (75°x50°). Classical anatomical methods and optometry were used to investigate the retinal architecture of the bird.

Our experiments indicate that the wulst caudal topographic map spans only part of the visual field of the contralateral eye, reaching horizontally from -5° in the ipsilateral visual field (beyond the beak tip =0°) to ~ 130°. Vertically, we detected activity only in a small strip from ~ 10° below the horizon up to 25°. The highest neuronal activity was always obtained from ~ 60° of the visual field, the direction of the fovea. Accordingly, the retinal ganglion cell density was highest at the foveal region and decreased towards the periphery. However, in contrast to the mammalian cortex, the size of the foveal representation in the wulst was not significantly different from that of peripheral parts of the retinotopic map.

Stimulation of the foveal region of the ipsilateral eye caused a wulst activation that was not retinotopically organized and much smaller than that induced by the contralateral eye. The location of the ipsilaterally induced activity spot overlapped with that of the contralateral eye. With frontal stimulation of either the contra- or the ipsilateral eye, only stimulation of the contralateral and not the ipsilateral eye elicited measurable wulst activation. Interestingly, however, when both eyes were stimulated simultaneously, the resulting activation was reduced compared to monocular stimulation of the contralateral eye, indicating a net inhibitory influence of the ipsilateral eye on wulst activation via the contralateral eye.

We are not able as yet to interpret the only partial overlap of the activation patterns induced by stimulation of the ipsi- and contralateral foveal region. The inhibition of the contralaterally induced activation by ipsilateral stimulation indicates binocular interaction in the representation of the frontal visual field. The lack of an overrepresentation of the fovea suggests that object identification, which needs a fine grain resolution of the foveal area, may not be the main task of the visual wulst representation. Alternative interpretations are discussed.

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The Yin and Yang of cortical plasticity: the role of postsynaptic density proteins 93 and 95 for mouse vision and visual cortical plasticity

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Activity in the binocular region of the mouse primary visual cortex (V1) is dominated by input from the contralateral eye. Monocular deprivation (MD) of this eye results in an ocular dominance (OD) shift towards the open eye. During a critical period (CP) in early life of mammals, V1 is particularly susceptible to plastic changes: In C57Bl6/J mice, the CP opens around postnatal day (PD) 20 and closes around PD 35. While 4 days of MD are sufficient to induce plasticity during the CP, “adult” OD-plasticity up to an age of PD 110 needs 7 days of MD. Beyond PD 110, OD-plasticity is absent in standard cage raised mice[1].

We recently observed that knockout (KO) mice for postsynaptic density protein 95 (PSD-95) retain a lifelong and juvenile OD-plasticity and have 9x more AMPA-silent synapses in V1 than wildtype littermates (Löwel et al. 2013)[2]. In contrast, the percentage of silent synapses dropped from ~55% (PD 10-12) to almost 0% already during the critical period (P21-30) in PSD-93 KO mice, indicating that PSD-93 and PSD-95 might have opposing functions for cortical network maturation and stabilization (Favaro, 2014)[3].

We therefore tested whether OD-plasticity declines more rapidly in PSD-93 KO mice, in line with the reduced number of silent synapses. To this end, we visualized V1-activity after MD in 3-4-weeks-old PSD-93 KO mice using optical imaging of intrinsic signals[4]. Indeed, PSD-93 KO mice showed an earlier closure of the CP: While during mid-CP (PD 24-28), most PSD-93 KO animals showed an OD-shift after 4 days of MD (OD-index (ODI) declined from 0.36±0.04 to -0.06±0.09 after MD, n=5; $p<0.01$; unpaired t-test), OD-plasticity was already largely absent during late-CP (PD 29-33; ODI decreased from 0.33±0.02 to 0.22±0.04, n=4; $p<0.01$, unpaired-test), while WT mice of the same age still showed OD-plasticity. To test whether the KO of PSD-95/PSD-93 influenced visual perception, we analyzed the mice in the visual water task (VWT) developed by Prusky et al.[5], a visual discrimination task based on reinforcement learning. While visual acuity was normal for both PSD-95 KO and PSD-93 KO-mice, orientation discrimination was severely impaired in both genotypes: KO-mice needed more than double the orientation contrast for a correct behavioral decision compared to WT-animals. In conclusion, we link PSD-95 and PSD-93 function to experience-dependent maturation of silent synapses in the critical period, demonstrating a critical function of silent synapses in neural network refinement and presumably their conversion into transmitting synapses as the terminating event for critical periods.

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Poster Topic

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T17-3D The Role of G protein Ga_i Isoforms in Hearing in Adult Mice
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T17-4D Analysis of the otoferlin I515T mutation causing temperature dependent hearing loss
Ellen Reisinger, Hanan Al-Moyed, Tina Pangrsic Vilfan, Alexandra Müller, Gerhard Hoch, Nils Brose, Tobias Moser, Carolin Wichmann, Nicola Strenzke

T17-5D The Role of LRBA (Lipopolysaccharide-Responsive, Beige-Like Anchor Protein) in Auditory Function
Nicola Strenzke, Christian Vogl, Tanvi Butola, Tzu-Lun Wang, Michael Leitner, Natja Haag, Britta Qualmann, Michael Kessels, Tobias Moser, Dominik Oliver, Manfred Kilimann

Analysis of Stability and Degradation of Otoferlin at normal and elevated Temperature

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Otoferlin, a protein of the ferlin family like myoferlin and dysferlin consists of several C2 domains which are said to be important for binding of Ca^{2+} and phospholipids as well as protein-protein interactions. More than 60 pathogenic mutations are known so far and lead to nonsyndromic autosomal recessive deafness DFNB9. The mutations we are focusing on are I515T, G541S, R1607W and E1804del, all of them leading to temperature-sensitive hearing loss in humans. In these patients already at normal body temperature the hearing is impaired resulting in decreased word discrimination although hearing thresholds in pure tone audiograms are almost normal. At elevated body temperature patients become profoundly deaf. The reason for that is not understood yet. We generated knock-in mice having the otoferlin I515T mutation to study the cause of temperature dependent hearing loss in more detail. Otoferlin levels were ~50% lower in inner hair cells of mutant mice. It could be shown with RealTime PCR that at least for this mutation mRNA levels were not reduced compared to wild type otoferlin. We next analysed the melting temperature of an otoferlin fragment comprising the first three C2 domains. The protein with the I515T mutation unfolded at 1K lower temperature compared to the wild type protein. For checking whether otoferlin in its mutated form is degraded faster than the non-mutated form, we transfected HEK cells with a vector containing either normal otoferlin or with one of the mentioned mutations. To observe degradation of otoferlin cells were treated with Cycloheximide which is blocking the protein biosynthesis so that no more protein can be generated. Cells were incubated either at 37°C standing for normal body temperature or 38.5°C feigning risen body temperature or fever. At time points from zero minutes on until 2h samples were taken and processed. The samples were loaded on polyacrylamide gels and the otoferlin bands quantitatively analyzed using mass spectrometry. This served us to determine the half-life-time of otoferlin and the mentioned mutated forms. Our data indicate a faster degradation of mutated otoferlin at 38,5°C. We conclude that the mutations render otoferlin less stable, and that a minimal amount of otoferlin in inner hair cells is required to ensure hearing.

Auditory Tuning in an Insect Ear

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The auditory system of bushcrickets has been the subject of many studies dealing with either, the anatomy, the mechanics or the neuronal tuning of receptor cells and higher neuronal levels. However, the mechano-electrical transduction process within these animals, especially the mechanical versus neuronal tuning of the high-frequency hearing organ (*crista acustica*, CA) and its tuning mechanisms remain unknown. To investigate the acoustic signal transduction, *in vivo* experiments were carried out on bushcrickets of the tropical Southeast-Asian species *Mecopoda elongata* measuring the mechanical tuning of CA motion and neuronal tuning of sensory cells.

First of all, we analyzed the sound-induced mechanical displacement of the CA using laser-Doppler vibrometry and found that the CA is able to respond to a frequency range from about 4 up to about 80 kHz by the generation of traveling waves along the organ. The mechanical measurements of CA motion revealed a tonotopical representation of the applied stimulus frequencies with low frequencies represented proximally and high frequencies represented more distally on the CA. Since mechanical displacement amplitudes induced by frequencies below 30 kHz were generally larger in magnitude than those induced by frequencies above 30 kHz, even the distal region was highly deflected by the extensions of the low-frequency traveling waves. Thus, the most sensitive responses of the mechanical tuning as well as the highest amplitudes induced by all presented frequency-level-combinations were restricted to frequencies below 30 kHz for all regions along the CA.

In a second step, we determined the resulting sound-induced neuronal response performing intracellular recordings of sensory cell activity. Again, stimulus frequencies were tonotopically represented along the sensory cells of the CA. However, distal cells did not respond to the low-frequency induced motion, but to their tonotopically assigned high-frequency induced traveling waves. Therefore, intracellular recordings of the distal cells revealed neuronal activity up to at least 60 kHz with most sensitive responses up to about 50 kHz. Comparing the mechanical and neuronal tuning along the CA, normalized and averaged mechanical frequency-tuning curves (FTCs) were tuned less sharply (~6 to 30 dB/oct from the proximal to the distal region) than the neuronal FTCs (~21 to 90 dB/oct from the proximal to the distal region). In further experiments, we examined the ions that are involved in acoustic signal transduction by applying certain channel blockers (e.g. TTX, TEAC, 4-AP, Isradipin, $CdCl_2$). These experiments revealed a significant role of voltage-dependent sodium and potassium channels in the acoustic signal transduction process, whereas calcium channel blockers did not influence the spiking process of *M. elongata*.

In summary, neuronal activity in the distal region of the CA is not directly caused by mechanical displacement magnitude. Thus, the distal region might be influenced by additional intrinsic tuning mechanisms that go beyond a simple signal transduction of the mechanical stimulus.

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BK Channels Are Not The Target Of The NO-cGMP Signaling Cascade In Mouse Inner Hair Cells

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Introduction

The big conductance, voltage and Ca^{2+}-activated K^+ (BK) channel is widely expressed and serves many functions, e.g. controlling smooth muscle tone and neuronal excitability. In mature mammalian inner hair cells (IHCs), BK channels underlie the fast-activating outward K^+ current, $I_{K,f}$, which is responsible for fast repolarization of the receptor potential and for the small time constant of the IHC. Recently, the NO-cGMP signaling pathway involving cGMP-dependent protein kinase type I (cGKI) has been found to protect IHCs from noise trauma (Jaumann et al., Nature Medicine 2012) but the targets of cGKI in IHCs are unknown. In smooth muscle cells, elevated cGMP levels activate cGKI, which phosphorylates and activates BK channels, thereby counteracting cellular depolarization (Lu et al., 1998). We therefore analyzed whether cGMP increases whole-cell BK currents in mature IHCs in a similar way.

Methods

Patch-clamp recordings of BK currents were performed on whole-mounts of the organ of Corti dissected from mature mice (P20). The patch-pipette contained the non-hydrolyzable analogue 8-bromo-cGMP. Because of an intrinsic Ca^{2+} dependence of PKGI, three intracellular free Ca^{2+} concentrations ($[Ca^{2+}]_i$), 1.8 nM, 22 nM and 209 nM, were used in the pipette. Further, the subcellular distribution of BK channels were analyzed using immunofluorescence labeling and image acquisition with two different excitation intensities.

Results

BK currents were isolated by their fast activation kinetics at 1.2 – 1.3 ms after depolarization, when other voltage-activated K^+ channels had not yet opened. Effects of 3 µM 8-bromo-cGMP on IHC BK current amplitudes and activation kinetics were subtle and showed a non-monotonic dependence on $[Ca^{2+}]_i$. Biophysical parameters such as $V_{1/2}$ and steepness obtained from fitting I-V curves to the product of a Boltzmann function times driving force were unaltered at the three $[Ca^{2+}]_i$ concentrations. Immunolabeling of BK channels revealed the known large BK channel clusters at the IHC neck and a smaller pool of BK-positive puncta at the synaptic pole of the IHCs.

Conclusion

Taken together, BK channels are not a major target of the NO-cGMP-cGKI pathway in IHCs. The subcellular distribution of BK channels suggest that the two subpopulations may follow different activation

mechanisms, which remains to be determined.

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Chronic radiofrequency exposure alters glycine receptor immunoreactivity in the mice auditory brainstem complex at SAR 1.6W/kg.

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Exponential increment of mobile usage has triggered the interest of possible effects on the regulation of neurotransmitter signals. Due to its close proximity during usage, there is a possibility that it could lead to a decrease in the ability to segregate sounds, leading to serious auditory dysfunction by long time exposure of radiofrequency (RF) irradiation. To interplay auditory processing, excitation and inhibition molecules interactions play a major role. Especially inhibitory molecule such a glycine is dominantly localized in the auditory brainstem. However, effect of RF exposure in the auditory function has not been reported till date. Thus, the present study investigated the effect of RF exposure on Glycine receptor (GlyR) immunoreactivity in the auditory brainstem region at 835 MHz with specific absorption rate 4.0W/kg for three months by using free floating immunohistochemistry. Compared with sham control (SC) group, significant loss of staining intensity of the neuropil as well as cells in the different subdivisions of the auditory brainstem regions were observed in the exposed (E4). A decrease in the number of GlyR immunoreactive cells was also noted in the cochlear nuclear complex (AVCN-31.09%, DCN-14.08%, PVCN-32.79%) and superior olivary complex (LSO-36.85%, SPN-24.33%, MSO-23.23%, MNTB-10.15%) regions of E4. ABR analysis performed also revealed significant elevation of threshold in the E4 group, which may implicate the auditory dysfunction. The present study suggests susceptibility of the auditory brainstem region to chronic RF exposure that might affect the function of central auditory system.

Electron microscopic study of assembly, maturation and heterogeneity of inner hair cell ribbon synapses

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Abstract

Hearing in mammals requires temporally precise and reliable transmission of sensory information mediated by inner hair cell (IHC) ribbon synapses. Synaptic ribbons are electron dense structures that tether numerous synaptic vesicles (SVs) and transmit signals by Ca^{2+}-dependent exocytosis of glutamate at the presynaptic active zone (AZ) to spiral ganglion neurons (SGNs).

Before onset of hearing, around postnatal day 12 (p12), IHCs fire spontaneous Ca^{2+}-dependent action potentials, while mature IHCs generate graded receptor potentials in response to sound stimuli. This stimulation of the auditory pathway in the immature stage is supposed to be essential for normal ribbon development (Johnson et al., 2013). During maturation IHC ribbons undergo several morphological changes: while they increase in size, their number decreases. Moreover, the ribbon appearance strikingly changes from round before onset of hearing to predominantly oval after hearing onset and ribbons become more connected to the AZ (Wong et al., 2014). The mechanisms underlying these changes are largely unknown.

To approach this question, we studied different developmental stages of IHCs before and after onset of hearing. Using electron microscopy, pre-embedding immunogold labelings and immunofluorescence microscopy we investigated five different age groups of wild type mice: p7-9 (immature), p12 (onset of hearing), p14-16 (short after onset of hearing), p19-21 (mature), p48-50 (older mature stage). Here, we will present changes in parameters such as size and number of SVs, number and size of ribbons and the postsynaptic density (PSD) length in each age group. Moreover, we are interested in the ultrastructural localization of AZ proteins such as piccolino or bassoon and how they rearrange during the maturation process.

Finally we will present first 3D reconstructions using serial block face scanning electron microscopy to systematically correlate synapse position within one IHC to attributes like ribbon and PSD size, number of SVs as well as SGN fiber diameter.

This way our study significantly contributes to the understanding of maturation processes and ribbon synapse heterogeneity within murine IHCs.

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Gender differences in the hearing organ of the bushcricket *Ancylecha fenestrata*

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All tibiae of bushcrickets (katydids) contain auditory organs. However, only the organs of the forelegs are sensitive to airborne sound. The sensory cells of the high-frequency hearing organ, called crista acustica, are tonotopically arranged. Low frequencies are represented in the proximal part, while high frequencies are represented in the distal part. This poster presents results of our study about gender differences in the morphology of the *crista acustica* of *Ancylecha fenestrata* (Ensifera – Tettigonioidea).

A. fenestrata were reared in first generation from commercially obtained larvae. For morphological analysis of the *crista acustica*, the tibiae of the forelegs were separated and perfused for fixation. After dehydration, the legs were embedded in araldite. Using a microtome (Leica Reichert-Jung Ultracut S) the tibiae were cut in 4 µm thick slices and stained with toluidine blue. Every slice that contains a sensory unit was photographed (Zeiss AxioPhot & ProgRes© C5) and different parameters, including the length of the dorsal wall and the area of the cap cells, were measured (ProgRes© CapturePro 2.5).

In comparison to other known bushcricket species, *A. fenestrata* possess an unusual high number of sensory cells in the *crista acustica*; 110-114 in males (n = 5) and 86-88 (n = 2) in females. The total length of the *crista acustica* reaches a maximum of 2 mm in males and 1.6 mm in females, despite the fact, that females are bigger in body size. The shape of the *crista acustica* is tapering along the proximo-distal axis. Both, males and females feature a decrease of the area of the cap cells in distal direction. In males this decrease is less prominent in the medial region of the *crista acustica*. In contrast, females exhibit nearly constant values for the cap cell area in the most distal region. Assuming that the size of the cap cells is essential for the generation of sound-induced responses, these regions with a constant area value might cause a disproportionate frequency representation in the *crista acustica*, which has to be tested by mechanical and neuronal investigations. Therefore the question arises, if there is a relationship between the found morphological gender differences in the *crista acustica* and the perception of the different sounds. First studies using laser Doppler vibrometry and intracellular recordings of sensory cells activity aim to answer this question.

We like to thank Manfred Kössl for technical support. This work is supported by the DFG (NO 841/1-2).

How Private is Private - Detection of Foreign Species Signals in Weakly Electric Fish *Apteronotus leptorhynchus*

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Communication in species-specific “private” communication channels, e.g. distinct frequency bands, is very common. For instance, different cricket species produce acoustic communication signals in distinct, non-overlapping frequency bands. A similar strategy is observed in weakly electric fish. These animals are nocturnal hunters that rely on a self-generated, oscillating electric field for prey detection, navigation, and communication. Cutaneous electroreceptors of the active electrosensory system are tuned to the oscillatory frequency of the electric organ discharge (EOD) and encode amplitude modulations of the field in their firing rate. Each individual fish has its own EOD frequency and each species of Gymnotiform weakly-electric fish covers a certain frequency band. Therefore, it is commonly assumed that the tuning properties of the electroreceptors establish a “private”, species-specific information channel in which individuals are undisturbed by the fields of other weakly-electric fish species in the same habitat.

We test this hypothesis experimentally by a combined electrophysiological, modeling, and behavioral approach. We record from electroreceptor afferents while stimulating with simulated fields of other species. We find that P-unit electroreceptors show phase locking not only to the frequency of the own field, but also to those of foreign fish whose EOD frequencies are far off the optimal tuning of the recorded fish’s receptors. This means that information about the presence of a foreign fish is encoded in these afferents. By means of an integrate-and-fire model that faithfully reproduces P-unit spiking activity we investigate the mechanisms and requirements for this unexpected response. In behavioral two-alternative-forced-choice experiments we test whether frequencies far off the optimal tuning of electroreceptors are actually used for behavioral decisions. Our electrophysiological data together with the behavioral experiments potentially indicate a so far neglected coding regime of the electrosensory system that also challenges the idea of a private communication channel of wave-type electric fish.

Investigation of long-term depression in the medial nucleus of trapezoid body-lateral superior olive synapses of developing circling mice

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The circling mouse, a spontaneous mutant with autosomal recessive inheritance is a model for congenital human deafness. In the present study, long term depression (LTD) and its involvement in synaptic silencing and strengthening at the medial nucleus of trapezoid body (MNTB) – lateral superior olive (LSO) synapses was investigated using circling mice. High frequency stimulation (10 Hz for 50 seconds) of MNTB inputs induced LTD of whole inhibitory postsynaptic currents (IPSCs) recorded at LSO neurons in P0~P3 mice which were: 59.03 ± 7.3 % (n = 7) in homozygous (cir/cir) mice and 58.35 ± 4.12 % (n = 9) in heterozygous (+/cir) mice. The magnitude of LTD declined dramatically to 32.18 ± 5.8 % in P8 ~ P12 heterozygous (+/cir) mice (n = 8), but was maintained in P8 ~P12 homozygous (cir/cir) mice (47.22 ± 1.0 %, n = 7). As the dominant transmitter of MNTB-LSO synapses is glycine in heterozygous (+/cir) mice and glutamate in homozygous (cir/cir) mice, I also recorded glycinergic or glutamatergic LTD. The glycinergic LTD declined age-dependently in both heterozygous (+/cir) mice and homozygous (cir/cir) mice. The extent of LTD in heterozygous (+/cir) mice was 46.52 ± 10.9 % (n = 5) in P0~P3 and 14.89 ± 5.29 % (n = 5) in P8~P12, while in homozygous (cir/cir) mice they were 42.34 ± 6.4 % (n = 7) in P0~P3 and 15.15 ± 4.0 % in P8~P12. The glutamatergic LTD in homozygous (cir/cir) mice showed a persisting LTD until P12, 44.87 ± 8.3 % in P0~P3 (n = 9) and 43.12 ± 7.8 % P8~P12 (n = 6), similar to that observed in whole IPSCs. This explained the maintenance of LTD in homozygous (cir/cir) mice and decline in heterozygous (+/cir) mice. Age-dependent decrease in convergence ratio of MNTB axons to a single LSO neuron was observed only in heterozygous (+/cir) mice, but not in homozygous (cir/cir) mice. The corresponding change of LTD with convergence ratio suggests that activity-dependent LTD might be involved in synaptic silencing of MNTB-LSO synapses.

Measuring single cell responses in the *Drosophila* hearing organ with two-photon microscopy

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Drosophila melanogaster uses its antennal ear to sense sound and wind.
These mechanical stimuli are transduced by the 500 neurons of the Johnston's organ (JO) which can be classified into wind- and sound-sensitive subgroups.
The transduction in these neurons relies on force-gated ion channels and is actively amplified, tuning the organ towards the frequency spectrum of the fly's courtship song. The transient receptor potential (TRP)channels NOMPC, Nanchung and Inactive are known to be involved in this process, but their specific role remains controversial.
To probe the function of these channels and other proteins involved in the transduction machinery, we are using two-photon calcium- and voltage-imaging. The optical sectioning of two-photon excitation allows single-cell resolution, enabling us to measure the response of single sensory neurons in the JO.
Imaging in wildtype flies shows diverse response characteristics of JO neurons also within the wind- and sound-sensitive subgroups, which could not be resolved with the population imaging and recording methods used in earlier studies.
By comparing the single-cell response of mutant flies, we can now investigate the influence of transduction-related proteins on the function of individual neurons.

Natural scenes of electrocommunication and their implications for sensory processing

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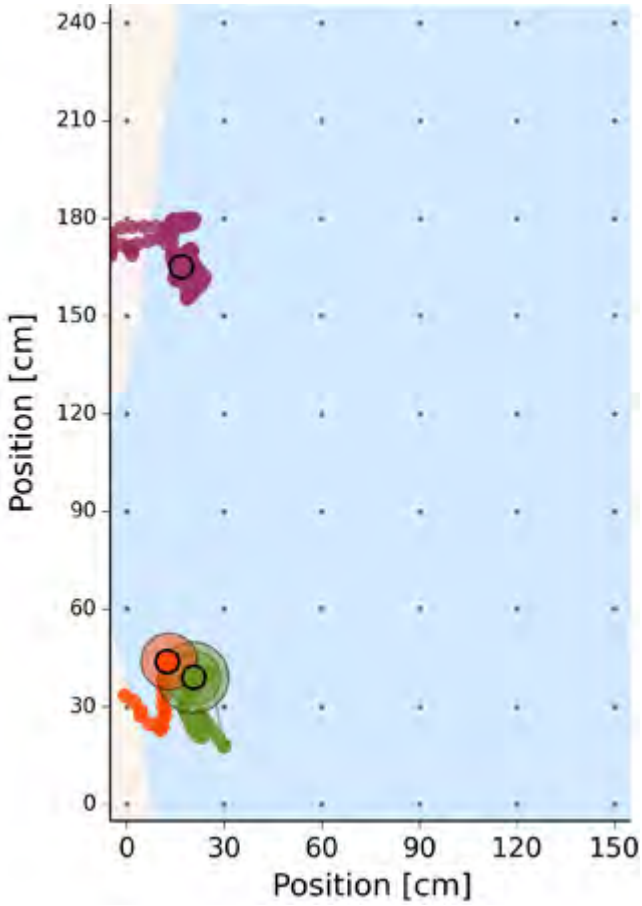
Sensory systems evolved to analyze behaviorally relevant natural scenes. Knowledge of these scenes and of the related natural stimulus statistics are a key requirement for understanding and correctly interpreting the functioning of neural sensory circuits (Lewicki et al, 2014). Difficult scenes, i.e. of low signal-to-noise ratio or with ambiguities, challenge sensory systems and in particular expose the complexity of neural processing. Field studies play a central role in identifying such challenging situations that then can be used in the lab for detailed electrophysiological studies.

The electric sense of the gymnotiform electric fish *Apteronotus leptorhynchus* is a successful model system in research on neural computations underlying both localization and, more recently, communication behavior (Sawtell et al, 2005). In order to assess the statistics of natural electrocommunication signals of these fish, we developed a novel, multi-electrode-based method for long-term monitoring of movements and communication behavior of populations of weakly electric fish (Figure). We applied this method in the fish's natural habitat in the Panamanian rainforest during the fish's reproductive season. In the recorded data we identified scenes with relevant social interactions between fish and analyzed the temporal and spatial structure of their communication interactions. In particular we measured critical distances of distinct behaviors and related them to the performance of the fish's electrosensory system. We found temporally highly stereotyped short-distance communication between individuals suggestive of reproductive behavior. The fastest responses to a communication partner's electric signals occurred within 40 ms, providing hard constraints for the timescale of sensory-motor integration. While most of the observed communication occurs on short distances implying relatively strong signals, resident-intruder interactions clearly show that detection of familiar conspecifics is possible over distances of up to at least 170 cm, demonstrating that the fish are able to process very small signals. In addition, we observed many interactions at frequencies that are much higher than the electroreceptor's preferred frequency. Our observations thus demonstrate that electrocommunication in *Apteronotus* challenges the limits of electrosensory processing. Electrophysiological studies so far completely neglected these regimes of difficult stimulus frequencies and amplitudes.

The figure shows a single snapshot of the study's recording area in Panama with the current and previous locations of three concurrently present *Apteronotus* (colored dots) and their respective communication signals (large circles). The positions of the recording electrodes are indicated with by dots. Two of the fish are engaged in dyadic communication, while a third fish holds position at a stable distance to the pair.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Neural basis of airborne vibratory signal processing of the honeybee *Apis mellifera*

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Honeybees can inform their nest-mates about the location of food sources by means of the waggle dance, and recruit them to nearby food sources. During the waggle dance the dancer generates airborne vibratory signals by wing beats, which is thought to be a key component of signal transmission. The followers (receivers of the waggle dance) detect air-particle movements with Johnston's organ (JO) in the pedicel as mechanical vibration of the flagellum. Sensory neurons of the JO project to the dorsal lobe (DL), the posterior protocerebral lobe (PPL) and dorsal part of the subesophageal ganglion (SEG). The dorsal lobe is a brain region where the other mechanosensory neurons on the antenna also project, therefore it is considered as the primary center of mechanosensory signal processing.
To investigate the neural mechanisms underlying vibratory signal processing in the honeybee brain, we identified antenna-vibration sensitive neurons using intracellular recording and staining technique. Among 55 types of vibration sensitive neurons 53 neurons had their dendrites in the DL and 25 neurons had dendrites in the SEG. The main output regions of these neurons were the lateral protocerebrum (LP) and the DL (25 and 23 neurons, respectively). These neurons responded to 50-400Hz vibration stimuli, which covers the frequency range of vibration pulses generated during the waggle dance. Furthermore, when pulse vibrations mimicking the dance signals were applied to the antenna, spike activities of a population of these neurons corresponded to the pulse pattern. These results suggest that the LP is the higher center of vibratory signal processing that encodes the temporal pattern of pulse vibration during the waggle dance.

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Optical stimulation of spiral ganglion neurons in vitro for laser pulse durations in the µs to ms range

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It has been shown that the inner ear can be stimulated in vivo by laser pulses resulting in cochlear potentials corresponding to auditory evoked signals [1]. Optical stimulation can be very site specific which coincides, due to the tonotopy of the cochlea, with a very frequency specific stimulation, possibly overcoming the limitations of conventional hearing aids as well as of electrical cochlea implants. To investigate the basic effects and mechanisms of the optical stimulation of the cochlea, single cell measurements were performed for different laser parameters.

Spiral ganglion neurons, isolated from the cochleae of P3 - P6 Sprague Dawley rats, were stimulated with laser pulses. Their electrophysiological reactions to different laser parameters such as pulse duration and optical peak power were detected by means of the whole cell patch clamp technique. Pulse durations from 10 µs up to 20 ms and optical peak powers from 50 mW up to 500 mW were investigated. Additionally, the corresponding temperature change was analyzed.

The irradiated cells show inward currents at resting potential, depending linearly on the peak power of the laser light. These reactions are clearly elicited by the laser beam and can be observed in voltage clamp measurements as current changes. For pulse durations of at least 200 µs the reaction saturates for all peak powers. For shorter pulse durations the cell reaction decreases. Corresponding current clamp measurements show only slight depolarizations of the membrane potential which are not sufficient to generate action potentials. The laser-induced temperature change depends highly on the employed laser parameters, namely pulse duration and optical peak power whereas the cellular response seems to depend mainly on the optical peak power, provided that the pulse duration is long enough.

The results show that the thermal effects of laser irradiation with pulse durations in the µs to ms range lead to cellular responses, but do not suffice to generate action potentials. In combination with in vivo experiments demonstrating positive stimulation results, performed with similar laser parameters, this suggests that direct stimulation of spiral ganglion neurons is not the main mechanism of optical cochlear stimulation. The results rather support the theory that the optical stimulation of the cochlea is based on an optoacoustic effect.

Support provided by the Seventh Framework Programme for Research (FP7) of the European Union, research project ACTION.

[1] Wenzel et al. Green laser light activates the inner ear. J. Biomed. Opt. 144, 044007 (2009)

Phonotactic behaviour of the cicada *Okanagana rimosa* (Cicadidae, Homoptera)

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Hearing plays an important role in several behavioural contexts. The sense of hearing is among others involved in intraspecific communication like mate attraction. Several groups of insects use this way of communication. For example the majority of male cicadas produce a species-specific calling song, whereby female cicadas perform phonotaxis towards these sounds.
In comparison to the male behaviour, much less is known about female phonotaxis. How do female cicadas locate their partners when they hear the specific calling song? Here we performed experiments of acoustic localization in the field with the cicada *Okanagana rimosa*, which is common in the northeast of USA and the south of Canada. Female cicadas have been acoustically attracted with the species-specific calling song to an arena.
In the arena the phonotacic threshold has been determined. The behavioural threshold has been determined with model songs containing different carrier frequencies (5 – 12 kHz), but with the time pattern of the calling song. The most sensitive response of female cicadas is at 7 – 9 kHz with a threshold of 68 dB SPL, matching the peak carrier frequency of the calling song.
Furthermore, the phonotactic localisation ability for elevated sound source positions was investigated. Several behavioural parameters like the distance to the target were analysed. Female *O. rimosa* landed most often above the loudspeaker with a mean distance of 44.5 cm ± 28.16 (SD; n= 68).
The data show, that female *O. rimosa* are adapted to the calling song of the males and are able to precisely locate a sound source in three dimensional habitats.

Phonotactic flight behaviour and vertical sound source localization of the parasitoid fly *Emblemasoma auditrix* (Diptera: Sarcophagidae)

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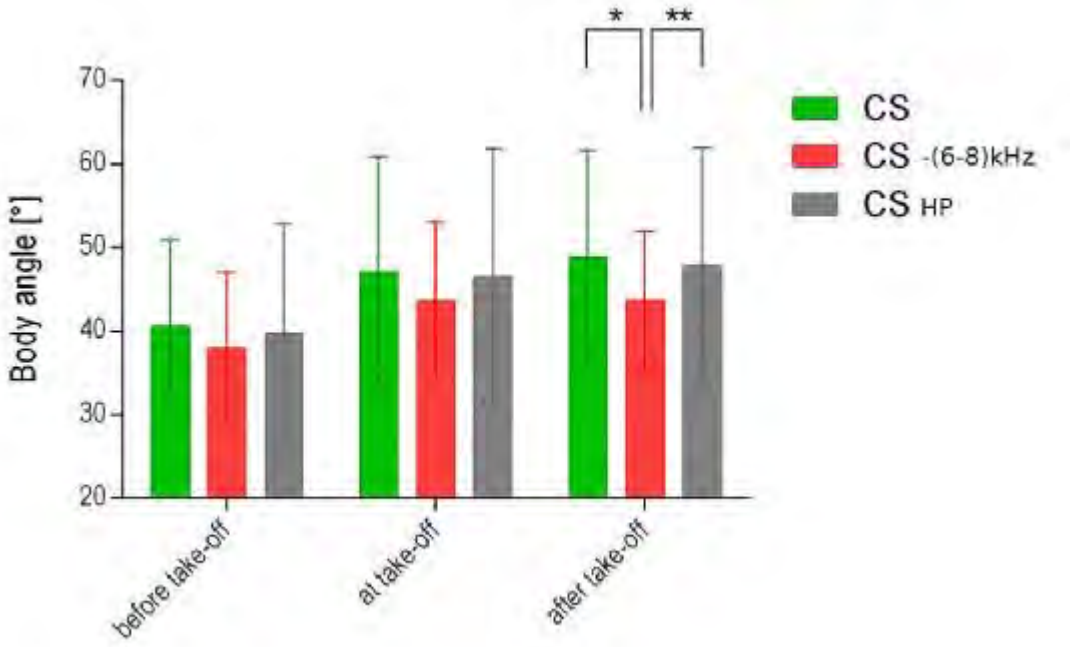
Acoustically directed movement in the three dimensional space is a complex task performed notably by birds, bats and insect species. The precision of acoustic orientation depends on directionality of the hearing system as well as on the auditory behaviour. The fly *Emblemasoma auditrix* is a parasitoid of the cicada *Okanagana rimosa* and locates its host in the complex habitat of a forest. In earlier experiments we could document that the flies are able to locate the sound source at start. The flies seem to determine the azimuth first and then they adjust to the elevation of the acoustic target (detection phase) before starting the phonotactic flight. The phonotactic flight is predominately two-dimensional. We performed experiments to analyse the detection phase and the flight phase.
For the detection phase a hypothesis is put forward, that frequency composition of the signal is important for orientation to elevated sound sources. Therefore we manipulated the frequency content of the attractive signal, the calling song of the host cicada *O. rimosa*. In field experiments the manipulated signals were broadcasted from a loudspeaker with different elevation and the behaviour of a fly was videotaped. Different behavioural parameters, like the orientation of the longitudinal body axis in respect to the acoustical target and the signal were analysed.
The acoustic localisation of the sound source is rather accurate and correlates with the inclination of sound. Interestingly, manipulation of the frequency content may lead to different body orientations (Fig. 1). This indicates that a frequency filter function serves as basis for detection of the elevation of a sound source.
Further experiments show the typical flight curves towards to acoustic target, with possible paths corrections during the flight.
In summary, *E. auditrix* is able to locate a sound source in the vertical axis and the frequency content may be an important parameter for correct orientation.

Figure 1: Longitudinal body axis angle at the start and sound incidence of 60°; the body angle was measured with video analysis for three steps: at one video frame before take-off, the angle at take-off and the angle at one frame after take-off. Tested were three different song models: CS = original calling song of *O. rimosa* (n=46); CS –(6-8)kHz = band pass filtered original calling song (6kHz to 8kHz reduced by 40dB (n=31); CS HP = high passed original calling song (9kHz cut off frequency (n=20). Kolmorogov-Smirnov t-tests: CS vs. CS –(6-8)kHz: P=0.0390; CS HP vs. CS –(6-8)kHz: P=0.0025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50
40
30
20
Body angle [°]
CS
CS -(6-8)kHz
CS HP
before take-off
at take-off
after take-off

Preferences for acoustic signals and decision making by female crickets *Gryllus bimaculatus* in choice situations

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Acoustic communication serves mate selection and pair formation on the basis of song signals. Male *Gryllus bimaculatus* produce calling songs made up from a series of pulses grouped into chirps and female crickets use these songs to localize the potential mating partner and to approach the singing male by phonotaxis. In the field, female crickets are confronted with several males singing at the same time at different distances which results in varying intensities of the songs at the position of the female. Thus a female has to make a decision and to choose the best male in the population by evaluating several available cues of the males' song such as the temporal pattern or intensity. Here we examined on which cues the decision of the female is based and how these cues are weighted in a choice paradigm.

We performed choice experiments during which two songs with different temporal pattern and/or sound intensities were presented from different speakers in a simultaneous or alternating fashion. The songs were played back at the same intensity or with a difference of 6dB. Furthermore we developed a simple balance model which assumes that females evaluate the songs based on quality of the temporal pattern and sound intensity. With the help of this model we predicted female preferences in a given choice situation and compared them with the preferences of the females in our experiments.

The results revealed that females were able to choose between two songs and that incoming signals seemed to be represented separately in the nervous system of the cricket. The simultaneous and the alternating playback led to similar results. Both temporal and intensity cues were reliable predictors of female decisions. For sound intensity not only the sound pressure level had to be taken into account but also the respective energy of the given signal. Our balance model demonstrated that the decision of a female can be explained by a simple weighting process for pattern quality and intensity.

Temporal structure of electrocommunication signals in the wave-type electric fish *Apteronotus leptorhynchus*

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Information content in animal communication is not only conveyed by different types of signals but also in their temporal structure. Here, we analyze the temporal statistics of electrocommunication signals of the Gymnotiform fish *Apteronotus leptorhynchus*. So called "type-2 chirps" are brief (15ms) increases of the frequency of the fish's electric organ discharge (EOD). They are also the prevailing signals during agonistic male-male encounters. However, the behavioral meaning of type-2 chirps still remains elusive: some studies classify them as aggressive signals, whereas others argue that type-2 chirps are used to avoid aggressive encounters, or may even be used as courtship signals. Creating mathematical models able to reproduce complex patterns of these communication signals can be helpful to quantitatively investigate the different theories.

While previous studies on this topic have examined the temporal structure of chirps between two conspecifics, a statistical baseline for the temporal patterning of chirps by a single individual is missing. In the present work we analyze the temporal structure of chirps produced by a fish who is stimulated with an oscillating electric field of constant frequency, mimicking the presence of a second, non-chirping individual. We find that the inter-chirp-interval (ICI) distribution (1) is scaled by the difference between the fish's own and the stimulation frequency (the beat frequency), and that (2) consecutive ICIs are positively correlated. We demonstrate that the statistical structure of two consecutive ICIs can be well approximated by a multivariate Log-Normal-Distribution whose scale deterministically depends on the beat frequency.

This model reveals previously unknown characteristics of the temporal structure of chirp production in *Apteronotus leptorhynchus* and can be used for more realistic playback stimuli in future behavioral and electrophysiological experiments.

The role of Ca^{2+} binding protein 2 (CaBP2) in synaptic sound encoding and hearing

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Auditory signal transmission to the brain is accomplished by synapses of inner hair cells (IHC) and with spiral ganglion neurons. At IHC ribbon synapses voltage gated Ca2+ channels translate membrane depolarization into Ca2+ influx, inducing vesicle fusion and transmitter release. In order to respond appropriately to receptor potentials, $Ca_V1.3$ Ca^{2+} channels activate at low membrane potentials and show weak inactivation kinetics. Proteins known to stabilize $Ca_V1.X$ channel openings are Ca2+ binding proteins (CaBPs), a family of EF-hand Ca^{2+} binding proteins. CaBP2 has been suggested to be crucial for faithful sound encoding, since a missense mutation in CaBP2, causing the truncation of the protein, leads to autosomal recessive non-syndromic hearing impairment DFNB93 (Schrauwen et al., 2011). In the present study we investigated the role of CaBP2 in $Ca_V1.3$ channel regulation and sound encoding. To identify the regulation of the biophysical properties of the $Ca_V1.3$ Ca^{2+} channel by CaBP2 we apply patch clamp recordings of transiently transfected HEK 293/SK3-1 cells co-transfected with either wt or mutant CaBP2. Furthermore a CaBP2 knock out (ko) mouse model was generated to investigate the function of CaBP2 in the auditory pathway. We used auditory brainstem recordings (ABR) to measure the auditory performance of the ko. To investigate synaptic transmission between IHCs and auditory nerve fibers (ANF) we performed patch-clamp recordings of IHC Ca^{2+} current and exocytosis of two different age groups and initiated extracellular recordings from single spiral ganglion neurons. Since the CaBP2 mutation induces moderate-to-severe hearing impairment, otocyst injections with an adeno-associated virus coding for CaBP2 are being performed to check whether the hearing impairment phenotype can be rescued.

The Role of G protein Ga_i Isoforms in Hearing in Adult Mice

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In developing mammalian cochlea, the migration of the primary cilium formed at the apical surface of the hair cells and the maintenance of the orientation of the developing stereocilia were shown regulated by heterotrimeric Gi-protein-dependent signaling. However, how these developmental steps affect the hearing function in adults is not well understood. In this study, mice with the deletion of heterotrimeric G protein Gai isoforms, i.e. Ga_i2 and Ga_i3, were generated and the auditory function, the cochlear, and the neuronal phenotypes of these mice were investigated.

We analyzed and compared the auditory function by measuring auditory brainstem response (ABR) and distortion product otoacoustic emissions (DPOAE) between the Ga_i KO and the control mice. The results are discussed in the context of the role of G-protein-dependent signaling and auditory function during hair cell development.

Analysis of the otoferlin I515T mutation causing temperature dependent hearing loss

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Sound waves elicit a depolarization of inner hair cells, the sensory cells of the inner ear. Ca^{2+} influx through voltage gated ion channels at the active zones of inner hair cells triggers fusion of neurotransmitter filled synaptic vesicles with the plasma membrane, a process that depends on otoferlin. Mutations in otoferlin lead to profound deafness, or, in rare cases, to mild hearing impairment at normal body temperature but profound hearing loss at elevated body temperature. In absence of otoferlin, Ca^{2+} triggered exocytosis is nearly abolished, suggesting a direct involvement in a late step of exocytosis. Studies on the deaf pachanga mouse line revealed an additional role for otoferlin in vesicle replenishment. Here, we generated a mouse line with a knock in of the I515T mutation in otoferlin causing temperature dependent hearing loss in humans. Auditory brainstem response thresholds in these mice were moderately elevated. ABR wave I only showed a mild linear decrease in amplitude upon local heating of the temporal bone. Using immunofluorescence, we found a 50% reduction in otoferlin levels in mutant inner hair cells. Patch clamp electrophysiological recordings revealed normal exocytosis for short (up to 20 ms) depolarization pulses. Sustained exocytosis, elicited by depolarization to -14 mV for 50 ms or longer, was significantly reduced, denoting a defect in vesicle replenishment and/or active zone clearance. At elevated temperature (~39°C), we found no difference in vesicle fusion rates for mutant and wild type inner hair cells, yet recovery of exocytosis at room temperature after this heat shock was impaired in mutants. Together with in vivo-electrophysiological and behavioral analysis of hearing impairment performed in the group of N. Strenzke and electron microscopy of inner hair cells synapses from the lab of C. Wichmann, our data confirm a role for otoferlin in vesicle replenishment and provide insight into the mechanism of fever induced hearing loss.

The Role of LRBA (Lipopolysaccharide-Responsive, Beige-Like Anchor Protein) in Auditory Function

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LRBA (LPS-responsive, beige-like anchor protein) belongs to the enigmatic BEACH protein family, members of which were shown to localize to subcellular membranous compartments and to participate in intracellular protein trafficking and pre- and postsynaptic function. LRBA is highly expressed in cochlear inner (IHCs) and outer hair cells (OHCs) and LRBA knockout mice show severe early-onset, progressive hearing impairment.

LRBA knockout mice had significantly elevated ABR thresholds alongside reduced ABR Wave I amplitudes and severely attenuated DPOAEs and cochlear microphonic and summating potentials. These findings indicate a major impairment of cochlear amplification, potentially aggravated by additional deficits in sensory receptor function. Electrophysiological recordings of prestin-driven changes in non-linear capacitance in OHCs indicated unchanged depolarization-induced electromotility in LRBA mutants. Moreover, IHC presynaptic calcium currents and exocytosis and suprathreshold auditory nerve fiber responses remained unaltered despite of LRBA loss. However, morphological studies revealed severe defects in hair bundle morphology accompanied by reduced FM1-43 dye uptake through stereociliary mechanotransduction channels. Finally, we show that LRBA localizes to the kinocilium basal body complex during development, where it might contribute to hair bundle anchorage and/or maturation.

Our data suggest a role for LRBA in active cochlear amplification but argue against a contribution to OHC electromotility or IHC presynaptic function. Rather, our findings suggest a major developmental defect in the formation and maturation of stereocilia, compatible with a role of LRBA in intracellular protein trafficking.

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Action potential conduction velocity in low- and high frequency globular bushy cell axons measured in vivo

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The timing of inputs in microcircuits plays a crucial role in information processing. The relative arrival time of inputs is largely determined by the relative conduction velocity of action potentials along individual axons. The conduction velocity, in turn, is effectively shaped by the axon diameter, the myelin thickness and the myelination pattern of the axon, which is the internode length. Traditionally, it has been thought that axon diameter and internode length are uniformly correlated with each other. However, recent data from our lab has revealed that the ratio of internode length and axon diameter deviates significantly in axons of low- and high frequency regions of globular bushy cell (GBC) axons in the antero-ventral cochlear nucleus (AVCN). Nevertheless, both the extent and the mechanisms by which the differences in internode length and axon diameter can actually contribute to adjust conduction velocity remain obscure.
Here, we investigated the structure-function relationship of axon morphology and conduction velocity. To this end, we measured the action potential conduction velocity in GBC axons in adult Mongolian gerbils in vivo. The measurements were performed by electrically stimulating GBC somata in the AVCN and simultaneously recording single cells extracellularly in the medial nucleus of the trapezoid body (MNTB). The precise measurement of the time period between the stimulation artifact and the initiation of the action potential allows for the calculation of the axonal action potential propagation latency between the GBC soma and the GBC-MNTB synapse.
Our experiments show that action potentials in the AVCN can be electrically elicited and simultaneously recorded in the MNTB in vivo. Thus, these recordings for the first time allow for the evaluation of the conduction velocity of morphologically characterized axons over a distance of as long as 5 mm in vivo. To relate the differences in axon morphology between low- and high frequency tuned GBC axons to axonal conduction properties, current work is concentrating on a frequency-specific characterization of conduction characteristics. Furthermore, comparing the latencies of electrically and acoustically evoked action potentials will give insight into the role of cochlear delays in shaping input timing.

Anaesthesia induced changes in neuronal response properties in mouse primary auditory cortex

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The majority of studies that concentrate on questions concerning the neuronal processing of acoustic stimuli in the central auditory system, have been conducted with anesthetized animals. It is broadly known, however, that many response properties of neurons can be influenced or even dramatically changed by anesthetic agents, especially in centers above the midbrain level (e.g. Zurita et al. 1994, Schulze and Langner 1997, Gaese and Ostwald 2001).
In this present study we examined the influence of a ketamine/xylazine anesthesia on the neuronal responses of single- and multi-units from the cortical layers III – IV (200- 400 µm depth) in the tonotopically organized primary auditory fields AI and AAF of the house mouse (Mus musculus). We presented pure tones (PTs) and 100% sinusoidally amplitude modulated (AM) tones to eight weeks old female mice. In line with previous studies on different species, we observed that neurons in both fields of anesthetized animals showed longer response latencies to PTs at the neurons best frequency and to AM tone stimulation compared to the respective responses in unanesthetized animals. In addition, neurons in anesthetized animals had a considerably lower spontaneous activity and a reduced evoked rate at their best frequency. The effect of the anesthesia was most obvious when comparing the capability of the neurons to synchronize their response to the time structure of the envelope of the AM tones. The neurons of anesthetized animals mostly showed no phase-locked discharges at all or were able to phase-lock only up to modulation frequencies of 18 Hz. Responses to AM tone stimulation recorded from unanesthetized animals showed phase-locking up to modulation frequencies of about 130 Hz. No significant differences with regard to the effects of anesthesia on neuronal responses were observed between fields AI and AAF. This study provides further evidence that the anesthetic state can change responses in the primary auditory cortex in a significant way, especially responses from time-domain processing as shown here for phase-locking to AM tones. Thus, neuronal data from anesthetized animals may not provide an unbiased basis for studying cortical mechanisms of auditory perception and recognition.

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Analysis of acute stress on long-term vulnerability after an acoustic injury in a mature rat model

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Excessive noise is a global health hazard that leads to noise-induced hearing impairment (NIHI) and presbycusis, currently with no successful clinical treatment. In the rising generation, NIHI will affect increasing numbers of people worldwide (NIDCD). Recent evidence suggests that endogenous or environmental stress has differential influence on the hearing ability and on the vulnerability of the ear for an acoustic insult *(Singer et al. [2013])*.

Categorization of Auditory Stimuli in the Behaving Mouse

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The ability to group different stimuli together based on a common property is called categorization. In a complex and changing world, this ability is essential for survival. Categorization of sensory information is believed to be a basic and common feature of sensory systems. To understand the factors contributing to categorization of auditory stimuli we tested it in the behaving mice under different conditions.
C57BL/6J adult female mice lived in an Audiobox (TSE) that allows continuous monitoring of individual behavior via an implanted subcutaneous transponder. The Audiobox consists of a home-cage connected by a long corridor to a specialized sound-attenuated corner. Food was available *ad libitum* in the home-cage and water was available in the corner. Visits to the corner were accompanied by the presentation of auditory stimuli. Mice were required to discriminate between one tone frequency (typically 7 or 14kHz) that was associated with access to the water and another tone (typically 1 octave above or below the 'safe' tone) associated with an air-puff. Subsequent exposure to new sounds upon entering the corner allowed us to estimate whether these sounds were categorized as 'safe' or conditioned. The new sounds were either tones with frequencies in between the safe and conditioned tone, or mixtures of the two in different ratios.
As has been shown before, mice were able to separate their responses to tones that were closer to the ‘safe’ or the conditioned tone into two categories with a sharp boundary in between. Sounds that were closer to the ‘safe’ tone in frequency were associated with reward and those closer to the conditioned tone were treated as dangerous. The shape of the psychometric curve was the same independently of whether the ‘safe’ tone was above or below the conditioned tone. Moreover, in trained mice, when a tone in between the boundaries became conditioned the categorizing behavior was not simply shifted sideways to respect the new boundaries but also downwards.
When tested with synthesized mixture of the two ‘prototype’ tones with thirteen equally spaced mixture ratios, mice treated the mixture sounds neither equivalently to the ‘safe’ tone nor to the conditioned tone. However, within the range of responses to the mixtures their performances gradually changed with the mixture ratio. The results suggest that categorization of mixture sounds has a very different shape, such that mixtures are grouped separately from both unmixed tones. The data suggest that the categorization of sound frequency is based on the existing internal/learnt categorical representations and that these representations are flexible.

Change of cortical activity patterns by selective apoptosis of auditory corticothalamic feedback projections

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The auditory cortex (AC) integrates sensory (e.g. spectral) information by temporally precise interactions of thalamocortical inputs and intracortical networks, as for example shown by dissociating their respective contributions to cortical activity patterns by pharmacological intracortical silencing (Happel et al., 2010, J. Neurosci. 30 ; 2014, J. Neurosci. 34). So far, the role of a further important circuitry in this information processing, namely corticothalamic (CT) feedback loops, is largely unknown and will be addressed here by a combination of several anatomical and physiological techniques.
First, we selectively eliminated CT neurons projecting from layer V/VI of the primary AC (field AI) to the medial geniculate body (MGB) using a chromophore-targeted laser photolysis method (Madison & Macklis, 1993, Exp. Neurol. 121; Bajo et al., 2010, Nat. Neurosci. 13). Mongolian gerbils received unilateral injections of the tracer solution containing the photosensitizer (chlorine e6) attached to rhodamine beads into the MGB. After retrograde transport of the tracer, the ipsilateral AI was illuminated transcranially by means of long-wave (670nm) laser light to initiate the apoptotic cascade within the local CT neurons. Then, the activity patterns in the ipsilateral lesioned AC evoked by electrical layer-specific intracortical microstimulation (ICMS) and by acoustic stimulation were investigated using current source density (CSD) analysis, before and after cortical silencing with muscimol (GABA agonist). Finally, brain slices were stained for neuronal nuclei (NeuN) to analyze the portion of degenerated CT neurons relative to the contralateral non-lesioned AI.
In animals with intact CT projections, infragranular and granular ICMS evoke a cross-laminar pattern of activation comparable to auditory stimulation. During cortical silencing, ICMS mainly activates thalamic input layer IV (Happel et al., 2014). In lesioned animals, which displayed variable strengths of neurodegeneration, we observed a negative correlation of signal strength, i.e. peak amplitudes and integrals of both granular CSD sinks and averaged rectified CSDs (AVRECs) with increasing lesion efficacy. Accordingly, in various animals with strong lesions, no significant sinks could be detected in layer IV following IG stimulation. After application of muscimol, the number of lesioned animals that had significant granular responses declined further.
Acoustically evoked CSD profiles of lesioned and non-lesioned animals did not differ notably. Also, the activation of layer IV did not significantly correlate with lesion strength at both BF and non-BF. However, the frequency tuning (calculated by the ratio of BF to non-BF) was less sharp in animals with stronger CT lesions. The same was true for the AVRECs. After application of muscimol, these correlations seem to be abolished.
Together, our results indicate that the generation and appearance of the cortical activity patterns induced by ICMS is highly dependent on fast-acting recurrent corticoefferent feedback. Furthermore, CT neurons seem to be involved in sharpening the frequency tuning within AI, possibly via egocentric selection of thalamic neurons.

Characterization of an exotic inhibitory synapse in the auditory brainstem

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Principal neurons of the lateral superior olive (LSO), a nucleus of the auditory brainstem, compute interaural level differences (ILD). These neurons receive excitatory input from the ipsilateral ventral cochlear nucleus (VCN) via the ventral acoustic stria (VAS). In combination with inhibitory input from the ipsilateral medial nucleus of the trapezoid body (MNTB), which carries information from the contralateral VCN, the classical neuronal circuit for computing ILD is thereby formed. In this study, we show that there is an additional exotic ipsilateral input into the LSO, which is inhibitory and of glycinergic nature. Electrophysiological measurements were performed in vertically tilted, acute coronal brainstem slices of C57BL/6 mice at P10-12 and 37° C. Whole-cell patch-clamp recordings and focal electrical stimulation of the VAS revealed evoked inhibitory postsynaptic currents (eIPSCs), which were identified as glycinergic by applying several receptor antagonists. A biotinylated dextran tracer was stereotacically injected into the VCN of adult mice, and fluorescence labeling against both biotin and the neuronal glycine transporter GlyT2 was subsequently performed, showing a co-localization of the tracer and GlyT2 in the LSO. Therefore, we conclude that the exotic glycinergic inputs arise from neurons in the ipsilateral VCN. The number of inputs per neuron was assessed by stepwise increasing the stimulation amplitude (min-max experiments). Preliminary data indicate 3-5 inputs per LSO neuron. The inhibitory VCN-LSO (VCN_{inhib}-LSO) synapses displayed slower kinetics than glycinergic MNTB-LSO synapses (Kramer *et al.*, 2014). As reliable high-frequency transmission and precision are hallmarks of synapses in the auditory brainstem, we stimulated unitary VCN_{inhib}-LSO inputs at frequencies from 1 to 333 Hz for 60 s each. In comparison to MNTB-LSO synapses, VCN_{inhib}-LSO synapses showed stronger depression of their eIPSC peak amplitudes at stimulation-frequencies > 20 Hz. By contrast, stimulation-frequencies < 10 Hz resulted in a weaker depression of eIPSC peak amplitudes. In addition, VCN_{inhib}-LSO synapses showed a lower fidelity during high-frequency transmission than MNTB-LSO synapses. Furthermore, the precision and onset latency of eIPSCs from both types of synapses will be compared. In summary, we conclude that VCN_{inhib}-LSO synapses are suited for a lower frequency spectrum of synaptic transmission compared to other, conventional synapses in the auditory brainstem (MNTB-LSO synapses: Kramer *et al.*, 2014; VCN-MNTB synapses: Scheuss *et al.*; 2007; excitatory VCN-LSO synapses: Janz *et al.* at this meeting). Therefore, we suggest that VCN_{inhib}-LSO synapses have a modulatory effect on the ILD computation, rather than a direct participation in ILD detection.

Characterization of optogenetic cochlea stimulation

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Severe hearing impairment or deafness can be treated by cochlear implants. These implants are by far the most successful neuroprostheses received by over 300,000 people worldwide and enable open speech comprehension in a majority of users. However, they suffer from low frequency resolution due to wide current spread from stimulation contacts, which limits the number of independently usable channels (usually to less than a dozen) and compromises speech understanding in noisy environments, music appreciation or prosody understanding. To ameliorate these drawbacks we are pursuing optogenetic cochlear implants in which spiral ganglion neurons are genetically modified to spike upon light stimulation. Optical stimulation can be spatially confined and thus promises lower spread of excitation in the cochlea. Accordingly, the increased number of independent stimulation channels is expected to enhance frequency resolution and intensity coding.

In our earlier proof-of-concept study we investigated optogenetic cochlea stimulation employing various transgenic rodent models as well as virus-mediated expression of channelrhodopsin variants in spiral ganglion neurons. Blue light stimulation of the spiral ganglion was capable of driving auditory activity, as demonstrated by recordings at several stages along the auditory pathway. Activation thresholds were found to be around 1 mW/mm², which is similar to thresholds for cortical neuron stimulation. The spread of excitation in the cochlea was probed using multielectrode recordings from the inferior colliculus of transgenic channelrhodopsin2 mice. Current source density based response maps were compared between optical, acoustic and electrical stimulation and indicated better frequency resolution of optical stimulation versus monopolar electrical stimulation. Current experiments investigate the spectral resolution of optical stimulation and test its interaction with normal acoustic processing to further characterize optogenetic stimulation of the cochlea. To this end, we employ single- and multi-unit recordings in the inferior colliculus and auditory cortex. In summary, our experiments demonstrate the feasibility of optogenetic cochlea stimulation to activate the auditory pathway and lay the groundwork for future applications in auditory research and prosthetics.

Cholinergic Signaling onto Spherical Bushy Cells in the Cochlear Nucleus of the Gerbil

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In the anterior part of the ventral cochlear nucleus of mammals, spherical bushy cells (SBC) receive direct input from the auditory nerve via specialized axosomatic synapses, the endbulbs of Held. The temporal precision of SBC output is critical for low-frequency sound source localization. Recent data have shown that inhibitory inputs tune SBC input-output function in a stimulus-dependent manner. In addition, collaterals of the olivocochlear bundle axons and fibers originating in the tegmentum form two cholinergic projections into the cochlear nucleus. We hypothesize that modulation through acetycholine (ACh) therefor plays an additional role in fine-tuning of SBC input-output function

In frontal and parasagittal brainslices of hearing gerbils (Meriones unguiculatus; P14-26), whole-cell recordings from SBC were obtained in the presence of glycine and GABA receptor antagonists. First, 1mM ACh or 500µM carbachol was locally applied onto the SBC with a Picospritzer device. This experiment was repeated in the presence of blockers specific to nicotinic and muscarinic acetylcholine receptors. As an additional confirmation of cholinergic signaling in the ventral cochlear nucleus, nicotinic acetylcholine receptors were stained with Alexa-conjugated Bungarotoxin (BTX). To identify presynaptic components of cholinergic transmission, immunostaining against choline acetyltransferase (ChAT) was performed.

We showed that ACh caused a transient depolarization of 3.23mV ± 1.7mV in 24 of 44 neurons, relaxing back to rest with a weighted time-constant of 282ms ± 311ms. Additionally, repeated ACh applications tonically shift the resting potential towards depolarized values by 1.34 mV ± 1.66 mV in 34 of 44 neurons. This tonic depolarization lasted for minutes after the last application, before fully recovering to the original resting potential. We could show that the transient depolarization was due to activation of a7-subtype containing nicotinic receptors, while the tonic depolarization was mediated by muscarinic ACh-receptors. Surpisingly, SBC hyperpolarized under atropine influence, indicating a tonic activation of muscarinic receptors by ambient levels of ACh. Immunohistochemical staining revealed colocalizing ChAT and BTX signals in the neuropil between SBC. The signal intensity and density was comparable to the staining in the dorsal cochlear nucleus, but less than in the posterior part of the ventral cochlear nucleus.

We have shown cholinergic transmission onto SBC in vitro, which influences the SBC membrane potential by both transient and long-lasting depolarization. In addition, we show with histological methods that cholinergic synapses most likely exist on SBC dendrites. We conclude that cholinergic innervation is an additional factor in the complex modulation of SBC excitability, acting by adjusting the resting membrane potential in a stimulus-dependent and -independent manner.

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Context dependent modulation of neuronal response in nucleus NCM of freely-moving Zebra finches

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In most songbird species much is known about learned vocalizations (generally songs), but very few studies have focused on unlearned vocalizations (calls) and we know even less about the mechanisms involved in their processing and production. Zebra finches (*Taeniopygia guttata*) have quite a vast repertoire of calls, which might have different functions. These birds even exhibit antiphonal communication patterns in established couples, which might indicate individual recognition through these vocalizations. When the couple goes into breeding state this antiphonal relation vanishes in one type of call and appears in another. NCM (nucleus Nidocaudal Mesopalium) is a secondary auditory area with a high concentration of estrogenic receptors, one of the reasons why it could potentially be related with changes in social context and breeding status. Our experiment tries to shed some light on how context dependent states and central mechanisms interact in order to effect a behavioral change such as the change in calling pattern. Established couples of Zebra Finches were implanted with tungsten microelectrodes and a miniature telemetric device transmitted the electrophysiological data. Apart from the electrophysiology transmitter the birds also carried a microphone transmitter over a period of 8 days, that allows to record all the vocalizations of each individual. During the first 3 days the aviary was impoverished and in day 4 the aviary was enriched with nesting material and nest boxes were provided. The neuronal responses of NCM to the mate calls, before, during and after the nesting behavior were analyzed.

Cross-modal plasticity of dorsal auditory cortex in congenital deafness

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Congenital and early deafness reorganizes the auditory system and leads to supranormal abilities in other sensory systems. In a previous study we could demonstrate that the field A1 (primary auditory field) is an unlikely site of visual cross-modal reorganization (Kral et al., 2003, Exp Brain Res). However, congenitally deaf cats behaviorally demonstrate supranormal performance in the visual domain (detection and localization in the periphery and movement detection, Lomber et al., 2010, Nat Neurosci). Furthermore, cooling deactivation localized the site of reorganization into secondary fields dorsal zone (DZ) and posterior auditory field (ibid.). To investigate the neuronal mechanism of such reorganization, the follow-up experiments focused on field DZ. Application of retrograde tracer into DZ and A1 demonstrated a differential recruitment of these fields in cross-modal reorganization in congenitally deaf cats: whereas A1 did not show significant cortical cross-modal reorganization, field DZ showed visual and somatosensory projections not observed in hearing controls (Barone et al., 2013, PLoS One). The reorganization was, however, only modest: only few % of all projections were identified as new crossmodal projections.

In the present experiments undertaken on 8 adult cats (4 deaf, 4 hearing), recordings were taken from field DZ and an adjoining visual field (PMLS) under cochlear implant (auditory) and visual stimulation in precisely-controlled isoflurane/N_2O anesthesia. Visual stimuli were flashes and, to include motion, phase-reversal gratings with different orientations and spatial frequencies. Simultaneous recordings were taken using two 16-channel Neuronexus probes inserted in both fields spatially as close as possible. The DZ penetration had an orientation parallel to the microcolumns. Electrodes were stained with DiI or DiO and the penetrations were histologically reconstructed after the experiment. Recording sites within DZ were confirmed using an SMI-32 staining.

From all recording sites, only the sites containing unit activity were processed further. Alltogether, ~1400 unit responses were evaluated, ~700 in each group. Spontaneous activity was significantly higher in PMLS than in DZ in both groups of animals. Unit responses showing statistically significant correlation with current level were considered responsive to auditory stimulation. There were significant auditory responses in both groups of animals in DZ at ~30% of all recording sites. In PMLS, only few units responded to auditory stimulation (< 10% in both groups). Visual stimulation led to responses in one third of units in PMLS in both groups of animals. In DZ, on the other hand, the deaf cats had more visual responses than hearing cats, although the proportion of these was modest (< 10%). In these responsive units, the evoked firing rate was higher in deaf cats, whereas hearing cats demonstrated more a modulation of ongoing activity than an evoked response in DZ.

The present results suggest a modest visual cross-modal reorganization of field DZ in congenital deafness and a generally preserved auditory responsiveness in the same field. The relatively small number of visually-responsive cells, corresponding to previous tracer studies (Barone et al., 2013), indicates that also other (possibly attentional) processes are involved in supranormal visual performance of deaf.

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DEEP BRAIN STIMULATION AT THE INFERIOR COLLICULUS: A NEW ANIMAL MODEL TO STUDY PARADOXICAL KINESIA?

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The inferior colliculus (IC), an important auditory midbrain relay, is also involved with defensive reaction since electrical stimulation of this structure induces aversive responses that mimic fearful behavior elicited by environmental challenges. It has been shown that the neural substrate of the IC can influence haloperidol-induced catalepsy suggesting that this structure plays a role in the sensorimotor gating triggered by emotional stimuli. Considering that (i) the paradoxical kinesia, observed in some parkinsonian patients, seems to be dependent of their emotional state and (ii) deep brain stimulation (DBS) has become an alternative therapeutic route for the relief of parkinsonian symptoms, the present study investigated the consequence of DBS at the IC on the catalepsy induced by systemic haloperidol in rats. Additionally, we investigated if DBS of the IC can elicits motor responses in anesthetized rats and whether DBS elicits distinct firing patterns of activity at the dorsal cortex (DCIC) or central nucleus (CNIC) of the IC in anesthetized rats. The results showed a significant reduction of the catalepsy response was seen in rats previously given haloperidol and receiving DBS at the IC. In addition, electrical stimulation to the ventral part of the CNIC induced immediate motor responses such as sideways postures, arching of the trunk, flexion of the forepaws, vibrissae movement, tunk movement to left or right, Straub-like phenomenon, tail movement to the right or left and positioning the tail between the hind legs in anesthetized rats. The electrophysiological results showed that neuronal spontaneous activity is lower in the DCIC compared to the CNIC. The ventral part of the CNIC is more active than the dorsal part. DBS to the ventral part of the CNIC increased the spike rate at neurons a few hundred microns away from the stimulation site. It is possible that the IC plays a role in the sensorimotor gating activated by emotional stimuli, and that DBS at the IC can be a promising new animal model to study paradoxical kinesia in rats.

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Differences in neural plasticity along the auditory pathway between animals with and without subjective tinnitus

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In a recent study we described differences between neural spiking plasticity in auditory cortex (AC) of animals that developed a subjective tinnitus percept (group T) after noise induced hearing loss (NIHL) and those that did not (group NT), as assessed by behavioural measurements. We put forward a model of a global inhibitory mechanism in AC that could be used by NT but not T animals in order to prevent tinnitus development (Ahlf et al., 2012). Here we extend our data analysis to the input activity of cortical neurons, based on the temporal and spectral features of local field potential (LFP) recordings, and an in-depth analysis of auditory brainstem responses (ABR). In response to NIHL we found in NT animals a significant reduction in overall cortical LFP activity with a drop of power in theta to beta frequency bands for stimulation frequencies up to 5.6 kHz and significant sound intensity dependent changes in all ABR wave (I to V) amplitudes. On the other hand, T animals showed no significant changes in overall cortical LFP activity but significant changes of power in specific frequency bands dependent on stimulation frequency, i.e., a power decrease in theta band frequency up to 5.6 kHz and a power increase in alpha frequency band for stimulation frequencies ranging from 8 to 16 kHz. We also found in these animals a significant increase in ABR amplitudes of wave V for all stimulation frequencies and intensities, corresponding to changes in the inferior colliculus (IC; ABR wave V). No significant changes were observed for waves I to IV. Based on these results we specify our model of tinnitus prevention (i.e., in NT animals) after NIHL: there, a top-down global (i.e. frequency-unspecific) inhibition seems to be able to reduce overall neuronal activity in AC and the brainstem, thereby counteracting NIHL induced bottom-up frequency-specific neuroplasticity suggested in current models of tinnitus development.

Ahlf S, Tziridis K, Korn S, Strohmeyer I, Schulze H (2012) Predisposition for and prevention of subjective tinnitus development. PLoS One 7:e44519.

Differential role of soluble guanylyl cyclase (NO-GC) isoforms in auditory function in adult mice

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The nitric oxide/cyclic guanosine 3'-5'-monophospate (NO/cGMP)-signalling cascade is involved in the regulation of a variety of physiological processes, e.g. in cardiovascular, skeletal and neural tissue (Arnold et al., 1977). In the inner ear, elevated cGMP levels were shown to have a sheltering role for cochlear hair cells and hearing function (Jaumann et al., 2012). However, it is unclear how cGMP generators contribute to this protective effect.

In mammals, cGMP can be generated from guanosine triphosphate (GTP) by two families of guanylyl cyclases (GCs): transmembrane (particulate) GCs and soluble, intracellular NO-responsive GCs (NO-GCs) (Chrisman et al., 1975). NO-GC is a heterodimeric protein consisting of an a- and a ß-subunit. Two isoforms of the protein (NO-GC1 and NO-GC2) are encoded by two different genes (a1 and a2) for the a-subunit. By generating transgenic mice strains lacking one of the isoforms of NO-GC (a1-KO and a2-KO) (Mergia et al., 2006) it has been shown that in the neuronal system the two NO-GC isoforms serve distinct functions (Haghikia et al., 2007, Taqatqehet al., 2009).

The aim of this study was to investigate whether deletion of the two NO-GC isoforms affects hearing function, vulnerability to noise exposure and recovery from acoustic trauma.
We analyzed and compared the auditory function by measuring the auditory brainstem response (ABR) and distortion product of otoacoustic emissions (DPOAEs) between the isoform-specific a1-KO, a2-KO and wildtype mice. The results are discussed in the context of the role of NO/cGMP-signalling in auditory function and noise vulnerability.

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Discrete representations in mouse auditory cortex and their stability in the presence of synaptic turnover

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Population imaging in mouse auditory cortex revealed clustering of neural responses to brief complex sounds: the activity of a local population typically falls close to one out of a small number of observed states [1]. These clusters appear to group sets of auditory stimuli into a discrete set of activity patterns and could thereby form the basis for representations of sound categories. However, to be useful for the brain, such representations should be robust against fluctuations in the underlying circuitry, which are significant even in the absences of any explicit learning paradigm [2]. Here we introduce a novel circuit model of mouse auditory cortex to study the emergence of the observed activity cluster states and their structural stability in the presence of synaptic noise. We find that generic random networks by virtue of their inhibitory recurrent connectivity can group complex sounds spontaneously into essentially discrete sets of activity states. Moreover, these states can display high degrees of stability, even when modifying a substantial fraction of synaptic connections, as long as the basic statistics of connectivity is maintained. We use the insights gained from the analysis of our model to interpret data gathered in a parallel effort, employing chronic two-photon imaging of population activity in the auditory cortex of awake mice.

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[2] Loewenstein Y, Kuras A, Rumpel S. Multiplicative dynamics underlie the emergence of the log-normal distribution of spine sizes in the neocortex in vivo, J Neurosci, 2011.

Does ginkgo biloba extract EGb 761® have a therapeutic effect on noise induced hearing loss and subjective tinnitus?

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This work is based on an earlier study, where we were able to show a protective effect of the ginkgo biloba extract EGb 761® against noise induced hearing loss (NIHL) and as a consequence a reduction in tinnitus development in Mongolian gerbils (meriones unguiculatus) [1]. Here we investigate if EGb 761® does also exert a curative effect on subjective tinnitus induced by NIHL in Mongolian gerbils, as assessed by behavioural and electrophysiological means. The acoustic trauma is caused by a 2 kHz pure tone at 115 dB SPL which is presented for 75 minutes. For verifying a possible tinnitus percept we recorded behavioural data, i.e. the pre-pulse inhibition (PPI) modified acoustic startle response (ASR) (gap-noise paradigm as developed by Turner et al. [2]). First, the auditory brainstem response (ABR) to click and pure tone stimuli of different frequencies and intensities were recorded. These data were used to determine the audiogram and to characterize the neuronal activity of the different auditory brainstem nuclei. Second, we performed single cell recordings of neurons in primary auditory cortex (AI) using a four by four array of 16 microelectrodes. Data of the behavioural tinnitus test and electrophysiological data verifying the hearing loss and reflecting the neuronal dynamics and plasticity along the auditory pathway are recorded before, immediately after and in weekly intervals for up to six weeks after the acoustic trauma. We applied EGb 761® or vehicle orally on a daily basis beginning 3 weeks after the trauma with the animals randomly assigned to one of the two groups. Preliminary results indicate that EGb 761® applied after otherwise permanent hearing loss has developed reduces hearing loss during the course of application as well as the tinnitus related behaviour. Our neurophysiological data indicate that during application of EGb 761® neuronal responses normalize back to pre-trauma characteristics, and are significantly different from those before the beginning of the treatment. These effects are not seen in the control group. These first results indicate that EGb 761® does not only have a protective but probably also a therapeutic effect on NIHL and tinnitus in our animal model.

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Dopamine-modulated recurrent corticoefferent feedback in primary auditory cortex: perceptual salience and memory function

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Dopamine modulates neural circuits throughout the brain and has important roles in motor control, reward, attention, and learning. In primary auditory cortex (AI), dopamine is required for learning and long-term memory formation. However, the circuit-effects of layer-dependent dopaminergic neurotransmission in sensory cortex and their possible roles in perception, learning and memory are largely unknown.

In a recent study, we gained first insights into the circuit functions of dopamine in auditory cortex by using current-source-density analyses to compare synaptic activation patterns evoked by auditory stimulation in the presence and absence of a D1/D5 dopamine receptor agonist in gerbil AI (Happel et al., 2014). Activation of D1/D5 receptors lead to sustained auditory (thalamocortical) input processing via a local and polysynaptic recurrent cortico-thalamocortical feedback loop originating from infragranular (corticoefferent) sub-circuits. A detailed circuit analysis of this dopamine-modulated corticoefferent feedback related its activation to the generation of behaviorally relevant signals and perception in a behavioral detection task. Dopaminergic modulation of such recurrent corticoefferent feedback might allow for learning-induced gain control promoting the read-out of task-related information from cortical synapses and improving perceptual salience and learning.

We could further emphasize the translational relevance of this corticothalamic feedback-gain circuitry involved in learning and memory malfunctions in the 5xFAD mouse model for Alzheimer's disease. Disruption of neuronal networks in the Alzheimer-afflicted brain is increasingly recognized as a key correlate of cognitive and memory decline in Alzheimer patients. In the 5xFAD model we found impaired functions of pyramidal cells in infragranular layers leading to a loss of the corticoefferent feedback-gain. This specific disruption of normal cross-laminar cortical processing coincided with mnemonic deficits in contextual and cued fear conditioning and preceded the occurrence of cell death, and hence, might reflect a possible circuit mechanism of memory deficits in early AD (Lison et al., 2013).

Dynamics and Precision of Temporal Responses in the Mouse Inferior Colliculus

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Neurons in the central nucleus of the inferior colliculus (ICC) respond to tone bursts with several temporal response patterns including phasic, phasic-tonic, tonic, pauser, and long-latency (buildup) responses. These responses may change in the neuron's frequency-receptive field. The present study tests the hypothesis that precise temporal coding of tone onsets may be provided only by a subset of neurons with stable temporal responses and stable latencies to tone onsets in the receptive fields.

Tone burst responses from the excitatory frequency-receptive field (3 - 80 kHz; -20 – 85 dB SPL) were extracellularly recorded and analyzed from 130 ICC neurons in ketamine-xylazin anesthetized NMRI mice. The receptive fields of the neurons, which are bordered by the frequency tuning curve, were divided in a core part covering frequencies at levels about 20 dB above the threshold at a given frequency and a peripheral part close to the frequency tuning curve.

Temporal response patterns of 37 neurons were stable in the core receptive field. 24 of these neurons had phasic responses. 23 of 29 neurons with constant (+/- 1 ms) tone response latencies in the core receptive field had phasic responses. 34 neurons, all with a phasic component in their tone response (phasic, phasic-tonic, pauser), showed only small changes of tone response latencies (+/- 6 ms) in their core receptive fields. The remaining 67 neurons (52% of the whole sample) had variable temporal response patterns often accompanied by large (up to 37 ms) changes of tone response latencies in the core receptive fields. Large changes in response latencies always correlated with changes in their response patterns, possibly due to changes of inhibitory influence, which could suppress the neuron's phasic onset component.

The results suggest that more than 50% of the neurons in the ICC are unsuitable to code the onset of tone bursts, maybe also of other sounds, with high precision. Only neurons with a phasic component in their response, especially the just phasically responding neurons, have stable response latencies necessary to code tone onsets reliably and with high precision. These results indicate a specialization of ICC neurons for certain tasks of sound analysis with phasically responding neurons being responsible for a precise coding in the time-domain. These neurons are more abundant than neurons with other tone response patterns in the periphery of the ICC (Reetz G, Ehret G, Brain Res. 816, 527-543, 1999; Hage SR, Ehret G, Eur J Neurosci 18, 2301-2312, 2003), specializing this ICC area for precision in time domain processing.

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Excitation - Inhibition integration in a binaural auditory circuit

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The Lateral Superior Olive (LSO) is the first nucleus within the auditory brainstem that integrates excitatory inputs arising from the ipsilateral ear with inhibitory inputs from the contralateral ear. This excitatory/inhibitory (E/I) integration is thought to underlie computation of sound localization based on interaural intensity difference (IID) cues. To understand how individual LSO neurons integrate excitation and inhibition, it is important to know how many excitatory, and inhibitory inputs converge onto a given LSO neuron and what are the underlying unitary synaptic strengths.

We made whole-cell recordings from LSO neurons in brain slices from P14 - P18 mice. Fiber stimulation lateral to the LSO, which should lead to the activation of excitatory fibers originating in Ventral Cochlear Nuclear (VCN), resulted in EPSCs and occasional IPSCs. We studied EPSCs in the presence of glycine and GABA-A receptor antagonists, and found that EPSCs increased overall smoothly with increasing stimulation voltages. The average EPSC amplitude at the threshold voltage for stimulation was ~ 50 pA. In contrast, medial stimulation aimed at activating inputs from the Medial Nucleus of the Trapezoid Body (MNTB) resulted in surprisingly large IPSCs, which showed clear jumps when the stimulation voltage was increased in small increments. Under conditions of high intracellular [Cl-], unitary IPSC (uIPSC) amplitudes as measured by the difference between subsequent jumps were remarkably large (up to 5 nA; mean 2.1 ± 1.8 nA). Under physiological intracellular [Cl-] (6 mM), the conductance of unitary IPSCs was still large (9.1 ± 6.9 nS), while the predicted conductance for unitary EPSCs was only ~ 0.7 - 3 nS.

To study the functional significance of these findings in excitation / inhibition (E/I) integration, we made a connectivity-based point neuron model. The model assumed a realistic number of excitatory and inhibitory inputs (n = 20 and 8) with the measured synaptic conductances (see above). Spike trains in the afferent excitatory - and inhibitory fibers were modelled stochastically based on the known in-vivo firing frequencies of the presynaptic neurons (Kopp-Scheinpflug et al., 2003). We modelled a 100 ms sound presentation by a step-wise increase in excitatory fiber spike frequency. At the same time, the spike frequency of inhibitory fibers was increased to either the same, or to smaller or larger frequencies, thus effectively modelling different sound-evoked firing rates in inhibitory and excitatory fibers caused by an IID cue. The model captured some properties of measured LSO tuning curves like transient AP responses at the onset of sound presentation, and the persistence of a transient but not sustained response when excitation is just balanced by inhibition (Magnusson et al. 2008). The membrane potential of LSO neurons showed strong negative excursions of ~ 8 - 10 mV amplitude, reflecting large unitary IPSPs. We will further use the computational model to investigate the functional significance of a few, but powerful inhibitory inputs in E/I integration in this auditory nucleus.

Expression profile of voltage gated K+ channels in the Medial Superior Olive

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Neurons in the Medial Superior Olive (MSO) code for the localization of azimuthal sound sources, by comparing the different arrival times of a sound at the two ears. This time difference, known as interaural time difference (ITD), is detectable in the sub-millisecond time scale. Such short integration times require specialized cellular adaptations. One adaptation is to decrease the membrane time constant by increasing the resting conductance e.g. the resting input resistance. The low input resistance of these neurons depends at least on the presence hyperpolarization-activated cation channels (Ih channels) and low voltage activated potassium channels. Studies concerning low voltage activated potassium channels focused on the expression Kv1.1 that is localized at the soma and proximal dendrites. However, the presence of other potassium channel subtypes has not been elucidated. As dendritic potentials of up to 80 mV were reported, even high voltage activated potassium currents might contribute to voltage signaling in MSO neurons.

The aim of this study was to determine the expression profile of low and high voltage activated K+ channels in the mature MSO of Mongolian gerbils (Meriones unguiculatus). Using immunohistochemical stainings and semi-quantitative distribution analysis we show the presence of both low and high voltage activated K+ channels in MSO neurons. In agreement with earlier studies, the expression of the low voltage activated channel Kv1.1 is biased to the soma and proximal dendrites of mature MSO neurons. Other Kv's irrespectively of voltage activation range are more biased towards the dendrites. Especially, the high voltage activating channels Kv2.1, Kv2.2 and Kv3.1b are present throughout the entire MSO neurons including the distal dendrites. This complementary expression profile could provide fast voltage signaling in MSO neurons and the linearization of the EPSP size and shape between the dendrite and soma. Thus, the variety of potassium channels in MSO neurons is larger than expected and is implicated to support ITD coding.

FM-FM neurons in the mustached bat encode target range depending of the level of the call: neural specializations for echo-level compensation

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Estimation of target range by echolocating bats is achieved by auditory neurons that compute time delay between the call and its echo, providing a direct measure of the distance to reflecting surfaces. The response of these so called “delay-tuned neurons” are multiparametric. The most common approach that captures at least some of the multiparametric nature of receptive fields of delay-tuned neurons is to calculate delay response fields (DRFs). DRFs represent the spike output of a neuron in the two-dimensional space of echo delay and echo level. Usually, the DRF of delay-tuned neurons is tilted such that the preferred echo delay decreases with increasing echo level. This feature could produce “tracking” of an object by the same neuron during approach of the bat towards the object while the levels of returning echoes are increasing. However, during flight, bats decrease the intensity of their emitted pulses when they approach a prey item or an obstacle. This echo-level compensation mechanism indicates the possibility of maintenance of echo intensity within an optimal sensitivity range. We hypothesized that DRFs obtained with echo delay and call level (cDRFs), instead of echo delay and echo level (eDRFs), would be tilted in a way that preferred echo delay increases with call level. This shape would reflect the echo-level compensation mechanism described so far. We tested this prediction in delay-tuned neurons of the auditory cortex (AC) of the mustached bat (*Pteronotus parnellii*). We found that when we tested eDRFs, in most of delay-tuned neurons in the AC of the mustached bat the echo level eliciting the maximum spike rate was about 80 dB SPL, which coincides with echo levels obtained from behavioral experiments in bats swinging in a pendulum. Following our prediction, in cDRFs the preferred echo delay increases with call level. This feature indicates that tracking of an object by the same neurons during approach could be done based in changes in the call level instead of the echo level. In addition, the population coding of target range is affected by call level. In the AC of the mustached bat, neurons tuned to shorter delays are maximally responsive at lower call levels while neurons tuned to longer delays showed maximum spike output at higher call levels. The relationship between best delay and best call level calculated in the population of neurons is similar to that obtained echo delay and call level from recordings obtained while the bats were swinging in a pendulum and echolocating towards a target.

Gaps of silence: how do they affect the performance of the glycinergic MNTB-LSO synapses during prolonged stimulation?

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During neurotransmission, synaptic strength often undergoes short-term depression (STD). STD has been extensively characterized in excitatory synapses, yet much less is known from inhibitory synapses. The glycinergic projection from the medial nucleus of the trapezoid body (MNTB) to the lateral superior olive (LSO) is well suited to address STD in inhibitory synapses. Here, we analyzed the performance and precision of MNTB-LSO synapses during prolonged stimulation. The stimulation protocol comprised continuous stimulation as well as intermittent stimulation patterns with inserted gaps of silence of various durations (50, 100, 150, 200 ms). Stimulation frequencies ranged from 50 to 333 Hz, and protocols lasted for 60 s. MNTB-LSO synapses were characterized in acute brainstem slices of P10-12 C57BL/6 mice. Inhibitory postsynaptic currents, evoked by electrical activation of MNTB axons (eIPSCs), were recorded from LSO principal neurons in the whole-cell patch-clamp configuration at 37 °C. Peak amplitudes of eIPSC were determined. At each stimulation frequency, we observed STD. At 50 Hz, amplitudes declined to about 48 % within the first 10 s and subsequently reached a steady state level of 30 %. Higher frequencies of continuous stimulation resulted in higher STD. For example, at 200 Hz, peak amplitudes declined to 5 % within the first 10 s, remaining at this low steady state value. eIPSC failures were observed at all frequencies, but they were very rare at 50 Hz (<15 %) at the end of the 60 s stimulation period. Analysis of temporal precision revealed accurate peak latencies of eIPSCs, which drifted from 2.3 ms to 2.7 ms within 60 s stimulation at 50 Hz. Introduction of gaps of silence led to increased eIPSC amplitudes. When the stimulation frequency was 50 Hz, gap durations of 50 ms or 200 ms resulted in steady-state levels of 40 % or 60 %, respectively, i.e., amplitudes could be boosted 2-fold (from 30 % to 60 %). The steady state levels never fell below 20 %, regardless of stimulus frequency. When further analyzing the data obtained from gap of silence experiments, we observed an unexpected rebound effect. This rebound effect occurred at stimulation frequencies >50 Hz. It was evidenced by increased eIPSC amplitudes which were observed after 30-40 s. During this time window, the amplitudes reached 29 % at 100 Hz and 100 ms gaps. Before this window, amplitudes had declined to 20 %. Thus, the rebound effect was about 1.5-fold. In summary, our results demonstrate a remarkably high performance of the inhibitory MNTB-LSO synapses upon prolonged stimulation. Gaps of silence resulted in a considerably increased performance. Furthermore, the synapses display high temporal precision and high fidelity during prolonged stimulation. We conclude that glycinergic MNTB axon terminals are tuned to faithfully transmit information to postsynaptic LSO neurons that are responsible for sound localization processing via computation of interaural level differences. In addition, the observed high frequency transmission is in line with the unique specializations present in the inhibitory pathway from the contralateral ear to the LSO, namely endbulbs in the cochlear nucleus, thick axons, and calyces of Held in the MNTB.

Hearing loss in adulthood modifies interneuronal interaction in the auditory brainstem: A Fos study of the rat.

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In the auditory system of the adult mammalian brain, the immediate-early gene *fos* (also known as *c-fos*) is one of the first genes activated by sensory activity evoked either by acoustical or electrical intracochlear stimulation (EIS). Its protein is a monomer of the heterodimeric Fos-Jun activator protein-1 (AP-1) transcription factor. AP-1 triggers the expression of many genes involved in neuroplastic remodeling, among them the growth associated protein Gap43.

In this study we investigated the pattern of Fos expression in anteroventral cochlear nucleus (AVCN), lateral superior olive (LSO), and central inferior colliculus (CIC) following EIS of rats that were deafened in adulthood. Bilateral hearing loss was induced at postnatal day 90 by a single injection of a loop diuretic (intravenous) in combination with an aminoglycoside (intramuscular). As a result, a rapid and permanent rise of hearing threshold by ~90 dB was measured. One month after hearing loss, anesthetized rats received unilateral EIS for 2 h by inserting a cochlear implant into the medial turn of the cochlea.

Following sustained tonotopic EIS, Fos positive nuclei were dispersed throughout the middle to ventral ipsilateral AVCN (AVCNi), the ipsilateral LSO (LSOi), and the whole dorso-lateral part of contralateral CIC (CICc). Only neurons in the upper dorsal part of AVCNi, medial LSOi, and ventro-medial CICc failed to express Fos. A direct comparison with our previous studies acquired in normal hearing as well as neonatally deafened rats (Rosskothen-Kuhl & Illing, 2010; 2012) reveals that the population of Fos positive neurons after stimulation is much larger in adultly deafened rats than in normal hearing rats. Additionally, it essentially disregards tonotopic order seen in normal hearing rats after 2 h EIS. In contrast, little differences could be observed between the Fos expression pattern of neonatally deafened or adultly deafened rats following EIS.

Overall, our data suggest that not only neonatal hearing loss but also a short period of hearing loss in adulthood has far-reaching consequences for the interneuronal communication within networks of the auditory brainstem. We show that even a hearing-experienced mature auditory brainstem modifies its neuronal responsiveness due to a permanent loss of sensory input.

Influence of Adaptation on the Representation of ITD in the Barn Owl's ICX

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Adaptation has been observed in many nuclei of the auditory pathway. One aspect of adaptation is that a neuron's response to a first stimulus (masker) is generally higher than to a second, succeeding stimulus (probe). This kind of adaptation is called response adaptation. We investigated response adaptation as a function of several parameters for masker and probe in the external nucleus of the inferior colliculus (ICX) in the American barn owl (*Tyto furcata*). This bird is a specialist in sound localization. For localizing sound sources in the horizontal plane it relies primarily on the interaural time difference (ITD). An auditory space map is formed in ICX by combining binaural cues for azimuth and elevation and integrating across frequency channels.

Extracellular recordings were obtained from neurons in ICX. The stimuli (de novo broadband noise, each 100ms duration, 5ms onset/offset ramps) were presented via earphones. The masker was adjusted to the most effective combination of binaural cues and the masker stimulus level was chosen to elicit 50% of the saturating response. Initially, the probe followed the masker without a silent interval. Its parameters were chosen identically to the masker but the ITD was varied randomly to span the physiological range. This enabled us to compare the resulting "2nd ITD tuning curve" (response vs. probe ITD) to a reference "ITD tuning curve" recorded by presenting the probe stimuli alone.

In additional experiments, either a silent inter-stimulus interval (ISI) between masker and probe was introduced, or the masker was presented with a less effective ITD corresponding to the slope or the trough of the main peak in the reference ITD tuning curve.

By comparing the 2nd ITD tuning curves to reference tuning curves, we observed response adaptation throughout the physiological range of ITDs. Adaptation was equal for all ITDs, including the masker ITD. The strength of adaptation depended on the rate a unit responded to the masker. Therefore, when non-best ITDs were used for maskers, the adaptation was weaker. Under all conditions tested, the shape of the ITD tuning curve remained similar under adaptive conditions as without adaptation. We quantified the impact of adaptation on accuracy (interpolated peak center) and precision (peak width) for each unit. Preliminary analysis did not reveal significant and systematic changes.

In conclusion, the representation of ITD in the ICX is robust against the influences mimicked by our double stimulation paradigm. A preceding sound induces response adaptation. The effect of adaptation observed in behavior is not reflected in the response adaptation observed in single ICX neuron. We speculate that population effects may then decrease or enhance the behavioral performance for repeated stimuli or enable novelty detection in higher brain areas.

Spatial update of the auditory world through vestibular and proprioceptive cues

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For stable sound localization in space a listener has to account not only for the motion of an auditory object but also for self-motion. This requires a head-to-world coordinate transformation by combining proprioceptive and vestibular inputs with the binaural cues for azimuthal sound localization. Proprioceptive and vestibular information about head-on-trunk position and head motion in space should therefore influence the evaluation of spatial auditory cues as indicated by the numerous proprioceptive and vestibular neural inputs into the auditory brainstem. Here we evaluate the relative influence of vestibular and proprioceptive cues on updating the world-centered position of auditory targets during active and passive head and/or body rotations in the horizontal plane. In a two-interval, forced-choice task, human subjects seated in a rotating chair judged whether the second of two noise bursts, emitted by one of nine closely spaced speakers was perceived to be to the left or right of the first burst in world coordinates. The first noise burst (reference) was presented from the central speaker at the midline, then the head and/or body was turned about the vertical axis by ~30 degrees, and then the second burst (probe) was presented. Performance was compared across three conditions: Vestibular-Proprioceptive (VP) in which subjects actively turned the head on the body, Vestibular-only (VO) in which subjects were turned passively by the rotating chair, and Proprioceptive-only (PO) in which subjects actively turned their heads while the chair simultaneously counter-rotated such that the body rotated under the stationary head. In the VP condition, the probe had to be shifted opposite the head turn to be perceived at the reference location, meaning that head rotation was overestimated relative to the perceived auditory eccentricity of the probe in head coordinates. Estimates of head rotation were reduced in the VO condition, i.e. when head rotation was passive rather than active. Interestingly, even in the PO condition, where the head remained stationary in space, the probe had to be shifted opposite the trunk turn to be perceived at the reference location. Taken together, results reveal that both signals are used to update the spatial representations of auditory targets, but that vestibular inputs contribute more than proprioceptive inputs. Results were used to derive computational weights attributed to vestibular and proprioceptive signals. Finally, a non-auditory control experiment confirmed that these weighted contributions are independent of the modality employed for object localization in world coordinates.

Influence of ear movements on spatial receptive fields in the bat superior colliculus

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Motion in space changes our sensory inputs even when, in world coordinates, the stimulus source does not move. In the auditory world spatial cues, such as interaural time- and level differences, are changed by self-motion. The auditory system has to compensate for these changes to stabilize perception in space. In most small mammals, including all those used as animal models for human hearing, the outer ears are movable. These pinna movements introduce dramatic variations in the binaural (and monaural) cues for sound localization. This is especially important for bats. Due to the combination of large moveable ears and ultrasound sensitivity, bats have much sharper auditory spatial receptive fields than other animal models. In neurophysiological experiments we use the bat *Phyllostomus discolor* as an animal model for auditory stabilization of space.

Our experiments investigated the influence of proprioceptive information (position of the pinnae) on the extent and location of auditory spatial receptive fields in neurons of the superior colliculus (SC) of anesthetized bats. A pinna muscle (adductor muscle) was stimulated electrically to evoke proprioceptive inputs through pinna afferents while presenting acoustic stimuli in virtual acoustic space via earphones. The acoustic input remained unchanged during this procedure. Acoustic stimuli were generated by convoluting naturalistic echolocation calls of *P. discolor* with the head-related transfer functions from the frontal hemisphere. Neural activity during electrical stimulation and acoustic stimulus presentation was recorded extracellularly. Trials with combined electrical and acoustical stimulation were randomly interspersed with trials in which only the acoustic stimulus was presented to generate control measurements.

Preliminary results show that 72% (23/32) of the recorded neurons in the SC exhibit a change of shape, size or position of the auditory spatial receptive fields during electrical stimulation of the ear muscles compared to the controls (Figure 1). In 52% (12/23) of these neurons direction of shift in size, shape or position correlated with the expected direction of pinna movement elicited by electrical stimulation of the respective ear muscle. The results suggest that the neuronal representation of space in the SC of bats can be influenced by proprioceptive information about pinna position.

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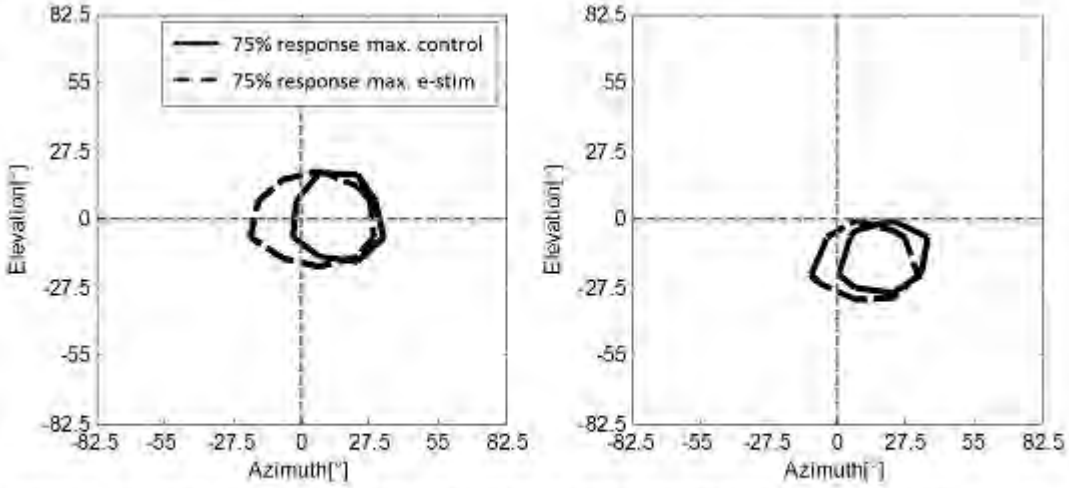

Figure 1: Examples for spatial receptive field changes in two SC neurons due to electrical stimulation of the pinna adductor muscle.

Integration of biosonar and visual information in the superior colliculus of bats.

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Integration of sensory information from different modalities can improve the object-detection and -recognition abilities of an organism. The underlying neural basis has been studied intensively for the combination of passive hearing and vision in a variety of animals. The impact of biosonar information on the neural processing of visual cues and vice versa in bats, however, has not been investigated so far.
Here we present novel neurophysiological data on biosonar-visual integration in the superior colliculus (SC) of the bat *Phyllostomus discolor*. We measured biosonar and visual spatial receptive fields of neurons in the SC of anaesthetized bats and investigated neural interactions of biosonar and visual stimuli presented within the particular receptive fields. As expected, neurons in the superficial layers of the bat's SC were only responsive to visual stimulation. But in contrast to other mammals, in the deeper layers neurons could only be excited by biosonar stimulation. Bimodal neurons responsive to biosonar and visual stimuli were rare and exclusively located in a small transitional region between superficial and deeper layers. Although most neurons in the bat's SC could only be driven by one stimulus modality, bimodal stimulation had extensive effects in the majority of neurons. In neurons responsive to biosonar stimulation additional visual information had mostly an enhancing effect, whereas, responses to visual stimulation were mostly depressed by additional biosonar information. Our results indicate that even though processing of biosonar information is dominant in the SC of bats, additional visual information can be integrated by the majority of neurons.

Investigation of auditory receptive fields in neurons of the optic tectum – do generalists have multimodal integration?

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The integration of multimodal information is an essential feature of sensory processing. It becomes increasingly obvious that, even on the earliest processing stages, input from other sensory modalities influence the computation of a given sensory channel, and that the integration of multimodal information has behavioral advantages in respect to speed and accuracy. However, the analysis of multimodal integration on the network, cellular, and subcellular level is still at an early stage. This is partly due to the fact that the animals that have been studied most intensely in respect to multimodal integration, i.e., cats and owls, are not ideal species to study anatomical circuit details. In addition, while the analysis of the strigiformes certainly has many benefits (Krogh-principle), the specialist layout of the system might lead to misassumptions of the “default” layout of the vertebrate brain.
We have thus started to investigate multimodal integration in chicken, a generalist animal highly amenable to anatomical and network analysis. Our focus lays on the bimodal integration of auditory and visual input in the optic tectum, a brain area that is considered to be a major multimodal processing area with an exquisite map of multimodal space. Previous work has demonstrated that the projection of the auditory midbrain into the tectum is not very pronounced and has a rather coarse topography. Here, we used extracellular recordings and virtual binaural sounds presented via earphones to assess the auditory receptive fields of neurons in various tectal layers. Our preliminary data suggest that auditory input can be found in the tectum; however, the tuning is rather weak, and the receptive fields are huge compared to the data reported for the specialized barn owl. We will discuss our finding in light of the network data and the putative role of the tectum in a generalist animal.

Layer-specific intracortical microstimulation of primary auditory cortex *in vivo*

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The electrical stimulation of cortical tissue is a valuable method to describe the functional properties of neural cells and the local networks they are part of. Here we designed a method that combines electrical stimulation with parallel neural recording on the same shank of a multi-electrode array *in vivo*. We modified a commercially available electrophysiology system by using a custom-built adapter to physically disconnect pre-defined channels from the recording headstage and connected them to a stimulating current-source. By that the stimulation channels safely bypass the recording equipment. That allows us to use the relatively high impedance electrodes on a typical multi-electrode array for stimulation without the danger of destroying the recording system through unusually high voltages.
We used this method to stimulate the (primary) auditory cortex of guinea pigs in the *in vivo* preparation and looked at the effects elicited through the stimulation between different channel combinations. For this, animals were anesthetized using Ketamine/Xylazin, tracheotomised, artificially ventilated and their temperature was kept at 38° C using a homoeothermic blanket. Following trephination and dura removal, multi-electrode arrays were inserted into the auditory cortex (field A1) perpendicularly to the cortical surface. By applying current pulses (200 µs/phase, charge-balanced) between two different channels (= electrodes) of a linear, single-shank multi-electrode array, we were able to stimulate cortical neurons in a layer-specific way. We alternatively stimulated either over the whole column, the supragranular, or the infragranular layers. The remaining, non-stimulating channels on the electrode array were, at the same time, used to record the evoked activity in the same cortical column without the constraints of a necessary lateral displacement. Furthermore, the recording contacts were used to determine the profile of excitation evoked by an acoustic stimulus (condensation clicks, 50 µs, 40 dB above hearing threshold). Stimulation with this system allowed us to investigate evoked local field potentials. The stimulation of the supragranular and infragranular layers resulted in different activation profiles, demonstrating the specificity of the stimulation sites and the specificity of the evoked responses. When comparing the response profiles similarities but also significant differences were observed. This demonstrates that the activation profile elicited by electrical cortical microstimulation differs from the response evoked by auditory input.
A potential application of this anatomically adapted stimulation protocol would be the development of improved neural prosthetics. Due to the crude monopolar or even only surface stimulation of sensory cortices, previous attempts to restore senses with cortical implants were unsatisfactory. Further research could show if a more focused, layer-specific stimulation is able to improve the perceptions gained from electrical pulses sent to the cortex and make them more similar to natural acoustic responses.
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Meaningful but passive sound exposure induces long lasting and spatially-restricted plasticity in adult inferior colliculus

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Incoming auditory information is filtered by its relevance. To test if passive but meaningful auditory experience affects sound processing at a sub-cortical level, we investigated the plastic changes elicited by behaviourally relevant auditory exposure in the inferior colliculus and cochlear nucleus of mice.

C57BL/6J adult female mice lived in an Audiobox (TSE) that allows continuous monitoring of individual behaviour via an implanted subcutaneous transponder. The Audiobox consists of a home-cage connected by a long corridor to a specialized sound-attenuated corner. Food was available *ad libitum* in the home-cage and water was available in the corner. Visits to the corner were accompanied by the presentation of fixed tone pips in the exposed group and by silence in the control group. A third group (random exposure group) was exposed to the same fixed tone pips but randomly and in the home-cage instead of the corner.

After 7 to 11 days of exposure we recorded sound-evoked neuronal activity in the inferior colliculus of acutely anesthetized mice and characterized tuning curves. The exposed group showed an increase in evoked multiunit activity. The augmented responses were specific to the dorsal part of the central nucleus independently of the exposed frequency (8 or 16 kHz). We also observed a shift in best frequency towards higher values along the tonotopic axis that was stronger when 16 kHz was the exposed frequency. The effect was specific to a meaningful sound, since in the random exposed group we observed augmented responses only in the area of the central nucleus where the best frequency matched the exposed frequency and not in the dorsal part. These changes were not due to a general adjustment upstream the inferior colliculus since responses in the cochlear nucleus were comparable in the exposed and control groups. Inactivating the auditory cortex with muscimol during acute recordings subtly increased the responses, but in equal magnitude, for both exposed and control groups.

These results indicate that behaviourally relevant, but not random, sound exposure induces sustained changes in a restricted area of the central nucleus of inferior colliculus. Plasticity takes the form of augmented evoked activity and a shift in best frequencies along the tonotopic axis. Its expression is independent of cortical activity. We conclude that, in adult animals, important sustained plastic changes in sound processing take place at a specific subcortical level that might contribute to the neural substrate of auditory expectations.

Model Predictions of How Cochlear Gain Loss and Neuropathy Affect Human Auditory Brainstem Responses

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Cochlear gain loss due to damaged outer-hair cells and noise-induced cochlear neuropathy expressed as a loss of the number of available auditory-nerve fibers both affect the levels of scalp-recorded auditory-brainstem responses (ABRs). To yield more targeted diagnostics and tailored treatment, it is crucial to understand which stimulus configurations inform about either of both cochlear deficits. Whereas outcome measures such as psychoacoustics and brainstem response measures suffer from the inability to separate hearing deficits, models are able to control each component independently. Through hearing-impaired model predictions of brainstem responses, this study investigates which stimuli can differentially diagnose cochlear neuropathy in patients with an audiometric hearing loss.

A human time-domain auditory brainstem response model that is designed to capture level-dependent features of human click-evoked otoacoustic emissions and ABR wave-V is adopted. Cochlear gain loss was modeled as frequency-dependent auditory filter gain loss (and associated filter widening), and cochlear neuropathy through selective loss of all low- and medium spontaneous-rate auditory-nerve fibers. Both brainstem responses to transients and sustained responses were simulated for different combinations of hearing pathologies.

Model predictions show that the slope of the ABR wave-V latency as a function of stimulus level is only very little affected by cochlear neuropathy, but that it relates to the slope of the audiometric loss as a function of frequency. Amplitude-modulated stimuli are a good predictor for the degree of cochlear neuropathy in simulated envelope-following responses (EFR) when cochlear gain is intact. Simulated EFR strength was stronger for the 100 than 400 Hz -4 dB deep amplitude modulated noise of 75 dB SPL, and neuropathy reduced the EFR strength to the 100 Hz modulation frequency more so than to the 400 Hz modulation frequency. Cochlear gain loss affected both modulation frequencies equally, and did not add up linearly with how cochlear neuropathy reduced the responses. EFR strength in itself may thus not be a good predictor of the degree of cochlear neuropathy in listeners with audiometric losses. Rather, a difference measure between EFR strength to different amplitude-modulated stimulus configurations in the same listener may be able to circumvent the cochlear gain loss component to differentially diagnose the degree of cochlear neuropathy in these listeners.

Neural processing of unlearned calls in secondary auditory areas in a songbird.

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Bird vocalisation research has principally focused on songs, whereas the study of unlearned calls has not been given proper attention. We use zebra finches to tackle the neuro-ethological study of vocal communication including both learned and unlearned vocalizations. Our group is engaged in demonstrating the importance of unlearned calls in a communication network and the relevance of the telencephalic areas during calling exchanges. In a previous behavioural experiment we have shown that members of a pair use specific types of unearned calls to answer to each other in a precisely timed way. This means that once a pair is established, they will answer each other in a stereotyped fashion, both in the type of calls and the tempo. This process is called antiphonal calling or duetting. During the antiphonal calling, a bird has to provide the specific response within a few milliseconds of an auditory stimulus. When the bird hears a call, it needs to process it: extract the type of call and the individual identity, recall the memory of the different individual, and extremely rapidly choose and produce an answer. It is clear that this system is ideal to study the basics of vocal communication.

We know that songbirds perform well in standardized auditory tasks, thus they can potentially recognize the small spectral differences of the different types of call. The secondary auditory area, specifically the Caudomedial Nidopallium (NCM) and Caudomedial Mesopallium (CMM), are thought to be involved in memory storage of the conspecific's identity. These areas are often considered analogues to the human auditory association cortex. Furthermore, it has been shown that female zebra finches are able to differentiate between the learned calls of the mate and of unfamiliar males, and that the NCM responds in different ways, both in awake and anaesthetized animals. However, we still do not know how secondary auditory areas process the unlearned calls. Moreover, it is unknown if and how NCM and CMM interact during these processes.

We have run a 2 staged experiment. First a behavioural stage in which we aim to extend to unlearned vocalizations a behavioural paradigm designed to show that learned vocalizations are recognized individually. This paradigm uses a series of playbacks with different levels of familiarity while monitoring the vocal response. Second we use the very same set of playbacks to monitor the response of single units electro-physiologically in the two secondary auditory areas. We monitored the activity in the NCM and CMM in parallel, recording from both areas in anesthetized animals simultaneously. We compared electrophysiological responses during the playback of distinct call types and calls' familiarity.

We expect to find a differential neuronal activity depending on the area examined (i.e. NCM and CMM), the site (i.e. where specifically within the examined areas), the call familiarity (i.e. whether it comes from the mate or an unknown individual).

The results will be helpful to complete and understand the pathway for antiphonal calling, clarify the function of these areas in birds. In the future a further comparison with recordings from awakes freely behaving animals will show the differences in the processing between awake and anesthetized animals.

Off-Response Facilitation in Mouse Auditory Midbrain Neurons to Models of Communication Calls

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Low-frequency harmonically structured calls (wriggling-calls, WCs) of mouse pups (Mus musculus) release maternal behavior in their mothers. WCs consist of three main harmonics below 20 kHz where the main sound energy is concentrated. The actual frequencies vary with the mouse strain, and the lowest harmonic is mostly near 3 – 5 kHz. WCs are suggested to be perceived as an auditory object if the three main harmonics are processed by three neighboring critical band filters, the outputs of which are summed in a nonlinear way to generate optimum call recognition (Ehret, In: The Auditory Processing of Speech. Schouten MEH, ed, De Gruyter, Berlin, pp 99-112, 1992). In order to study mechanisms of auditory object formation by critical band filtering in the auditory midbrain, we recorded single-unit responses from the central nucleus of the inferior colliculus (ICC) to natural wriggling calls, to synthetic models of the calls composed of 5 + X + 15 kHz frequency components (X varied between 5.4 and 13.6 kHz), to single and pairs of these frequency components. In addition, excitatory and inhibitory frequency receptive fields of the neurons were characterized by tone responses (see Egorova et al., Exp Brain Res 140, 145-161, 2001).

The data analysis of 120 neurons in the ICC showed extreme heterogeneity of responses to the natural WC, the WC models and frequency pairs in the WC frequency range. In general, the responsiveness of the neurons was determined by the amount of integration of the frequency components in and their overlap with the excitatory and inhibitory receptive fields. Interestingly, off-responses of 37 units (31%) were facilitated by two-component signals, natural WC and/or WC models due either to the appearance or the significant increase of the off-response to the sound. The strongest facilitation occurred, if the frequency components were separated so that they could be processed by non-overlapping critical bands (the critical bandwidth is about 4 kHz in the respective frequency range; Ehret, Biol Cybernet 24, 35-42, 1976). In 14 neurons (12%), the off-component appeared only in response to pairs of harmonic components of the WC (5, 10, 15 kHz), the WC models, or the WC itself.

The data suggest that ICC neurons contribute to auditory object formation via the critical band mechanism, and off-responses of the neurons may help to integrate the auditory objects, WCs in this case, into a single perceptual stream (rhythm).

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Optimizing temporary inactivation methods to selectively define the importance of different rat auditory cortical areas

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To investigate the functional significance of a certain brain area, namely the auditory cortex (AC), former studies often used permanent inactivation approaches. Lesion experiments of the AC, for example, revealed its important role in the discrimination of frequency-modulated (FM) tones while the discrimination of pure tones was not impaired.
Various studies temporary inactivated the AC using different pharmacological methods in order to prevent other areas taking over certain functions of the inactivated region. This was repeated using different behavioral paradigms, however, with usually rather high dosages. In parallel several approaches were pursued: Talwar et al., 2001, J Nphys. 85:2350 showed a muscimol-induced impairment (up to loss of function) in tone detection and tone frequency discrimination tasks. Furthermore, Rybalko et al., 2010, Behav Brain Res 209:123 used muscimol-induced inactivation of the AC to impair temporal discrimination.
However, what general statements can be made by using different methods and behavioral parameters? Moreover, only the inactivation of the whole AC led to significant results in rodents. Thus, we started to investigate important factors influencing the temporary pharmacological inactivation of the rat AC with a focus on the functional significance of individual areas of the AC.
All animals were first trained in a 2-alternative-forced-choice paradigm (2AFC) either testing sound localization of a broadband noise (easy task) or to test discrimination between pure tone and narrow band noise (hard task). Afterwards, guide cannulas were bilaterally implanted in the AC. After a short retraining phase different portions of the AC were temporary inactivated in different animals.
In general, we revealed that also a temporary inactivation of a small portion of the AC can affect discrimination ability, reaction times (RT) and also the general performance (trials/min).
While optimizing the method we saw that the use of different drugs for inactivation can cause huge differences in effect size. Data from individual animals suggest that muscimol application leads to significantly stronger effects than the infusion of a comparable dose of a local anesthetic (lidocaine or ropivacaine).
It is remarkable, that even in a simple discrimination task the inactivation can cause different behavioral outcomes across animals. Some animals showed higher fault rates but nearly unchanged RTs or vice versa. Interestingly, all animals had a significantly increased number of trials/min. Any effect of the injection side (L/R) could not be shown so far.
Current approaches test whether these differences among behavioral outcomes are either caused by slightly different cannula positions (i.e. different inactivated areas) or by different behavioral strategies of individual animals during inactivation (of the same area).
The few data from testing performance in the hard discrimination task revealed that individual animals, again, showed only slightly changed fault rates, RTs or a higher number of trials/min.
Animals have so far not been tested in other perceptual tasks like FM discrimination. Such tests are presumably probing discrimination abilities closely related to auditory cortical processing.

Performance of glutamatergic synapses from the cochlear nucleus to the lateral superior olive during prolonged stimulation

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Ongoing neuronal activity can alter synapse function in several ways. One form of altered synapse function is short-term depression (STD), which is characterized by reduced amplitudes of the postsynaptic events. Principle cells of the lateral superior olive (LSO) receive glutamatergic input from neurons in the ipsilateral cochlear nucleus (CN). This projection is well suited to analyze the stability and/or vulnerability of neurotransmission in fast excitatory synapses during ongoing activity, including STD behavior. We here characterized the glutamatergic CN-LSO connection with the focus on the performance under prolonged stimulation comprising low and high frequencies. CN-LSO synapses were characterized in acute brainstem slices of P10-12 C57BL/6 mice. Excitatory postsynaptic currents (EPSCs) were recorded from LSO principal neurons in the whole-cell patch-clamp configuration at 37°C under strychnine blockade (500 nM). EPSCs were evoked by focal electrical stimulation of axons in the ventral acoustic stria. The challenging protocol consisted of 60 s of continuous stimulation, with stimulation frequencies ranging from 1 Hz to 333 Hz. Challenging phases alternated with 60-s-long recovery phases of 0.2 Hz stimulation. Besides basic kinetics, the peak amplitudes of evoked EPSCs (eEPSCs) were analyzed. Even at the lowest stimulation frequency of 1 Hz, the initial amplitude decreased to 80% after 60 s, thus demonstrating weak STD of 20%. STD also occurred at 2, 5 and 10 Hz and increased with stimulation frequency (22% at 2 Hz, 25% at 5 Hz, 30% at 10 Hz). At 20 Hz, the STD increased as evidenced by peak amplitudes of 65% within the first 20 s which reached steady state levels of 45% by 35 s. At 50 Hz, the STD was even more pronounced, mounting to peak amplitudes of 30% after 10 s and reaching steady state amplitudes of <20% at 40 s. At the highest frequency of 333 Hz, eEPSC amplitudes decreased within the first 10 s to values <20% and reached a steady state level below 10% after 15 s. The basic kinetics of eEPSCs did not change during prolonged stimulation. Furthermore, the number of failures increased as a function of stimulation frequency. For example, the fidelity rate dropped to <95% within the first 10 s at 10 Hz and to <70% within the first 10 s at 50 Hz. In summary, excitatory CN-LSO synapses are able to reliably follow stimulation frequencies from 1 to 10 Hz with no or little failures. Previous work of our group (Kramer et. al 2014, Front Neural Circuits) has shown that high frequency stimulation of the inhibitory projection from the medial nucleus of the trapezoid body to the LSO also leads to STD, but less profound (45% of initial amplitude at 50 Hz after 10 s, steady state level of 30%at 25 s). By contrast, inhibitory CN-LSO synapses (Weingarten et al, this meeting) show an even stronger STD (steady state level of 35% after 60 s at 20 Hz, <20% at 50 Hz). We conclude that the CN-LSO synapses maintain reliable synaptic transmission up to 50 Hz under prolonged stimulation conditions.

Phasic and tonic changes of neuronal activity in primate auditory cortex induced by the dopaminergic ventral midbrain

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This study was motivated by recent findings that activation of auditory cortex is related to motivational and cognitive aspects of auditory tasks. Because dopamine is involved in both aspects, we studied the effects of electrical stimulation of dopaminergic cell structures of the ventral midbrain on neuronal activity and sensory processing in primate auditory cortex.

We found that electrical stimulation of the dopaminergic ventral midbrain affected auditory cortex on two timescales. It induced phasic (< 1 second) activation and tonic (> 1 second) modulation of auditory cortex. Phasic activation was reflected in electrically evoked field potentials and excitatory and inhibitory multi- and single-unit responses, all of which could be reversibly altered by the dopamine D1-receptor antagonist SCH23390. Thus, we speculate that the dopaminergic ventral midbrain exerts a temporally precise, phasic influence on auditory cortex using fast-acting non-dopaminergic transmitters and that their effects are modulated by dopamine on a longer timescale. Tonic modulation was reflected in a decrease of the spontaneous firing and in a bidirectional modification of the power of auditory evoked potentials. We consider that the increase in the dopamine tone in auditory cortex induced by electrical stimulation of the dopaminergic ventral midbrain is responsible for these tonic modulations.

Our findings suggest that information such as the motivational value or the motivational salience, which is encoded in the neuronal firing in the dopaminergic ventral midbrain, could be conveyed to auditory cortex. Thus, the mesocortical pathway may contribute to the representation of non-auditory events in the auditory cortex and to its motivational and cognitive functions.

Pre- and postsynaptic refinements in the medial superior olive (MSO) during late postnatal development

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Binaural coincidence detector neurons of the MSO process sound localization cues with microsecond precision by integrating excitatory and inhibitory inputs. The inhibitory input to these neurons has been shown to refine around hearing onset in an activity-dependent manner, and is accompanied by severe developmental changes in the cell's biophysical properties. To further understand the refinement mechanism, we investigated the developmental regulation of calcium entry in MSO neurons. Calcium is an important second messenger potentially associated with developmental alterations. Whole-cell recordings were carried out in acute slices from Mongolian gerbils around hearing onset at postnatal day (P) 10-18 and at mature stages (P60) to image calcium transients evoked by synaptic and action potential (AP) stimulations. Even after hearing onset, a strong calcium influx could be evoked by both APs and EPSCs. However, from P13 onwards the AP-evoked calcium influx decreased rapidly to undetectable levels at P60 as a consequence of reduced AP amplitude and a downregulation predominantly of a T-type calcium current. Moreover, the NMDAR component of the EPSC declined drastically from P13 onwards. These developmental changes in calcium influx were accompanied by a refinement in the arrangement of presynaptic input fibres. However, at mature stages a significant dendritic calcium transient was still driven locally by the AMPAR component. Our data suggest that calcium entry shifts from its pre- and postsynaptic activity dependence to predominantly presynaptic activity dependence. However, the presence of AP- and EPSC-evoked calcium signals just after hearing onset suggests the persistence of refinements after hearing onset.

Role of microRNA-183-96 for development and function of the auditory brainstem

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The miR-183 cluster consists of three microRNAs, miR-183, -96 and -182, and is highly expressed in sensory cells. Inactivation of the miR-183 cluster in mice leads to retinal degeneration (Lumayag et al., 2012). Mutations in the miR-96 sequence are associated with deafness in both humans and mouse, caused by arrested hair cell development (Mencía et al., 2009; Lewis et al., 2009). We recently showed that miR-96 is postnatally upregulated in the mouse brainstem (Rosengauer et al., 2012). To further investigate the role of miR-96 in the auditory brainstem we performed anatomical studies of homozygote *dmdo* mice, which harbor a miR-96 point mutation and show peripheral deafness. Young-adult (P25-30) *dmdo* displayed a 25 to 35% volume reduction of the auditory cochlear nucleus complex and the superior olivary complex. Determination of the cross sectional area and cell countings identified reduced cell size as the main factor for this volume reduction. In contrast to adult mice, neonatal *dmdo* mice showed no significant volume reduction, while an intermediate reduction was observed in P4 mice. In order to examine, whether the observed reduction is confined to the auditory brainstem, volume measurement of the non-auditory 7th nerve nucleus was performed. Only a slide reduction of 7.5 % was seen in young-adult *dmdo* mice. On the back of this, deaf *claudin14*$^{-/-}$ mice were included to examine the contribution of peripheral deafness to the volume reduction in the auditory brainstem of *dmdo* mice. These mice display a cochlear phenotype similar to *dmdo* mice. No significant volume change of auditory structureswas observed in *claudin14*$^{-/-}$ mice. This indicates that the observed volume reductions in *dmdo* mice are due to an on-site effect of the mutated miR-96 and do not merely reflect a general degeneration of auditory brainstem structures in deaf mice. To explore an evolutionary role of miR-96 for central auditory structures, RNA *in-situ* hybridization in chicken was performed. A miR-183-96 probe revealed strong expression in the chicken auditory brainstem. All data together demonstrate that mutation in miR-96 affect postnatal development of the mouse auditory brainstem, implying an important role of miR-96 throughout the auditory brainstem. This assumption is supported by the expression pattern of miR-183-96 in the chicken auditory brainstem that points to an evolutionary conserved role of miR-96 in central auditory structures across different vertebrate groups.

Role of particulate guanylyl cyclase B/natriuretic peptide receptor 2 (GC-B/Npr2) in auditory function in adult mice

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The cyclic guanosine 3'-5'-monophosphate (cGMP) signalling pathway is involved in a variety of cellular processes, especially in cell growth and survival (Beavo and Brunton [2002], Kemp-Harper and Feil [2008]). In mammals, two types of cGMP generators (guanylyl cyclases, GCs) are known: the soluble NO-responsive GCs (NO-GCs) and the membrane-bound 'particulate' GCs (pGCs) which are sensitive for natriuretic peptides (Friebe and Koesling [2003], Kuhn [2003]).

This study elucidates the abundance and characteristics of the cGMP generating particulate guanylyl cyclase B/natriuretic peptide receptor 2 (GC-B/Npr2) in the auditory system and examines how a global deletion of this receptor protein affects the physiology and function of the adult peripheral (cochlea/auditory nerve) and central auditory pathway components (brainstem/thalamus/auditory cortex).

It was already shown that a global deletion of GC-B/Npr2 leads to a failure in sensory axon bifurcation at the dorsal root entry zone (DREZ)(Schmidt et al. [2007]) and results in a deficient bifurcation of all cranial nerves including the vestibulocochlear nerve (CN VIII) during embryonic development of the central nervous system (CNS) while growing into the hindbrain (Ter-Avetisyan et al. [2014]).

Using auditory evoked brainstem responses (ABR), distortion product otoacoustic emissions (DPOAEs) and immunohistochemical approaches, we examined the functional auditory phenotype of a global GC-B deficient mouse strain ($Npr2^{lacZ/lacZ}$). First data will be presented and discussed regarding the developmental deficits and the absence of GC-B/Npr2 protein expression in the adult auditory system, depicting the role of cGMP signalling on auditory processing *in vivo*.

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Sensorimotor feedback maintains auditory objects formation in zebra finches (*Taeniopygia guttata*)

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Sound cannot be known as an object independently without sensory experience (*Griffiths & Warren, 2004*). The brain must have a role in auditory object formation from acoustic events. Both birdsong and human language are learned vocalization and auditory objects. They are complex, diverse and rarely heard in isolation. Birdsong or speech is often heard in a competing acoustic environment. In an international cocktail party for instance, people are able to identify those who speak the same languages from background noise easier than those who speak other languages. It suggests that there must be neural mechanisms for a listener to maintain the sensitivity of his own language.

Patients with damage to the Broca's area suffered from aphasia, but also had difficulty in speech comprehension, which implies that sensorimotor feedback may influence speech perception. In zebra finch, the sensorimotor nucleus HVC (higher vocal center) corresponds functionally to Broca's area. Both HVC and Broca's area were mostly studied in sensory-motor interaction for speech production and learning, whereas sensorimotor feedback in speech perception is less known. The aim of this study is to investigate the influence of HVC on auditory object formation.

Short-term auditory adaptation to continuous and interrupted motion in human

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A lot of moving sound sources in natural environment has interrupted time structure. In spite of the fact majority of investigations is devoted to continuously moving sound sources' perception and to processing of information about the sources. Auditory adaptation to the type of movement or continuous motion aftereffect was investigated. The aftereffect manifested itself in changing of velocity's and direction's perception of moving sound source (test stimulus) after listening to another moving source (adapting stimuli). The auditory motion aftereffect is commonly associated with activity of motion detection neurons, which are responsible to a particular direction and range of velocities of movement. Do this type of neuronal structures also responsible to interrupted sound sources' localization too or the information is processed in different way? To response the question our investigation was undertaken.

Nine subjects with normal hearing (seven women and two men) were tested in our experiments. The illusion of sound source approaching or withdrawing was made by sequences of broadband noise bursts, which were presented through two loudspeakers placed in front of the subject at distances 1.1 and 4.5 m. Simultaneous increase of the amplitude of the noise bursts on the one loudspeaker and its decrease on the other one led to illusion of approaching or withdrawing sound source which had determined trajectory. Two types of signals were used in the experiment: one of them made illusion of interrupted motion, the other – illusion of continuous motion. Two adapting stimuli (one interrupted and one continuous) and four test stimuli (two interrupted and two continuous) were made. Two of four test stimuli had rhythmical structures not coinciding with structures of adapting stimuli, another two test stimuli had the same structures as adapting stimuli. It allowed us to estimate contribution of adaptation to rhythm into the aftereffect. In our experiments we used short-term adaptation to approaching sound source, because as it is known the type of adaptation is connected with sensory processing of information, while long-term adaptation is commonly associated with response bias (Grantham, 1979). Changes in perception of motion direction of test stimuli after 5 s adaptation to continuous and to interrupted motion were registered. The estimation of the effect was made by subtracting percentage of answers «test stimulus withdraws» to all test stimuli after adaptation and without it (control conditions). Statistical reliability of the estimation proceeded by using non-parametric Wilcoxon matched pair test. Adaptation to motion was observed for both types of auditory motion. Statistically reliable changes in perception of motion direction of continuous and interrupted sound sources were shown only after adaptation to the same type of motion ($p<0.01$). Changes in perception of direction of test stimuli were reliably larger in case of coinciding rhythms of test and adapting stimuli ($p<0.01$). These results indicate that localization of interrupted and continuous auditory motion is processed by different neuronal structures.

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Short-term plasticity in the auditory brainstem and the hippocampus: a comparative study of three synaptic systems

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Short-term plasticity (STP) plays an important role in modulating synaptic transmission by facilitation or depression in the millisecond to second range. STP is use-dependent and has to be adapted to the functional requirements of the particular neuronal circuit. The superior olivary complex (SOC) is part of the auditory brainstem where signals originating from both ears are analyzed. This process is characterized by fast and precise neurotransmission, even at very high frequencies (>100 Hz), and ensures correct signal processing. SOC neurons display continually high rates of spontaneous activity *in vivo*, with firing rates of 35 spikes/s and up to 107 spikes/s in few cases (Sonntag *et al*. 2009, J Neurosci). In contrast, *in vivo* firing rates of neurons in the hippocampal formation, one of the most intensely studied brain structures, are drastically lower (0.14 spikes/s, Okamoto *et al*. 2014, J Physiol). A direct comparison of the synaptic performance to various stimulation frequencies, including STP behavior, is lacking between the SOC and the hippocampus. To fill the gap, we here characterize three synaptic systems in a comparative approach: 1.) The inhibitory, glycinergic connection between the medial nucleus of the trapezoid body (MNTB) and the lateral superior olive (LSO) in the SOC. 2.) The inhibitory, GABAergic connection between the entorhinal cortex (EC) and the dentate gyrus (DG) in the hippocampal formation. 3.) The excitatory, glutamatergic synapses between CA3 region and CA1 region in the hippocampus. To do so, whole cell patch-clamp recordings were performed in acute brain slices of P10-12 C57BL/6 mice at 37° C. Neurons were challenged via focal electrical stimulation of the presynaptic fibers at frequencies of 1-100 Hz for several tens of seconds. Each challenge period was followed by a recovery period at low frequency stimulation (0.2 Hz or 1 Hz). Paired pulse facilitation occurred in CA3-CA1 synapses at interstimulus intervals of 20 ms to 100 ms. In contrast, none of the two inhibitory connections showed facilitation of ePSC peak amplitudes. All synaptic systems showed a frequency-dependent depression of the ePSC peak amplitudes during challenge periods. Only minor differences were observed between both hippocampal systems and the MNTB-LSO synapses if stimulation frequencies were <10 Hz. At stimulation frequencies =50 Hz, the MNTB-LSO synapses showed 3-fold higher steady state ePSC peak amplitudes (50 Hz: MNTB-LSO, 30 %; CA3-CA1, 10 %; EC-DG, 10 %), implying much weaker depression. Full recovery (>90 %) of ePSC peak amplitudes was observed after the 50 Hz challenge at the MNTB-LSO synapses, whereas the hippocampal synapses recovered only partially (CA3-CA1, 25 %; EC-DG, 65 %). The fidelity analysis (ratio of responded stimuli/given stimuli) also showed a better performance of the MNTB-LSO synapses compared to the hippocampal systems. At stimulation frequencies <10 Hz, differences were only minor. In contrast, MNTB-LSO synapses showed a 6- to 18-fold higher fidelity than the hippocampal synapses when challenged at 50 Hz (MNTB-LSO, 90 %; CA3-CA1, 15 %; EC-DG, 5 %). In summary, moderate depression and high fidelity during high frequency transmission, a hallmark of auditory brainstem synapses, was not found for the hippocampal systems. We conclude that the extent of STP differs considerably between various synaptic systems as an adaption to the functional requirements of the particular neuronal circuit.

Startle-based tinnitus assessment in rodents: improving effectiveness and reliability

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Gap-prepulse inhibition (gap-PPI) measurements of the acoustic startle response (ASR) is a common method for objective tinnitus assessment in laboratory animals. Animals with tinnitus have deficits in gap detection and therefore show lower gap-induced startle response inhibition values. Repeated startle measurements are required for tracking long-term changes of trauma-induced alterations finally leading to the formation of tinnitus. Thus, it is necessary to ensure reliable results from gap-PPI measurements that can be compared over an extended period of time. For that purpose, we investigated the optimization of important stimulus parameters, like gap duration, that influence gap-detection measurements. Further we analyzed frequency-dependence of the ASR in the gap paradigm and the spontaneous activity. Mongolian gerbils (Meriones unguiculatus) and Sprague Dawley rats, two established animal models in tinnitus research, were used in this project for comparison.

Beside the used frequency range and sound pressure level, the duration of the gap-prepulse is important for reliable detection of tinnitus. Therefore, we performed startle measurements with gerbils (n=8) using 50, 200, 500 and 1000 ms long gaps in the background noise. We found that gap-PPI was more stable over all measured frequencies (8, 10, 12, 18 kHz and broadband noise, BBN) with 500 ms long gaps than with 50 ms gaps, meaning that the variance of values was smaller. This could results in more reliable results for tinnitus investigations. Therefore longer gap durations, like 500 ms, are more suitable to detect trauma-induced effects such as tinnitus. Experimentally-induced tinnitus, as it seems, is a rather weak signal which is less noticeable in a short gap and which might be detected more certain in longer gap durations than the regular ones.

Another point concerns the dependency of the absolute startle response in the gap paradigm on the used frequency band (± 0.25 oct) of the background noise and across measurements over time. Gerbils (n = 8) showed a continuous amplitude decrease of the ASR from low (4 kHz) to high frequencies (18 kHz), whereas rats (n = 8) showed now frequency-dependency of the amplitude. However, always salient in both animal groups was the high absolute startle response in gap-PPI measurements with BBN (1.5 – 20 kHz) as background noise. Long-term habituation was not observed which indicates comparable startle measurements over a long period. Spontaneous motor activity was also analyzed in both species. We found that gerbils show a nearly two-fold higher spontaneous activity than rats which was higher in gap paradigms with either BBN or narrowband (± 0.125 oct) background noise. This could cause misleadingly low gap-PPI values.

Taken together, gap-PPI-based tinnitus assessment can further be improved by optimizing stimulation parameters. We found that longer gap duration can significantly improve tinnitus detection by less variable base line values. Comparability between the necessarily repeated measurements over an extended time of tinnitus development can be ensured by tracking ASR amplitudes and spontaneous motor activity.

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Structural and Functional Changes in the Mouse Central Auditory Pathway after Noise Exposure

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A noise trauma leads beside damage in the periphery to profound changes in the central auditory system as well. Recent work demonstrated that noise exposure has a strong and rapid impact on central neuroanatomy and neurophysiology. A modified spontaneous activity, changes in cell density and neurotransmitter action were reported for several auditory structures. It is still unclear how far these changes are based on the reduced input from the noise-damaged organ of corti (deprivation) or on a direct traumatizing impact on central auditory structures. It is therefore highly important to identify the temporal pattern of the underlying mechanisms to understand the development of noise-induced hearing loss. It was the aim of our studies to investigate structural and functional changes in auditory brain structures at different time points after noise exposure. Normal hearing mice (NMRI strain) were exposed to a broadband noise (5-20 kHz, 115 dB SPL) for 3 hours under anaesthesia and have been investigated immediately (acute) or 7 or 14 days after trauma. Hearing thresholds were determined by auditory brainstem response audiometry (ABR). To investigate changes in spontaneous neural activity in vitro, electrophysiological single-unit recordings were carried out in brain slices of auditory brainstem and midbrain structures. Further, calcium-dependent neural activity was measured using manganese-enhanced magnetic resonance imaging (MEMRI). Signal strengths in several central auditory structures were analysed in noise-exposed animals. In addition, cell densities as well as cell death pathways (HE and TUNEL staining) were investigated in auditory brain areas to identify central neurodegeneration. For all investigations, unexposed mice served as normal hearing controls. The ABR results demonstrated that hearing loss in the animals was highest immediately after noise exposure and recovered significantly within one week. However, a significant permanent threshold shift was present even at day 14 after noise exposure. Spontaneous neuronal activity was significantly increased in brainstem structures of the acute group, but declined within one week after noise trauma. After 14 days, spontaneous activity was significantly elevated in the lower auditory pathway. In contrast, calcium-dependent activity showed a significant increase immediately after trauma in the brainstem, followed by an enhancement in higher structures within one week. Interestingly, MEMRI signal decreased to control levels until day 14 in most investigated brain areas. Histological investigations further demonstrated strong neurodegeneration in the entire auditory pathway. Cell death mechanisms started immediately after noise exposure and seemed to persist for days or weeks.The data indicate that a noise trauma is acutely affecting central cellular properties and induces short-term plasticity, particularly in brainstem structures. Furthermore, long-term physiological alterations seem to appear slowly and might thus differ from the early effects. It could be hypothesized that noise-induced neurodegeneration during the first days after trauma induces compensating neuroplasticity, which in turn could lead to pathophysiological changes in central neuronal transmission of auditory information. The present findings might help to better understand the complex changes after noise-induced hearing loss, playing an important role in several psychoacoustic phenomena like tinnitus or hyperacusis.

Temporal Precision of sound-onset coding in the Mouse Auditory Brainstem

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Auditory brainstem evoked responses (ABR) usually lead to 5 waves. The peaks of the waves reflect the summed synchronized excitatory postsynaptic potentials (EPSPs) to sound onsets from the cochlea (peak 1, P1), the cochlear nucleus (P2), the superior olivary complex (P3), the superior olive plus lateral lemniscus plus inferior colliculus (P4), and the lateral lemniscus plus inferior colliculus (P5). The better the EPSPs are synchronized, the higher are the wave amplitudes and the narrower are the wave widths, which means less jitter of wave latencies. Therefore, the degree of variation of wave latencies can be taken as measure for the precision of temporal coding of sounds of certain properties in the auditory brainstem centers. Here, we report data of ABR recordings from 7 ketamin-xylazin anesthetized adult NMRI mice per experiment.

Silver wire skin electrodes were placed over the contralateral colliculus inferior and the bulla ipsilateral to the loudspeaker emitting the following sounds freefield. Experiment 1: Series of four 50 kHz tones (duration 10 – 150 ms including 1 ms rise/fall time, 200 ms interval between the tone bursts, 75 dB SPL), mimicking mouse pup ultrasounds. Experiment 2: Pairs of a noise burst (7 ms including 1 ms rise/fall, 50 dB SPL) followed by a 50 kHz tone (50 ms including 1 ms rise/fall, 75 dB SPL) with variable intervals (0 – 100 ms) between the two sounds, mimicking a phoneme of a stop consonant and a vowel. Experiment 3: Series of four harmonic complexes (frequencies of 3.8 + 7.6 + 11.4 kHz, 100 ms duration including 2 ms rise/fall times, 60.5 dB SPL of each harmonic) with variable intervals (10 – 700 ms) between the complexes, mimicking series of mouse pup wriggling calls. The series or pairs of the sounds were repeated 500 times with 1 s interval between the repetitions.

Among our results are the following: (1) Standard deviations (SDs) of average peak latencies to 50 kHz series are 10 – 15% of the peak latencies for all waves. Average latency differences between the wave peaks are about 0.7 – 1.2 ms with SDs of only 2 – 5%. (2) SDs of average peak latencies to noise bursts are only 1 – 2% of the peak latencies for all waves. SDs of average latencies to the 50 kHz tone in the noise-tone pair are about 10 – 30% of the peak latencies for all waves and independent of the interval length. Average latency differences between the wave peaks are about 0.8 – 1.2 ms with SDs of 1 – 6% for the noise and 10 – 30% for the 50 kHz tone. (3) Average latencies of P2 – P5 in response to series of harmonic complexes may significantly be prolonged by about 10% depending on the interval between the sounds for intervals of 50 ms and shorter. This latency adaptation neither influences the average precision (SD) with which the peaks occur nor the precision of the latency differences between the wave peaks.

We conclude that the absolute and relative temporal precision of synaptic transfer in auditory brainstem centers is high, especially in response to noise bursts. SDs of the average latencies of P1 and P2 and the latency differences between both are near 10 µs or 1%. The latencies may be influenced by preceding sounds which, depending on the interval length between and the kind of sounds, may reduce the precision of coding or delay the response.

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Temporal response properties in the receptive fields of mouse primary auditory cortex neurons

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The mouse auditory cortex (AC) contains the three primary fields: AI (primary field), AAF (anterior auditory field) and UF (ultrasonic field) (Stiebler et al. J Comp Physiol A 181, 559-571, 1997). Different from higher-order fields, neurons in primary fields are thought to be important for coding of sounds in the time domain (Joachimsthaler et al. Eur J Neurosci 39, 904-918, 2014). Here we test whether neurons in the primary fields code sound onsets with high stability and precision.

Tone-response latencies and temporal response patterns across frequency-level combinations in the excitatory frequency-receptive fields of 141 left-hemisphere primary AC neurons were studied by extracellular single-unit recordings in ketamine/xylazine anesthetized mice. Based on the mapping of isofrequency lines, 77 units were referred to AI, 50 to AAF, and 14 to UF. Tone bursts were 110 ms long (including 5 ms linear rise and fall times) and had 900 ms inter-burst intervals. Frequency-receptive fields were analyzed from response threshold up to 85 dB SPL (see Egorova J. Evol Biochem Physiol 41, 476-480, 2005). Among our results are the following: (1) Temporal response patterns of all recorded units in the three fields were the same, i.e. only phasic responses occurred. (2) Only 5 neurons (3.5%) had constant (+/- 2 ms) tone-response latencies through the whole frequency receptive field (4 units were located in the AAF, one – in A1). (3) Response latency within the receptive field of the other 136 neurons was very divers, varying between 8 and 96 ms. In 40% of the units (56 neurons), receptive fields could be divided in a peripheral belt close to the frequency tuning curve with latencies exceeding 40 ms and a core part covering frequencies at levels about 20 dB above the threshold at a given frequency. In the core part latencies were more stable but still varied between 8 and 47 ms.

These data show that despite the phasic discharges, which are reliably coupled to the tone onsets, the great majority of neurons in the primary fields AI, AAF and UF do not have stable response latencies in their frequency-receptive fields. Since stability of response latencies to tone onsets seems to be necessary for coding tone series with high precision, we suggest that this precision is lost in the AC compared to the midbrain level (central nucleus of the inferior colliculus) where nearly 50% of the neurons had latency variations of less that +/-7 ms (see poster by Egorova et al. Dynamics and precision of temporal responses in the mouse inferior colliculus).

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The continuous motion perception thresholds for approaching sound images with different rhythmic structures

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Biologically significant signals are rhythmic or quasi-rhythmic structures with different time characteristics, for example, steps of man and animals, communicative signals produced by animals during movement (Catchpole, Slater, 1995; Wilden et al., 1998), human speech (Zellner, 1994). Localization of moving sources that emit sounds with different ratios of the pulse duration and pause almost not investigated. The aim of our study was to determine the continuous motion perception thresholds in terms of pause and period of pulse repetition for approaching sound images with different rhythmic patterns and comparison of perceptive conditions required for sequences of short and long pulses. The illusion of approaching sound source was created by the noise pulses sequences with linearly increasing amplitude. The sequences were emitted by one loudspeaker, placed at the level of the head of the subject at a distance of 1.1 m in free field. The modeling of sound source approaching were performed by using sequences of broadband noise pulses which had duration of 5 and 10 ms (short), as well as 70 and 100 ms (long), and separated by pauses in the range from 10 to 150 ms in steps of 10 ms. The amplitudes of the sequence pulses were linearly increasing or decreasing up to 40 dB to imitate approaching or withdrawing sound images. The maximum intensity level was the same at the subject's place for all stimuli and accounted for 67 dB SPL. The duration of the sequences was 1.0 or 0.4 s. Comparative analysis of the individual pause thresholds for the continuous motion perception had the highest values in case of short noise pulses compared to the long, regardless of the total sequence duration. For sequences with short pulses thresholds were 50 and 40 ms, and for ones with long pulses they had fallen to 20 and 15 ms, respectively. The period thresholds for sequences of short pulses were 55 and 50 ms and the thresholds did not significantly differ between themselves. For sequences of long pulses the thresholds were almost twice bigger and amounted 90 and 115 ms. Large individual variability of continuous motion perception thresholds was observed when the illusion of approaching sound source was created with short noise pulses. When the duration of noise pulses was 5 ms the pause thresholds varied in the range from 10 to 90 ms, and when the duration of the pulses was 10 ms the range was from 10 ms to 70 ms. According to the model of integration of information about sound source's position in a fixed time window ("snapshot theory"), we proposed that in case of short noise pulses the time during which the auditory system received this information was quite small compared to the duration of the window and as a result the perception of motion could be ambiguous. This ambiguity was reflected in the variability of the continuous motion perception threshold for sequences of short noise pulses. For the long pulses (about tens of ms) subject's thresholds were invariable. Perhaps, non-simultaneous masking plays a critical role in the perception of the quality of movement, which is confirmed by the coincidence of the values of the continuous motion perception thresholds and the effective time of the masking for the sequence of noise pulses. The work was supported by RFBR, grant N 15-04-02816.

The role of L-Type Ca^{2+}-channels for development and function of the auditory brainstem

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The auditory brainstem harbours essential centres for sound processing. The cochlear nucleus complex (CNC) is the first centre to process acoustic information and represents the obligatory gateway to the central auditory system. From there, the information is distributed to distinct ascending pathways in the brainstem such as the superior olivary complex (SOC), which represents the first binaural processing centre.

The molecular mechanisms that underlie correct formation and function of the auditory brainstem are poorly understood. Our data reveal an essential role of the two L-type Ca^{2+}-channels $Ca_V1.2$ and $Ca_V1.3$ for proper development and function of auditory brainstem nuclei in mice. Both channels, encoded by the genes *Cacna1C* and *Cacna1D*, show an overlapping expression pattern in auditory brainstem neurons. But lack of isoform specific inhibitors precluded the elucidation of their individual role for a long time. To clarify their precise contribution to the development and function of the auditory brainstem, we used a conditional knockout approach in mice, using an *Egr2::Cre* driver line for channel-specific deletion in major auditory brainstem nuclei. Targeted deletion of *Cacna1D* resulted in abnormal morphology and approximately 45% volume reduction in the nuclei of the SOC and strongly altered auditory brainstem responses (ABRs), demonstrating an essential on-site role of $Ca_V1.3$ in the auditory brainstem (Satheesh et al., 2012).

Here, we report on our investigation of $Cacna1C^{Egr2}$ mice, lacking $Ca_V1.2$ in the auditory brainstem. Similar to $Cacna1D^{Egr2}$ mice, nuclei of the CNC and SOC of $Cacna1C^{Egr2}$ mice showed a significant volume reduction of 35% – 40% at postnatal day (P) 25 compared to control mice. Cell counts of selected nuclei identified a reduction of neurons as the underlying mechanism. Interestingly, histological analysis of to $Cacna1C^{Egr2}$ and to $Cacna1D^{Egr2}$ animals at P0 demonstrated normal volume and cell number of the MNTB, revealing an essential role for L-type Ca^{2+}-channels in postnatal survival rather than birth or migration of brainstem neurons.

Analyses of biophysical properties of LSO neurons in $Cacna1C^{Egr2}$ mice demonstrated normal firing pattern and unchanged action potential characteristics such as peak amplitude and afterhyperpolarization. However, the half width of action potentials was reduced by 36%. In order to analyze also functional consequences of deleting $Ca_V1.2$ in the auditory brainstem, ABRs were measured. They showed nearly normal wave forms. The only difference was an increase in the interindividual variation of the latency of negative peaks III – V. This contrasts the strong alteration in ABRs observed in $Cacna1D^{Egr2}$ mice.

In summary, these results identify overlapping as well as distinct functions of L-type Ca^{2+}-channels $Ca_V1.2$ and $Ca_V1.3$ in the developing auditory brainstem.

Tinnitus development after repeated acoustic overstimulation

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A general cause of tinnitus induction is the exposure to (very) loud noise. We are investigating these aspects of tinnitus induction in Mongolian gerbils, an animal model with low frequency hearing in a range similar to humans. The goal of this study was to find important parameters of noise stimulation determining the degree of noise-induced hearing loss (NIHL) and the development of tinnitus over time with repeated acoustic overstimulation.
The NIHL was detected using auditory brainstem measurements before and after the overstimulation. Tinnitus-related changes were studied by behavioral measurements. Noise stimuli for overstimulation around a center frequency of 8 kHz (1h, 105 dB SPL) had different bandwidths (0.25 and 0.5 oct) and were applied repeatedly with eight weeks in-between on 29 adult Mongolian gerbils (Meriones unguiculatus).
Independent of the noise bandwidth used, all applied overstimulation generated comparable threshold shifts immediately after their application by about 15 (at 8 kHz) to 30 dB (at 12 kHz). A repetition of the overstimulation after five weeks led in case of the small bandwidth noise (0.25 oct) to a permanent threshold shift while the number of animals developing tinnitus was low. In contrast, the use of the broader trauma of 0.5 oct only led to a temporary threshold shift, however a higher number of animals showed tinnitus-related changes. In addition, the frequency distributions of the found tinnitus-related changes were different in both groups. While the changes in the group stimulated with the small bandwidth of 0.25 oct were found only at 10-12 kHz, changes in the group stimulated with a bandwidth of 0.5 oct exhibited two maxima with changes in many animals at 8-10 kHz and at 16-18 kHz. The second overstimulation again induced a temporary threshold shift. However, only overstimulation with a small bandwidth of 0.25 oct led to an increase in the number of animals with tinnitus-related changes. In summary, our study shows that the noise bandwidth of overstimulation can strongly affect the long-term changes related to tinnitus.

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Poster Topic

T19: Chemical Senses: Olfaction, Taste, Others

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Monika Gorin, Marc Spehr

T19-2A “Low energy balance” hormones modulate the olfactory responsiveness
Diana Loch, Heinz Breer, Jörg Strotmann

T19-3A 3D-reconstruction as a tool to study postmetamorphic changes in neuropeptide pattern in the antennal lobe of *Tribolium castaneum*
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T19-4A A CD36 expressing subset of murine olfactory neurons is involved in fatty acid detection.
Eva M. Neuhaus, Sonja Oberland, Tobias Ackels, Stefanie Gaab, Thomas Pelz, Marc Spehr

T19-5A A_1 receptor-mediated modulation of recurrent inhibition in mouse olfactory bulb mitral cells
Kristina Schulz, Natalie Rotermund, Christian Lohr, Daniela Hirnet

T19-6A Activation of the OR37 subsystem coincides with a reduction of novel environment induced activity within the paraventricular nucleus of the hypothalamus
Anna-Maria Maier, Bettina Klein, Verena Bautze, Jörg Strotmann

T19-7A Adenosine receptor A_1 modulates the potassium conductance of olfactory bulb mitral cells
Svenja Winandy, Nadine Breitkreutz, Natalie Rotermund , Christian Lohr, Daniela Hirnet

T19-8A Adenosine-mediated modulation of olfactory bulb network activity and odour information processing
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T19-9A Anatomical and Functional Organization of the *Drosophila* Antennal Lobe: Glomerular Convergence and Divergence
Veit Grabe, Amelie E. E. Baschwitz, Bill S. Hansson, Silke Sachse

T19-10A Brain composition in *Godyris zavaleta,* a diurnal butterfly, reflects an increased reliance on olfactory information
Swidbert Roger Ott, Stephen H Montgomery

T19-11A Ca^{2+} Dependent K^+ Currents in Uniglomerular Olfactory Projection Neurons of the Antennal Lobe
Viktor Bardos, Cathleen Bradler, Ben Warren, Sabine Schleicher, Andreas Klein, Peter Kloppenburg

T19-12A Ca^{2+}-activated Cl^{-} currents in the vomeronasal organ are mediated by Anoctamin 1 and Anoctamin 2
Jonas Münch, Gwendolyn Billig, Thomas J. Jentsch

T19-13A Chemo- and thermosensory signaling in the Grueneberg ganglion
Joerg Fleischer, Katharina Schellig, Sabrina Stebe, Ying-Chi Chao, Ruey-Bing Yang, Heinz Breer

T19-14A Chemosensory characterisation of antral G-cells and its nutrient-induced modulation of genexpression
Amelie Therese Rettenberger

T19-15A Coexpression of Anoctamins in cilia of olfactory sensory neurons
Daniela Ricarda Drose, Bastian Henkel, Tobias Ackels, Marc Spehr, Eva Maria Neuhaus

T19-16A Co-expression of six tightly clustered olfactory receptor genes in the antenna of the malaria mosquito *Anopheles gambiae*
Jürgen Krieger, Tim Karner, Isabelle Kellner, Anna Schultze

T19-1B Compartment-specific calcium handling properties in olfactory projection neurons
Debora Fuscà, Ben Warren, Andreas Pippow, Christophe Pouzat, Peter Kloppenburg

T19-2B Compound valence is conserved in binary odor mixtures in *Drosophila melanogaster*
Michael Thoma, Bill S. Hansson, Markus Knaden

T19-3B Disruption of Kcc2 from mitral cells of the olfactory bulb leads to specific odor discrimination defects due to disregulated GABAergic inhibition
Kathrin Gödde, Dmytro Puchkov, Carsten K. Pfeffer, Thomas J. Jentsch

T19-4B Effect of piperine and other sensory active compounds on human KCNK channels of the TASK and TRESK subfamilies
Leopoldo Raul Beltran, Madeline Beltran, Caroline Flegel, Sascha Titt, Günter Gisselmann, Hanns Hatt

T19-5B Effects of neonicotinoid exposure on the honey bee odor coding
Mara Andrione, Martina Puppi, Quentin Badouin, Albrecht Haase, Renzo Antolini, Giorgio Vallortigara

T19-6B Effects of sublethal doses of a neonicotinoid insecticide on the olfactory system of a moth
Kaouther RABHI, Kali Esancy, Elodie Demondion, Philippe Lucas, Hélène Tricoire-Leignel, Sylvia Anton, Christophe Gadenne

T19-7B Electrophysiological Analysis of Concentration Coding and Learning in the Honeybee Dual Olfactory System
Maren Reuter, Martin Strube-Bloss, Martin Brill, Wolfgang Rössler

T19-8B Entrained Slow Oscillatory Activity in Mitral Cells of the Mouse Accessory Olfactory Bulb
Chryssanthi Tsitoura, Katja Watznauer, Monika Gorin, Marc Spehr

T19-9B Evidence for daytime- and pheromone dose-dependent metabotropic signal transduction cascades in the hawkmoth *Manduca sexta*
Petra Gawalek, Robin Schumann, Thomas Schendzielorz, Andreas Nolte, Monika Stengl

T19-10B *Ex vivo* functional imaging of olfactory sensory neurons in *Drosophila melanogaster*
Fabio Miazzi, Sabine Kaltofen, Bill S. Hansson, Dieter Wicher

T19-11B Expression of gustatory receptors in chemosensory organs and the alimentary tract of *Heliothis virescens*
Jennifer Reuck, Heinz Breer, Jürgen Krieger

T19-12B Functional imaging of cortical feedback projections from the anterior olfactory nucleus to the mouse olfactory bulb
Markus Rothermel, Matt Wachowiak

T19-13B Glomerular structure of the central nervous projections from scorpion pectines: Structural details and gender differences
Harald Wolf

T19-14B High conservation of family size and genomic structure of *ora* olfactory receptor genes in twelve teleost species
Veronika Zapilko, Sigrun Korsching

T19-15B High fat feeding affects the number of GPR120 cells and enteroendocrine cells in the mouse stomach
Patricia Widmayer, Hannah Goldschmid, Helena Henkel, Markus Küper, Alfred Königsrainer, Heinz Breer

T19-16B Identification of a natural source for the OR37B ligand
Verena Bautze, Wolfgang Schwack, Heinz Breer, Joerg Strotmann

T19-1C Illuminating the function of inhibitory microcircuits in the zebrafish homologue of olfactory cortex
Thomas Frank, Rainer W Friedrich

T19-2C Imaging molecular vibration sensing in honeybee olfactory circuit
Marco Paoli, Elisa RIgosi, Roberto Canteri, Giorgio Vallortigara, Renzo Antolini, Albrecht Haase

T19-3C Involvement of the GPCR, DopEcR, in the modulation of olfactory-guided behaviour in a moth
Sylvia Anton, Antoine Abrieux, Line Duportets, Stéphane Debernard, Christophe Gadenne

T19-4C Kenyon cell plasticity in adult red flour beetles
Björn Trebels, Joachim Schachtner

T19-5C Magnetic field-driven induction of ZENK in the trigeminal system of pigeons (Columba livia).
Nele Annika Lefeldt, Dominik Heyers, Nils-Lasse Schneider, Svenja Engels, Dana Elbers, Henrik Mouritsen

T19-6C Metamorphotic remodeling of the olfactory organ of the African clawed frog *Xenopus laevis*
Katarina Dittrich, Thomas Hassenklöver, Ivan Manzini

T19-7C Molecular basis of olfactory receptor evolution in closely related *Anopheles* mosquitoes
Stefanie Blankenburg, Sharon R. Hill, Rickard Ignell

T19-8C Molecular elements in the detection of the minor sex pheromone component in *Heliothis virescens*
Monika Zielonka, Pablo Pregitzer, Jürgen Krieger

T19-9C Morphological analysis of mitral cell populations in the mouse accessory olfactory bulb
Katja Watznauer, Monika Gorin, Marc Spehr

T19-10C Multimodal coding in the gustatory system of the Drosophila larvae
Lena van Giesen, Simon G. Sprecher

T19-11C NEUROCELLULAR BASIS OF OLFACTORY ACUMEN IN THE AFRICAN GIANT RAT (Cricetomys gambianus)
Matthew Ayokunle Olude, James Olukayode Olopade, Amadi Ogonda Ihunwo

T19-12C Neuropeptides and Blood Feeding Behavior – A Quantitative Analysis in *Aedes aegypti*
Peter Christ, Anna Reifenrath, Jörg Kahnt, Sharon R. Hill, Frank Hauser, Rickard Ignell, Joachim Schachtner

T19-13C Neuropeptides and Mating Behavior – A Quantitative Analysis in *Aedes aegypti*
Anna Reifenrath, Peter Christ, Jörg Kahnt, Sharon R. Hill, Frank Hauser, Rickard Ignell, Joachim Schachtner

T19-14C Octopamine Modulates the Excitability of Identified Olfactory Interneurons in the Cockroach Antennal Lobe
Sandra Wendler, Cathleen Bradler, Peter Kloppenburg

T19-15C Odor responses of *Drosophila* receptor neurons – Response profiles, mixtures and individual response dynamics
Daniel Münch, Thomas Laudes, Jennifer S. Ignatious Raja, Anja Nissler, C. Giovanni Galizia

T19-16C Olfactory imprinting and pERK related cellular activity in the zebrafish olfactory system
Daniela Biechl, Kristin Tietje, Iori Namekawa, Rainer Friedrich, Mario F. Wullimann, Gerlach Gabriele

T19-1D Orco expression during larval and pupal development of the red flour beetle *Tribolium castaneum*
Joachim Schachtner, Carolin Knoll, Stefan Dippel, Björn Trebels

T19-2D Organization of the blood–brain barrier in the olfactory nerve layer
Antonia Benita Beiersdorfer, Janine Grawe, Melissa Malekpour, Kristina Buddrus, Hartwig Wolburg, Christian Lohr

T19-3D Origin of high variability in colony odor representation in the ants' brain

Stefanie Neupert, Bernd Meyer, Christoph J. Kleineidam

T19-4D Physiological characterization of formyl peptide receptor expressing cells in the mouse vomeronasal organ
Tobias Ackels, Benoît von der Weid, Ivan Rodriguez, Marc Spehr

T19-5D Postmetamorphic plasticity in the intrinsic neuropeptide repertoire of the *Tribolium castaneum* antennal lobes
Stefan Ries, Christoph Nolte, Marlene Binzer, Jörg Kahnt, Joachim Schachtner

T19-6D Post-stimulus activity in the olfactory pathway of *Drosophila*
Alja Lüdke, Georg Raiser, C. Giovanni Galizia, Paul Szyszka

T19-7D Regeneration in the insect olfactory system
Hannah Wasser, Michael Stern

T19-8D Representation of odor information in higher brain centers of the vinegar fly
Amelie Erika Elfriede Baschwitz, Jan Sölter, Antonia Strutz, Bill S. Hansson, Silke Sachse

T19-9D Sulfated Steroids are Chemosensory Stimuli of Both the Main and Accessory Olfactory System of *Xenopus laevis*
Alfredo Sansone, Thomas Hassenklöver, Ivan Manzini

T19-10D Temporal processing of odor stimuli from Olfactory Receptor Neurons to Projection Neurons
Carlotta Martelli, Andre' Fiala

T19-11D The neuronal and molecular basis of caffeine taste signaling in *Drosophila* larvae
Anthi A. Apostolopoulou, Saskia Köhn, Michael Lutz, Alexander Wüst, Lorena Mazija, Anna Rist, C. Giovanni Galizia, Alja Lüdke, Andreas S. Thum

T19-12D To go or not to go? - Behavioral responses to binary mixtures of attractive and aversive odors
Tom Retzke, Ahmed A. M. Mohamed, Markus Knaden, Silke Sachse, Bill S. Hansson

T19-13D To go or not to go? Olfactory processing of odor features: Good vs. Bad
Ahmed A.M. Mohamed, Tom Retzke, Markus Knaden, Bill S. Hansson, Silke Sachse

T19-14D Two octopaminergic VUM neurons bilaterally innervate muscles and sensory epithels of the honeybee antenna
Stephan Shuichi Haupt

T19-15D Unique connectivity from OR37 expressing olfactory sensory neurons to higher brain centers
Jörg Strotmann, Andrea Bader, Bettina Klein, Heinz Breer

T19-16D Vasopressin depresses excitatory synaptic transmission in mitral cells of the olfactory bulb
Michael Lukas, Veronica Egger

"Slow spontaneous oscillations in mitral cells of the mouse accessory olfactory bulb"

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The accessory olfactory bulb (AOB) represents the first stage of central information processing in the rodent accessory olfactory system. Social chemosignals activate sensory neurons in the vomeronasal organ, which project to the AOB and form synaptic connections with apical dendrites of mitral cells (MCs). As the main excitatory AOB projection neurons, MCs bypass the thalamo-cortical axis and project directly to the amygdala and hypothalamus.

Despite their physiological significance, the intrinsic properties of MCs and their role in social information coding are not fully understood. Here, we investigate the biophysical properties of AOB mitral cells using both voltage- and current-clamp recordings from optically identified neurons in acute mouse AOB tissue slices. We identify a population of MCs that display slow oscillatory discharge which persist after pharmacological inhibition of fast synaptic transmission (AP5, NBQX and gabazine). The underlying subthreshold membrane potential fluctuations display a high degree of periodicity and are mainly driven by a TTX-sensitive persistent sodium current. Moreover, our data demonstrate a complex interplay of multiple voltage-gated ion channels, such as TTX-sensitive sodium channels, SNX-sensitive calcium channels, and calcium-dependent potassium channels, that maintain and modulate rhythmicity.

Together, the oscillatory discharge patterns observed in AOB mitral cells could play an important role in both sensory coding and gain control along the accessory olfactory pathway.

“Low energy balance” hormones modulate the olfactory responsiveness

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The sense of smell significantly affects the amount of food consumption and the frequency of food intake. Most animals, such as rodents, rely on the olfactory systems in their daily foraging. The necessity to find suitable food sources is particular urgent when the internal energy reservoir, the body fat content, is running low. The level of body fat is reflected in the concentration of two adipokine hormones, adiponectin and leptin, which are considered as long-term modulators of energy balance. Whereas the leptin concentration correlate positively with body fat content, the level of adiponectin in the circulation is increasing as the fat reserves are declining. The finding that olfactory neurons have a receptor for adiponectin has led to the hypothesis, that adiponectin may alter their responsiveness. In fact, we have found that exogenously applied adiponectin renders the olfactory sensory neurons (OSNs) more responsive to odorants. This increased activity was also reflected on the next level of olfactory information processing, in the olfactory bulb; after hormone pre-treatment significantly stronger neuronal responses to odor stimuli were registered at receptor-specific glomeruli, indicating that in fact the stronger response at the periphery was conveyed to the brain. An application of leptin, in contrast, did not lead to any change in the responsiveness of olfactory neurons to odorants.
Food finding and food consumption is also crucial in an acute hunger situation. The gastric hormone ghrelin is known as an indicator for an acute hunger and supposed to elicit food intake behavior. In the approaches to assess whether ghrelin may also affect olfactory neurons we found that they do express the receptor for ghrelin, GHSR1a. Moreover, monitoring the olfactory responsiveness revealed that after a pre-treatment with nasally applied ghrelin, a standardized olfactory stimulus activated a significantly higher number of sensory neurons and also led to an increased activity in the related glomeruli. The short-term satiety hormone PYY, in contrast, did not show any effect on the olfactory responsiveness. Together the results demonstrates, that hormones which represent conditions of scarce energy reserves or conditions of an acute hunger both render the olfactory system more responsive to odor stimuli and may thereby contribute to cover the energy demand by an improved discovery of food sources.

3D-reconstruction as a tool to study postmetamorphic changes in neuropeptide pattern in the antennal lobe of *Tribolium castaneum*

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Neuropeptides represent the largest and most diverse group of signaling molecules in the CNS. They are thought to be involved in processes related to neuronal plasticity, the substrate for learning and memory. But, how plastic is neuropeptide expression in adult insects? To answer this question, we examine the pattern of tachykinin related peptides (TKRP) in the antennal lobes (ALs) of the red flour beetle Tribolium castaneum during the time period after adult eclosion when the adult animals are for the first time confronted with odors from the environment. The paired ALs of insects are the first integration centers for the processing of olfactory information in the insect brain.

TKRP immunohistochemical staining reveals a dense innervation of all glomeruli by a set of about 40 local AL interneurons. By means of 3D-reconstruction of the TKRP staining, we counted the olfactory glomeruli and analyzed volumes and volume distribution. In contrast to earlier studies with synapsin staining as a basis for glomeruli counts, the TKRP label allows for a better discrimination between single glomeruli, resulting in higher glomeruli numbers. Further, first results, comparing ALs of freshly eclosed animals and 7 day old adults suggest volume increases in several selected glomeruli. In an attempt to further characterize changes of neuropeptide/ glomerular distribution pattern in the AL, we started to analyze distances between glomeruli using the maximum inscribed spheres method (Silin & Patzek, 2006, *Phys. A*, 371(2), 336–360).

A CD36 expressing subset of murine olfactory neurons is involved in fatty acid detection.

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Olfactory signals influence food intake in a variety of species. To maximize the chances of finding a source of calories, the preference for fat foods and triglycerides in animals takes already place in the process of food location by the olfactory system. Also humans are able to detect the fat content of food via odors alone. The molecular identity of receptors and ligands mediating olfactory-dependent recognition of fatty acids are not described so far. We started to elucidate the molecular requirement for fatty acid recognition in mice, and report a subset of olfactory neurons expressing the fatty acid receptor CD36. These neurons are required for the detection of oleic acid, a major milk component. CD36-positive olfactory neurons share olfactory-specific transduction elements and project to numerous glomeruli in the ventral olfactory bulb. Ca2+ imaging showed that olfactory responses to oleic acid are drastically reduced in mice lacking CD36. In addition, we demonstrate a receptor-like localization of CD36 in olfactory cilia by STED microscopy.

A_1 receptor-mediated modulation of recurrent inhibition in mouse olfactory bulb mitral cells

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Besides their predominant role in energy metabolism, purines such as ATP, ADP and adenosine play a major part in the communication between cells in various areas throughout the whole nervous system. In the olfactory bulb (OB), ATP is released as a neurotransmitter and stimulates neuronal network activity as well as glial calcium signalling. In addition, ATP-degrading enzymes are highly expressed in the OB, highlighting a pivotal role of purinergic modulation in olfactory information processing.

In this study, we analyzed adenosine-mediated modulation of signal transmission between neurons of the OB in acute mouse brain slices. Signal transmission was studied on the basis of recurrent inhibition in mitral cells (MCs), the principal neurons projecting to higher brain centers, by using whole-cell patch-clamp recordings. Recurrent inhibition in MCs is thought to be mediated mostly by recurrent synapses between glutamatergic MCs and GABAergic granule cells (GCs) and plays a major role in the processing of olfactory information. Recurrent inhibition was evoked by a brief depolarizing voltage step and was greatly enhanced by magnesium-free extracellular media and application of cyclothiazide to enhance NMDAR- and AMPAR-mediated synaptic transmission, respectively. Bath application of adenosine (100 µM) led to a 20 % reduction of recurrent inhibition in wild type MCs, but not in A_1 receptor (A_1R) knock out mice.

Adenosine also reduced self-excitation in MCs by 35 %, which was used as a quantification of glutamate release from MC dendrites. Additionally, adenosine produced a 20 % decrease of MC calcium currents consisting of mainly P/Q-, but also L- and N-type calcium currents. By using different combinations of calcium channel blockers, we found that adenosine seems to predominantly affect P/Q- and N-type currents.

Adenosine reduced NMDAR-mediated recurrent inhibition characteristic for the GC-MC reciprocal synapse by 30 %. Moreover, adenosine decreased isolated AMPAR-mediated recurrent inhibition by 15 %. These currents were blocked by 80 % using NASPM (100 µM), a specific antagonist of calcium-permeable AMPARs, which are particularly expressed on parvalbumin interneurons (PVNs). This illustrates an impact of adenosine not only at GC-MC synapses, but also at PVN-MC synapses.

Adenosine acts through a presynaptic modulation at recurrent synapses involving an A_1R-mediated inhibition of presynaptic Ca^{2+} influx and thus reduces the release of glutamate at MC lateral dendrites. Hence, adenosine influences the performance of recurrent synapses between mitral cells and different classes of interneurons, thereby modulating processing of olfactory information.

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Activation of the OR37 subsystem coincides with a reduction of novel environment induced activity within the paraventricular nucleus of the hypothalamus

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Members of the OR37 subfamily differ from other vertebrate odorant receptors due to a variety of special features. They are exclusively found in mammals, are highly conserved during evolution and exhibit a unique structural element, indicating that OR37 receptors are tuned to special ligands. In the search for compounds that can activate receptors of the OR37 subfamily it was found that bodily secretions from conspecifics elicited an activation of glomeruli of the OR37 subtypes A, B and C. Monitoring simultaneously the activity of the paraventricular nucleus of the hypothalamus (PVN), a target region of projection neurons from OR37 glomeruli, revealed a significantly reduced level of activity in comparison to controls. The large number of c-Fos positive cells in the PVN of mice which were kept in a clean test box (controls) turned out to be corticotropin-releasing hormone (CRH) cells, which points to an activation of the hypothalamic-pituitary-adrenal axis and a stress response due to the novel environment. The much lower number of activated cells of mice in a box containing bodily secretions from conspecifics indicates a reduced stress response, a phenomenon related to a social buffering effect. Since bodily secretion from conspecifics activated the OR37 system and simultaneously reduced stress-induced activation of the PVN, it was hypothesized that long chain aliphatic aldehydes, the ligands for OR37 receptors, may be able to induce this effect. Indeed, a similar reduced activity in the PVN was found in mice kept in a clean test box and exposed to a mixture of penta-, hexa- and heptadecanal delivered via air stream. These data indicate that the OR37 system may play a role in mediating social buffering phenomena.

Adenosine receptor A_1 modulates the potassium conductance of olfactory bulb mitral cells

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Adenosine is an ubiquitous modulator of neuronal activity and synaptic transmission. In the olfactory bulb, however, the impact of adenosine on neuronal performance has not been investigated so far. We found that adenosine hyperpolarises olfactory bulb mitral cells, thereby reducing network activity. We investigated the mechanism of the adenosine-evoked hyperpolarisation using whole-cell patch-clamp recordings in acute olfactory bulb slices of mice. Adenosine induced an outward current in voltage clamp in wild type mice, but not in A_1 receptor knock out mice. Adenosine failed to evoke membrane currents in the presence of GDP-ß-S and GTP-γ-S and when intracellular Ca^{2+} was buffered by BAPTA, indicating that A_1 receptors mediate the outward current via a G protein-coupled and Ca^{2+}-sensitive pathway. Blocking the adenylate cyclase had no effect on the adenosine-induced current. The outward current was enhanced in amplitude at a holding potential of -30 mV compared to a holding potential of -70 mV and was reduced when K^+ in the pipette solution was replaced by Cs^+. The adenosine-mediated hyperpolarisation reversed close to the calculated K^+ reversal potential. These results suggest that adenosine leads to an activation of a K^+ conductance. However, the canonical K^+ channel blockers TEA and 4-AP as well as the G protein-coupled inward rectifier blocker Ba^{2+}, the KCNQ (M-current) blocker Xe 991, and the ERG channel blocker E-4031 had no effect of the adenosine-evoked hyperpolarisation. By using TRAM-34 and UCL 1684 as blockers we could additionally exclude that adenosine modulates Ca^{2+}-dependent K^+ channels in mitral cells. In summary all K^+ conductances described so far as being regulated by A_1 receptors could be ruled out in this study pointing to a novel interaction partner of A_1 receptors in mitral cells of the olfactory bulb.
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Adenosine-mediated modulation of olfactory bulb network activity and odour information processing

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In the olfactory bulb, ATP is released as a co-transmitter together with glutamate from axon terminals of sensory neurons. The mitral cells, the principal neurons in the olfactory bulb, receive this input and convey the information after intense processing via local circuits to higher brain centres such as the piriform (olfactory) cortex. Accordingly, mitral cells receive synaptic input from, and give synaptic input into most if not all types of neurons in the olfactory bulb. Brief and rapid application of ATP excites the neuronal network of the olfactory bulb measured as spontaneous synaptic currents in voltage-clamped mitral cells. Sustained application of ATP, in contrast, results in a reduced network activity and in hyperpolarisation and hence inhibition of mitral cells. This hyperpolarisation can be mimicked by adenosine, suggesting that ATP degradation to adenosine accounts for the inhibition of mitral cells. A1 receptor-deficient mice lack the inhibitory effect of adenosine, and adenosine fails to hyperpolarize mitral cells in the presence of the A1 antagonist DPCPX, indicating that the adenosine-dependent inhibition is mediated by A1 receptors. Similar to the slow application of ATP adenosine significantly reduced the frequency of synaptic events, indicating that the inhibition of mitral cells reduces the activity of the entire network. The direct synaptic input from sensory neurons to mitral cells, in contrast, was not affected by adenosine. The unchanged sensory input in conjunction with the reduced background network activity results in an increased signal-to-noise ratio upon stimulation of sensory neurons in the presence of adenosine. This renders adenosine as a good candidate to modulate odour information processing in the olfactory bulb.

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Anatomical and Functional Organization of the *Drosophila* Antennal Lobe: Glomerular Convergence and Divergence

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Chemical environments consist of a vast, complex and fast changing array of volatile cues with differing ecological relevance. To orient within this plethora of volatile cues, the vinegar fly *Drosophila melanogaster* possesses an elaborate olfactory system consisting, on the periphery, of a set of four sensilla classes present in stereotypical patterns on the 3rd antennal segment and the maxillary palp. Each sensilla type, either antennal basiconic, trichoid, coeloconic or palp basiconic, houses one to four olfactory sensory neurons (OSN), each expressing a known repertoire of one or two olfactory receptors with a unique molecular receptive range. OSNs expressing the same receptor converge in the protocerebrum forming 54 discrete spherical structures, the glomeruli as units of the antennal lobe (AL), where they synapse with omniglomerular local interneurons and uni- or multiglomerular projection neurons.

In our study we investigate the concise basis of this neuronal network. Most glomeruli, except the putative pheromonal examples, are often thought to be part of a very homogeneous cluster of AL subunits solely separated by the receptor repertoire of innervating OSNs. Although an increasing group of non-pheromonal glomeruli serving very specific tasks is described, their potentially differing anatomy is usually neglected. Here we display the heterogeneous nature of the glomeruli regarding their specific neuronal input and output quantity as well as their *in vivo* volume. Based on the morphological data we show that described functional differences can also be correlated or derived from anatomical alterations.

Brain composition in *Godyris zavaleta,* a diurnal butterfly, reflects an increased reliance on olfactory information

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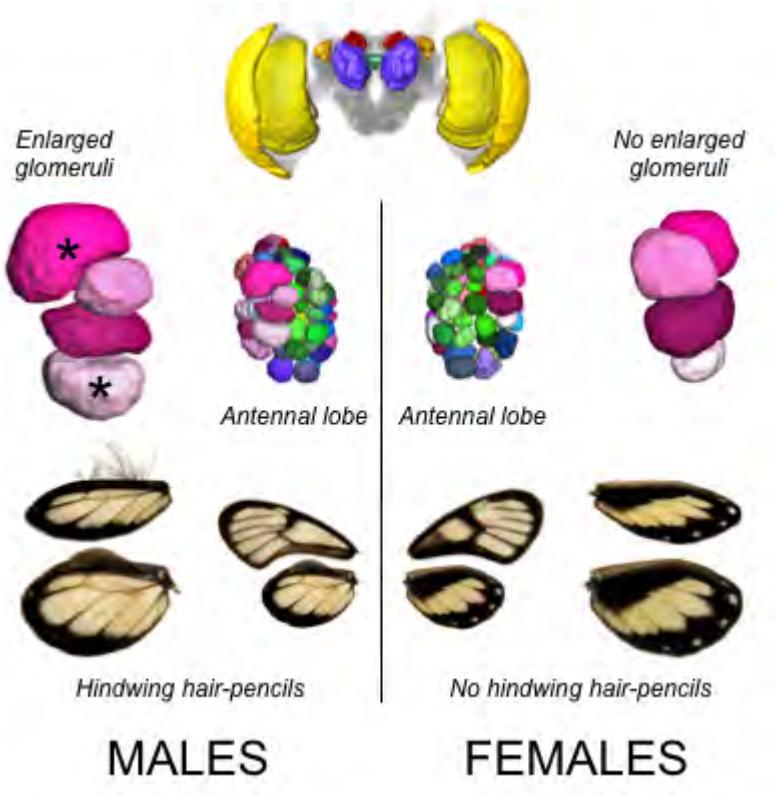
The size and structure of nervous systems are shaped by selection in the context of developmental and functional constraints. Understanding how and to what extent selection negotiates these constraints to bring about adaptive evolutionary change that enhances the fitness of an animal's behaviour is key to understanding the principles of brain evolution. The principal way to tackling these questions has been to compare brain size and structure across multiple species with divergent ecologies. The Lepidoptera have long been a favoured model in evolutionary biology, but descriptions of brain anatomy have to date largely focused on the few species that are commonly used as model organisms in neurobiological research. We describe the brain of the Zavaleta Glasswing, *Godyris zavaleta,* a member of the Neotropical butterfly sub-family Ithomiinae with enhanced reliance on olfactory information and a derived mating behaviour. This entails sex-specific pheromone communication mediated by hind-wing 'hair-pencils' and a sex-specific motivation to locate specific plant allelochemicals (pyrrolizidine alkaloids) that serve as precursors for pheromone synthesis. We test two hypotheses: i) that the derived mating behaviour of *G. zavaleta* results in sexual dimorphism in the antennal lobes not observed in other butterflies; and ii) that the generally enhanced role of olfaction in *G. zavaleta* is supported by shifts in the relative investment in sensory neuropiles. We demonstrate for the first time the presence of sexually dimorphic glomeruli within a distinct macroglomerular complex (MGC) in the antennal lobe of a diurnal butterfly. This presents a striking convergence with the well-known moth MGC, prompting a discussion of the potential mechanisms behind the independent evolution of specialized glomeruli. Interspecific analyses across four Lepidoptera further show that the relative sizes of their sensory neuropiles closely mirror interspecific variation in sensory ecology, with *G. zavaleta* displaying levels of sensory investment that are intermediate between the diurnal Monarch butterfly *(Danaus plexippus),* which invests heavily in visual neuropile, and night-flying moths which have a greater investment in olfactory neuropile, and diminished visual neuropile. We identify several traits that distinguish butterflies from moths, and several that distinguish *D. plexippus* and *G. zavaleta.* Our results illustrate how ecological selection pressures mould the structure of invertebrate brains, and exemplify how comparative analyses across ecologically divergent species can illuminate the functional significance of variation in brain structure.


Enlarged glomeruli
No enlarged glomeruli
Antennal lobe
Antennal lobe
Hindwing hair-pencils
No hindwing hair-pencils
MALES
FEMALES

Ca^{2+} Dependent K^+ Currents in Uniglomerular Olfactory Projection Neurons of the Antennal Lobe

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Ca^{2+} activated potassium currents ($I_{K(Ca)}$) are an important link between the intracellular signaling system and the membrane potential, shaping intrinsic electrophysiological properties. To better understand the ionic mechanisms that mediate the intrinsic firing properties of olfactory uniglomerular projection neurons (uPN), we used whole-cell patch-clamp recordings combined with single cell labeling in an intact, adult brain preparation of *P. americana* to analyze $I_{K(Ca)}$.

In the insect brain uPNs form the principal pathway from the antennal lobe to the protocerebrum, where centers for multimodal sensory processing and learning are located. While the morphology and the odor response profiles of uPNs are generally well documented we have only limited information about the intrinsic firing properties and the cellular mechanisms that determine them. However, an in depth knowledge of the cell type specific intrinsic electrophysiological properties and the membrane conductances that mediate them is an important prerequisite towards a detailed understanding of the cellular basis of olfactory information processing. Here we analyze the Ca^{2+} dependent K^+ outward currents ($I_{K(Ca)}$) of uPNs, which play a crucial role in controlling neuronal firing characteristics.

The time course for activation was clearly voltage and Ca^{2+} dependent. Thus, under physiological conditions $I_{K(Ca)}$ is strongly dependent on the Ca^{2+} influx kinetics and on the membrane potential. $I_{K(Ca)}$ was sensitive to charybdotoxin (CTX) and iberiotoxin (IbTX) but not to apamin. The functional role of $I_{K(Ca)}$ was analyzed in occlusion experiments under current clamp, in which portions of $I_{K(Ca)}$ was blocked by CTX or ibTX. Blockade of $I_{K(Ca)}$ by CTX and IbTX showed that $I_{K(Ca)}$ contributes significantly to the intrinsic electrophysiological properties such as the action potential waveform and membrane excitability.

Ca^{2+}-activated Cl^- currents in the vomeronasal organ are mediated by Anoctamin 1 and Anoctamin 2

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The mammalian olfactory system comprises two major parts, the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). Whereas the MOE is responsible for sensing and discriminating volatile compounds, the VNO plays an essential role in instinctive decisions. Its sensory entities, the vomeronasal sensory neurons (VSNs), are able to detect intraspecific pheromones and interspecific kairomones. In the VNO chemical cues bind to G protein-coupled receptors which are located in the microvilli of VSNs. Activation of those receptors leads to a phospholipase C mediated production of diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG opens the non-selective cation channel TRPC2 (transient receptor potential channel 2), leading to a depolarization of the membrane through an influx of Na^+ and Ca^{2+}. The intracellular Ca^{2+} transients lead to an opening of Ca^{2+}-activated chloride channels. Due to the high Cl^- concentration in VSNs, this opening causes an outwardly directed Ca^{2+}-activated chloride current (CaCC) which is thought to amplify the VSN response. Strangely, genetic ablation of TRPC2 does not completely abolish stimulus-induced VSN activity. By immunohistochemistry in isolated neurons and intact VNO slices we show that the Ca^{2+}-activated chloride channels Anoctamin1 (Ano1, TMEM16A) and Anoctamin2 (Ano2, TMEM16B) are co-expressed in microvilli of VSNs. This finding was confirmed by *in situ* hybridization. To further elucidate the role of these two channels we generated a double knock-out mouse with constitutive deletion of Ano2 and conditional deletion of Ano1 in the olfactory system. Electrophysiological whole-cell recordings in acute slices of the VNO show that CaCCs are absent in the double-knockout, yet persisting in single knock-outs of either Ano1 or Ano2. Hence, the double-knockout mouse model serves as a validated tool to investigate the role of CaCCs in functional electrovomeronasograms and behavioral assays such as aggression and mating tests. Besides the double knock-out mouse, we generated a triple-knockout mouse, lacking TRPC2 in addition to Ano1 and Ano2. This model will help to find out if the described remaining activity in TRPC2 deficient mice is due to the action of a secondary transduction pathway via CaCCs.

Chemo- and thermosensory signaling in the Grueneberg ganglion

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The Grueneberg ganglion (GG) - a cluster of neurons in the anterior nasal region – is considered as an olfactory organ. We have recently identified odorants activating GG neurons, in particular given pyrazine analogues. Responsiveness to these compounds occurred in GG neurons characterized by the expression of the olfactory receptor V2r83, the guanylyl cyclase GC-G and the cyclic nucleotide-gated ion channel CNGA3. Experiments with knockout animals disclosed that GC-G and CNGA3 are important for odor-evoked GG responses.
GG neurons were also found to be activated by cool temperatures. Investigating the relevant signaling mechanisms revealed that almost all V2r83-/GC-G-/CNGA3-positive GG neurons responded to coolness, i.e, the same subset of GG neurons is activated by coolness and the above mentioned odorants. Searching for thermosensory proteins in the GG, the thermosensitive ion channel TREK-1 was found to be expressed in numerous GG neurons. Moreover, in TREK-1-deficient mice, GG responsiveness to coolness was reduced. However, even in the absence of TREK-1, GG neurons clearly responded to cool temperatures, suggesting that in addition to TREK-1, another thermosensor must be present in the GG. In this context, we observed that that the guanylyl cyclase GC-G is an unusual enzyme which is directly activated by cool temperatures. Consistently, in GC-G-deficient GG neurons, responsiveness to cool temperatures was largely reduced, indicating that this enzyme serves as a major thermosensor in cells of the GG.

Chemosensory characterisation of antral G-cells and its nutrient-induced modulation of genexpression

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A continuous assessment of ingested food in the gastric lumen is essential for fine-tuning the digestive activities, including the secretion of the regulatory hormones such as gastrin. It has been proposed that G-cells may be able to sense the amounts of ingested proteins and adjust the secretion of gastrin accordingly. Our previous studies have shown that G-cells do in fact express suitable receptor types, most notably the peptone-receptor GPR92 and the amino acid receptors CaSR and GPRC6A; however, their relative importance remained unclear. Therefore, in this study a more quantitative estimation for each receptor type was performed by analyzing individually isolated G-cells from the transgenic mouse line mGAS-EGFP. Real time PCR analysis revealed that the amount of mRNAs for the peptone receptor GPR92 was highly abundant, and significantly higher than for the amino acid receptor GPRC6A. To determine the relative amount of the actual receptor protein, isolated G-cells were analyzed by means of a Liquid Chromatography Tandem-Mass Spectrometry (LC/MS/MS) procedure. The results indicate that the amount of receptor protein for GPR92 was much higher than for the other receptor types and supports the notion that the peptone receptor GPR92 may be particularly relevant for sensing partially digested protein products in the gastric chime. To further support this view we set out to investigate, whether a high-protein (HP) diet may affect the GPR92 expression in the gastric antrum. The results showed that after 48 h of HP feeding the amount of antral GPR92 mRNA was significantly decreased but was strongly increased after 5 weeks. In contrast, in the circumvallate papilla the mRNA level of GPR92 was significantly increased after 48 h HP feeding and reduced after 12 weeks. These results indicate that GPR92 expression was differentially regulated in gastric and gustatory tissue.

Coexpression of Anoctamins in cilia of olfactory sensory neurons

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Vertebrates can sense and identify a vast array of chemical cues. The molecular machinery involved in chemodetection and -transduction is expressed within the cilia of olfactory sensory neurons. Currently, there is only limited information available on the distribution and density of individual signaling components within the ciliary compartment. Using superresolution microscopy, we show here that CNG channels and calcium-activated chloride channels of the Anoctamin family are localized to discrete microdomains in the ciliary membrane. In addition to ANO2, a second Anoctamin, ANO6, also localizes to ciliary microdomains. This observation, together with the fact that ANO6 and ANO2 colocalize indicates a role for ANO6 in olfactory signaling. We show that both ANO2 and ANO6 can form heteromultimers and that this heteromerization alters the channels' physiological properties. Thus, we provide evidence for interaction of ANO2 and ANO6 in olfactory cilia, with possible physiological relevance for olfactory signaling.

Co-expression of six tightly clustered olfactory receptor genes in the antenna of the malaria mosquito *Anopheles gambiae*

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Females of the malaria mosquito, *Anopheles gambiae*, highly depend on their sense of smell in various behaviors, including seeking out blood hosts or selecting oviposition sites. In their olfactory organs, the detection of behavioral relevant odorants is mediated by olfactory sensory neurons (OSNs) which comprise distinct odorant receptors in their dendritic membrane. In the genome of *A. gambiae* 76 genes have been annotated encoding putative odorant receptors (AgORs), with the majority of the genes arranged in clusters. To assess whether the expression of clustered AgOR genes may be uniquely regulated we have explored six tightly adjoined genes. By using whole mount fluorescence *in situ* hybridization approaches with AgOR-specific probes we determined the number and distribution of OSNs, which express a distinct AgOR-type and analyzed the co-expression patterns of all six receptor types in the female antenna. It was found that on the antennal segments 2-13 about 75 OSNs co-transcribe mRNAs of the four types AgOR13, AgOR15, AgOR17 and AgOR55. Moreover, in about half of these OSNs also mRNA for AgOR16 and AgOR47 could be detected, indicating the existence of two populations of OSNs with transcripts for four, respectively, six ORs of the same cluster. Subsequent RT-PCR experiments with antennal cDNA and primer pairs spanning the coding regions of adjacent AgOR genes revealed the existence of polycistronic mRNA, indicating that not individual genes from the cluster were transcribed but that rather mRNAs were generated, which comprises the coding sequence of several genes. It is still unclear, whether all encoded receptor types are in fact translated, however, the data indicated a unique principle for the expression of OR genes.

Compartment-specific calcium handling properties in olfactory projection neurons

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The antennal lobe of insects constitutes the first synaptic relay and processing center of olfactory information, received from olfactory sensory neurons located on the antennae. Complex synaptic connectivity between olfactory neurons of the antennal lobe ultimately determines the spatial and temporal tuning profile of (output) projection neurons to odors. Using paired whole-cell patch-clamp recordings in the cockroach *Periplaneta americana* we recently characterized the excitatory synaptic interactions between cholinergic uniglomerular projection neurons (uPNs) and GABAergic type I local interneurons, both of which are key components of the insect olfactory system (Warren and Kloppenburg, 2014,J Neurosci 34(39):13039-13046). Between uPNs and type I local interneurons we found short latency, rapid, strong excitatory cholinergic synaptic transmission that was coincident with single presynaptic action potentials. These results clearly show that uPNs have not only an important role in relaying processed olfactory information from the antennal lobe to higher brain centers, but also provide synaptic input to antennal lobe neurons during olfactory processing.

Since highly localized cytosolic Ca^{2+} dynamics are involved in pre- and postsynaptic signal processing, we investigated the Ca^{2+} handling properties in the glomerular neurites of uPNs. Using the added buffer approach combined with Ca^{2+} imaging and electrophysiological recordings we observed significantly different Ca^{2+} handling properties in the different functional compartments of uPNs. For example: Compared to the soma the Ca^{2+} extrusion rates were significantly larger in the glomerular neurites, while the Ca^{2+} binding ratios where similar in both compartments. Electrophyiological recordings combined with fast multi-photon Ca^{2+} imaging showed that the neuritic Ca^{2+} handling properties were sufficient that even fast electrophysiological activity was reflected in the voltage dependent cytosolic Ca^{2+} dynamics.

Since Ca^{2+} regulates a multitude of cellular functions, and many aspects of information processing in single neurons are dependent on highly localized calcium domains, we consider the characterization of cellular parameters that determine cytosolic Ca^{2+} dynamics, as an important step towards a detailed understanding of the cellular basis of olfactory information processing at the single cell level.

Acknowledgments

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Compound valence is conserved in binary odor mixtures in *Drosophila melanogaster*

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Most naturally occurring olfactory signals do not consist of monomolecular odorants but, rather, are mixtures whose composition and concentration ratios vary. While there is ample evidence for the relevance of complex odor blends in ecological interactions and for interactions of chemicals in both peripheral and central neuronal processing, a fine-scale analysis of rules governing the innate behavioral responses of *Drosophila melanogaster* towards odor mixtures is lacking. In this study we examine whether the innate valence of odors is conserved in binary odor mixtures.

We show that binary mixtures of attractants are more attractive than individual mixture constituents. In contrast, mixing attractants with repellents elicits responses which are lower than the responses towards the corresponding attractants. This decrease in attraction is repellent-specific, independent of the identity of the attractant and more stereotyped across individuals than responses towards the repellent alone. Mixtures of repellents are either less attractive than the individual mixture constituents or these mixtures represent an intermediate. Within the limits of our data set, most mixture responses are quantitatively predictable on the basis of constituent responses.

In summary, the valence of binary odor mixtures is predictable on the basis of valences of mixture constituents. Our findings will further our understanding of innate behavior towards ecologically relevant odor blends and will serve as a powerful tool for deciphering the olfactory valence code.

Disruption of Kcc2 from mitral cells of the olfactory bulb leads to specific odor discrimination defects due to disregulated GABAergic inhibition

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The potassium-chloride cotransporter 2 (Kcc2) is the main chloride extruder in adult neurons and is also described to play a substantial morphogenic role in the development of the CNS. Its deletion leads to an elevated intracellular chloride concentration in neurons and hence a reduced GABA(A) receptor-mediated hyperpolarization. We selectively deleted Kcc2 in mitral cells of the olfactory bulb of mice to investigate the consequences of decreased GABAergic hyperpolarization in the excitatory projecting neurons of the main olfactory bulb on processing and perception of odor information. We could show that the reversal potential of GABAergic currents is indeed shifted to more positive potentials in mitral cells after Kcc2 disruption, thereby decreasing hyperpolarization-mediated GABAergic inhibition. Unexpectedly, we further found a significant higher number of perisomatic reciprocal synapses in Kcc2 depleted mitral cells, while the overall number of synapses appears unchanged. These changes in electrical and morphological properties of mitral cells and its connected interneuron network after Kcc2 disruption translate to impaired ability in the discrimination of similar odors as well as odor mixtures.

Effect of piperine and other sensory active compounds on human KCNK channels of the TASK and TRESK subfamilies

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Black peppercorns constitute one of the most consumed spices worldwide. Although several sensory active compounds have been isolated from them, their pungent oral impression has been mostly associated with the alkaloid piperine. This compound has been proved to activate TRPV1, the receptor for capsaicin, when heterologously expressed on HEK293 cells. However, we recently demonstrated that the two-pore domains K+ channels (K2P), TASK-1 and TASK-3 also constitute a target for piperine as well as for other pungent compounds. In order to gain a better understanding of the effect of these substances we used a primary culture of mouse trigeminal neurons and invesigated the effect of piperine as well as other sensory active compounds by means of the calcium imaging and patch-clamp techniques. we observed that the vast majority of neurons presented a decrease in their background current after the application of piperine, which was compatible with the inhibitory effect on K2P channels we previously reported. Interestingly, in some neurons, piperine elicited both a decrease in the outward current, and also an increase in the inward current, compatible with an effect on both TRP and K2P channels. Additionally, we performed reverse transcriptase PCR as well as next generation sequencing experiments that confirmed the expression of KCNK3 and KCNK9 on human trigeminal ganglia. Our results suggest a complementary role for both families of ion channels in the perception of pungency and probably other trigeminal sensations.

Effects of neonicotinoid exposure on the honey bee odor coding

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Exposure to neonicotinoid pesticides is among the main causes of colony collapse disorder and bee decline. At sub-lethal doses these chemicals have shown to be effective in disrupting a number of basic behaviours, ranging from navigation to olfactory learning and memory. For what concerns the latter, it is hypothesized that interference of neonicotinoids with the acetylcholine signalling in mushroom bodies plays a key role. However, we have studied effects of acute exposure to these substances on the functional output of the honey bee antennal lobes, revealing huge effects already at the level of odor coding, that might subtend and precede effects on memory storage and retrieval. Calcium imaging of the projection neurons shows indeed altered odor responses within the glomeruli. We have defined critical concentration ranges for this effects, and investigated, on the other hand, ability of the exposed animals to discriminate between stimuli in a differential conditioning paradigm that was run in parallel to the imaging experiments.

Effects of sublethal doses of a neonicotinoid insecticide on the olfactory system of a moth

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Pheromone communication is crucial for mating in moths, including many agricultural pest species. In general, male moths locate their mating partners by following the plume of a species-specific female-emitted sex pheromone. This behaviour is mediated by highly specific olfactory receptor neurons and sex pheromone information is processed in a dedicated area of the primary olfactory brain centre, the macroglomerular complex of the antennal lobe. Recent studies have shown that sex pheromone communication is strongly submitted to modulation by physiological and environmental factors. One such environmental factor is the extensive use of neurotoxic molecules, including neonicotinoid insecticides, in actual pest management strategies.The widespread use of these insecticides results in residual accumulation of low concentrations in the environment. Neonicotinoids are known to disrupt neurotransmission through nicotinic acetylcholine receptors (nAChRs) and therefore kill insects at high doses. At low doses, however, neonicotinoids might also affect sensory systems. Olfactory processing in insects is reliant on nAChRs and sublethal neonicotinoid exposure could therefore be expected to modulate pheromone communication. Whether neonicotinoids disrupt or enhance pheromone communication is a question largely unexplored. Adaptation processes to environmental pollution have previously been shown to occur in some insects after exposure to low insecticide doses, leading to increases in reproduction. As pheromone processing is intrinsically linked to reproduction, potential beneficial effects of low neonicotinoid doses on the olfactory system might contribute to these observations, and were in the focus of our study. The black cutworm, *Agrotis ipsilon* (Hufnagel) (Lepidoptera: Noctuidae), is an occasional pest of maize, *Zea mays* L., that may cause severe stand losses and injury to corn seedlings. The neonicotinoid insecticide clothianidin is widely used against A. ipsilon in foliar soil and seed treatments.

In this study we explored the impact of low clothianidin doses on the olfactory system of adult male *A. ipsilon* . We determined the toxicity of oral clothianidin treatments and subsequently tested behavioural responses to the sex pheromone 24 h after intoxication with low doses of clothianidin. Surprisingly, we observed an increase in pheromone responses after intoxication with one low dose corresponding to the lethal dose 20 (LD20) in the surviving males and a decrease in pheromone responses after intoxication with a very low dose (*A. ipsilon* males and compared their expression level in the brain of clothianidin-treated with that of control males, using qPCR experiments.

Electrophysiological Analysis of Concentration Coding and Learning in the Honeybee Dual Olfactory System

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In insects, odors are perceived by an elaborate neural system comprising components remarkably similar to olfactory structures in the vertebrate brain. Insect 'mini-brains', therefore, provide excellent model systems to study principles of olfactory coding and learning. In the Honeybee, olfaction plays a crucial role in communication and orientation behaviors. Many odors need to be memorized over a bee´s lifetime. Using established conditioning paradigms, olfactory learning and behavior can easily be studied under controlled laboratory conditions. This allows for simultaneous invasive monitoring of neural activity along the olfactory pathway.

An interesting and unique feature of the olfactory system in Honeybees and other Hymenoptera is that information is sent via easily identifiable parallel pathways from the first order olfactory neuropil, the antennal lobe (AL), to higher order processing centers like the mushroom body (MB) and the lateral horn (LH). This includes the medial and lateral antennal lobe tracts (m- and l-ALT). The m-ALT projects first to the MB and then into the LH, whereas the l-ALT runs in the opposite direction.

Using electrophysiological recordings as well as imaging techniques, recent research has focused on functional differences of these dual pathways leading to partially contradictory conclusions. Thus, the functions as well as evolutionary benefits of odor processing via parallel pathways are still not fully clarified, which represents the motivation of our present study. We test whether odor identities and concentrations are coded differently by projection neurons of both AL tracts. Furthermore it is conceivable that both tracts differ regarding their neural plasticity. We therefore include classical conditioning experiments to investigate tract dependent changes following learning and memory formation. To be able to analyze our data with high temporal resolution over long periods of time we have established a method for extracellular multi-unit long term recordings in various recordings sites along the olfactory pathways (Brill et al. 2014 J Vis Exp).

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Entrained Slow Oscillatory Activity in Mitral Cells of the Mouse Accessory Olfactory Bulb

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The accessory olfactory bulb (AOB) is the first stage of information processing in the mouse vomeronasal pathway. As the main AOB projection neurons, mitral cells (MCs) receive sensory input from peripheral vomeronasal neurons and relay this information to the vomeronasal amygdala and the hypothalamus. A subpopulation of MCs exhibits slow oscillatory discharge that persists upon pharmacological inhibition of fast synaptic transmission. Here, we identify an excitatory circuit within the AOB network that entrains oscillatory activity in a second MC subpopulation. Using patch clamp recordings from mitral cells in acute AOB tissue slices we investigate the synaptic mechanisms underlying these entrained discharge patterns. Entrained MCs display periodically increased excitatory synaptic input that correlates with their own 'spontaneous' discharge patterns. Block of fast glutamatergic synaptic transmission reveals that (a) neural entrainment depends on an intact glutamatergic network, and (b) the rhythmicity in intrinsically oscillating MCs is stabilized by this glutamatergic circuit. By contrast, block of fast GABAergic transmission reveals that tonic GABAergic MC inhibition frequently masks entrained slow oscillations. On-going experiments aim to unravel the network mechanisms underlying MC entrainment and the role of slow rhythmic burst activity in AOB sensory information processing.

Evidence for daytime- and pheromone dose-dependent metabotropic signal transduction cascades in the hawkmoth *Manduca sexta*

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At night female hawkmoths release a pheromone blend with the main component bombykal, which triggers pheromone-dependent upwind flight of their conspecific mates. *Manduca sexta* males detect the pheromones with olfactory receptor neurons (ORNs) which innervate long trichoid sensilla on their antennae. Pheromone transduction in insects is still under debate. Odor-binding olfactory receptors (ORs) are inversely inserted into the dendritic membrane which locates the known C-terminal G-protein binding side on the extracellular side. In addition, ORs appear to form heteromeric complexes with a conserved 7-TM cation channel, named Orco. Thus, it was suggested that all insects employ ionotropic odor transduction via the OR-Orco odor receptor ion channel complex. However, in *M. sexta*, so far, we found no evidence for an ionotropic pathway in pheromone transduction employing Orco agonist VUAA1 and antagonist OLC15. In contrast, pheromone application in patch clamp experiments of primary cell cultures of *M. sexta* ORNs elicited a sequence of inward currents which was mimicked via inclusion of IP_3 in the patch pipette. Thus, we searched for G-protein-dependent activation of a phospholipase Cß (PLCß) which generates DAG and IP_3, resulting in rises of intracellular Ca^{2+} levels in the bombykal-transduction cascade. In tip-recordings of single long trichoid sensilla of the intact hawkmoth application of G-protein antagonists as well as PLC-antagonists decreased responses to brief, physiological pheromone pulses. Furthermore, PKC-activity, which is regulated by DAG and Ca^{2+} appears to modulate pheromone responses daytime- and odor-dose-dependently. The PKC-agonist PDBu increased the pheromone response during the hawkmoths' resting phase, only. Accordingly, in the presence of PDBu pheromone application significantly increased IP_3 levels in hawkmoth antennae during rest. Finally, inhibition of PKC resulted in a decrease of the pheromone response with stronger effects during the resting phase. In contrast, activation of PKC during the activity phase could adapt pheromone responses at low but not at high pheromone concentrations. Thus, PKC could either adapt or enhance pheromone responses daytime- and odor-dose-dependently. Based on different studies we conclude that pheromone transduction in the hawkmoth does employ G-protein-dependent activation of a PLCß cascade with IP_3-dependent activation of a calcium channel as the first transduction channel. The resulting influx of Ca^{2+} in turn activates directly and indirectly via PKC activation ion channels underlying pheromone responses. In the hawkmoth activation of Orco appears not to affect the first 100 ms of the pheromone response but controls the late, long-lasting pheromone response which possibly underlies casting behavior. Current experiments challenge our hypothesis of metabotropic odor transduction also in the fruitfly *Drosophila melanogaster*. [Supported by DFG SPP 1392, STE531/21-1,2 to MS]

Ex vivo functional imaging of olfactory sensory neurons in *Drosophila melanogaster*

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Insects smell using two pairs of olfactory organs, the antennae and the maxillary palps, located in the anterior part of their head. These organs are covered with hair-like structures called sensilla, which house the dendrites of olfactory sensory neurons. Volatile compounds enter the sensillum lymph via tiny pores in the cuticula and with the help of odorant binding proteins they reach the neuron dendrites, where they bind to the olfactory receptors.

Insects possess three types of olfactory receptors: Ionotropic Receptors (IRs), Gustatory Receptors (GRs) and Odorant Receptors (ORs). ORs, in particular, are involved in the perception of a plethora of behaviourally relevant organic compounds, like food odours, danger odours (e.g. Geosmin) and pheromones. Each olfactory neuron houses a specific odorant receptor (OrX) and a ubiquitous coreceptor (Orco), which is necessary in order to target the OrX to the dendritic membrane and to transduce the olfactory signal. Despite significant progress in the knowledge of the ORs structure, the signal cascade that leads to olfactory perception and its regulation is still controversial. Studies involving functional imaging and patch-clamp techniques have been so far conducted expressing insect ORs in heterologous systems, due to the difficulty to access native olfactory neurons inside the antennae or to establish cell cultures of these neurons.

Here, we present a new preparation that allows exposing olfactory neurons in *Drosophila* antennae. We show how this technique makes possible to stimulate these cells and to monitor the response of individual olfactory neurons expressing genetically encoded fluorescence indicators using functional imaging techniques. Moreover, coupling imaging analysis with pharmacological experiments it is possible to study olfactory transduction in *ex vivo* conditions. We present two study cases; first, using flies expressing the Ca^{2+} indicator GCamP3.0 in olfactory neurons, we show that calmodulin affects odorant receptor function. Furthermore, using Epac1-camps cAMP indicator, we show that ORs stimulation induces cAMP production in olfactory neurons. These results demonstrate the effectiveness of our new technique and indicate its potentialities to advance in the study of olfactory transduction mechanisms in insects.

Expression of gustatory receptors in chemosensory organs and the alimentary tract of *Heliothis virescens*

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The detection and evaluation of appropriate food sources as well as the chemosensory control of ingested food to regulate digestion is crucial for most organisms. In insects, the registration of gustatory signals in the environment is achieved through contact chemosensilla on the body surface. These sensilla harbor gustatory sensory neurons with gustatory receptors (GRs) on their dendrites, which detect tastants like sugars or bitter compounds. Beside the expression of GRs in gustatory neurons, GR transcripts have been detected in the alimentary tract of insects, where the receptor proteins are supposed to be involved in nutrient chemosensing and regulation of digestive processes. In the pest insect, *Heliothis virescens*, we previously have identified three cDNA sequences, HR1, HR4 and HR5, encoding proteins which are highly related to functionally characterized sugar receptors in other insects. In this study we set out to explore their expression in different gustatory tissues and the alimentary tract of adults and larvae. RT-PCR experiments showed the expression of all three HRs both in classical gustatory organs, the proboscis and the legs, as well as in female and male antennae of *H. virescens*. Moreover, by applying the *in situ*-hybridization (ISH)-method the HR5-expressing cells could be visualized in the antennae and were assigned to gustatory sensilla chaetica. In contrast, in the proboscis the results of ISH-approaches and RT-PCR experiments support the expression of HR5 in neurons of the gustatory sensilla styloconica and sensilla basiconica type 1. Further RT-PCR experiments indicated expression of HR1 and HR4 along the proboscis, with lower HR4-expression at the tip. In addition, expression of the three putative sugar receptors was proven in the alimentary tract of adult and larval *H. virescens*. Transcripts of all receptor types were found in fore-, mid- and hindgut tissues of adult animals and in different parts of the larval gut. Together, the results indicate a widespread expression of HR1, HR4 and HR5 and suggest that in *H. virescens* the three putative sugar receptors are active in the detection of gustatory signals from the environment and chemosensory processes in the alimentary tract.

Functional imaging of cortical feedback projections from the anterior olfactory nucleus to the mouse olfactory bulb

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Processing of sensory information is substantially shaped by centrifugal, or feedback, projections from higher cortical areas. However, the functional properties of these projections are poorly characterized. Here, we used genetically-encoded probes to functionally image centrifugal projections targeting the olfactory bulb (OB). The OB receives massive centrifugal input from cortical areas but there has been as yet no characterization of their activity in vivo. We focused on projections to the OB from the anterior olfactory nucleus (AON), which constitutes a major source of cortical feedback to the OB. We expressed GCaMP3 selectively in AON projection neurons using cre-dependent AAV vectors and a mouse line expressing Cre in a large fraction of principal AON neurons. Virus injection into AON or the OB lead to GCaMP expression throughout AON projection neurons, including their axon terminals in the OB. Electrical stimulation of AON evoked large, transient optical signals at the level of the OB. Surprisingly, odorants also evoked large signals that were transient and coupled to odorant inhalation both in the anesthetized and awake mouse, suggesting that feedback from AON to the OB is rapid and robust across different brain states. The strength of AON feedback signals increased during wakefulness, suggesting a state-dependent modulation of cortical feedback to the OB. Two-photon GCaMP imaging revealed that different odorants activated different subsets of centrifugal AON axons and could elicit both excitation and suppression in different axons, indicating a surprising richness in the representation of odor information by cortical feedback to the OB. Finally, we found that activating neuromodulatory centers such as basal forebrain drove AON inputs to the OB independent of odorant stimulation. Our results point to the AON as a multifunctional cortical area that provides ongoing feedback to the OB and also serves as a descending relay for other neuromodulatory systems.

Glomerular structure of the central nervous projections from scorpion pectines: Structural details and gender differences

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Primary chemosensory organs of scorpions are the pectines (which also bear numerous mechanosensors not considered here). These appendages are located on the ventral side of the abdomen on the 2nd segment behind the walking legs (fig.s A, B). Depending on scorpion species, between about 20,000 and 1,000,000 afferent neurons arise from a pectine appendage (1, 2). Afferent axons enter the main central nervous projection area, the (posterior) pectine neuropil (fig. C), from the outside, and they terminate in glomerular structures (fig. D) reminiscent of the glomeruli in the antennal and olfactory lobes of other arthropod groups. However, there are also several idiosyncratic features, including very different sizes and shapes of the individual glomeruli. Most glomeruli are not globular but flattened to sausage-shaped, or even donut-shaped. These glomeruli are arranged in four to five concentric layers, giving an onion-like structure to the pectine neuropils (fig. D). In adult scorpions, the layers are about 20 to 30 micrometers thick, which is thus also the thickness of the glomeruli along their shortest axis.

The present study compares the glomerular structure of the posterior pectine neuropils between scorpion species with distinctly different pectine sizes, and between genders. The pectine neuropils appear to be rather conservative in their structure, with small differences between genders, except that males' pectine neuropils are often considerably larger, in keeping with the larger pectines in the males of the respective species. The structures of the pectine neuropils are also similar between different scorpion species, with prominent glomeruli appearing in similar positions even in distantly related species. However, delimiting different smaller glomeruli is often difficult and glomeruli numbers and identities were not yet assessed precisely.

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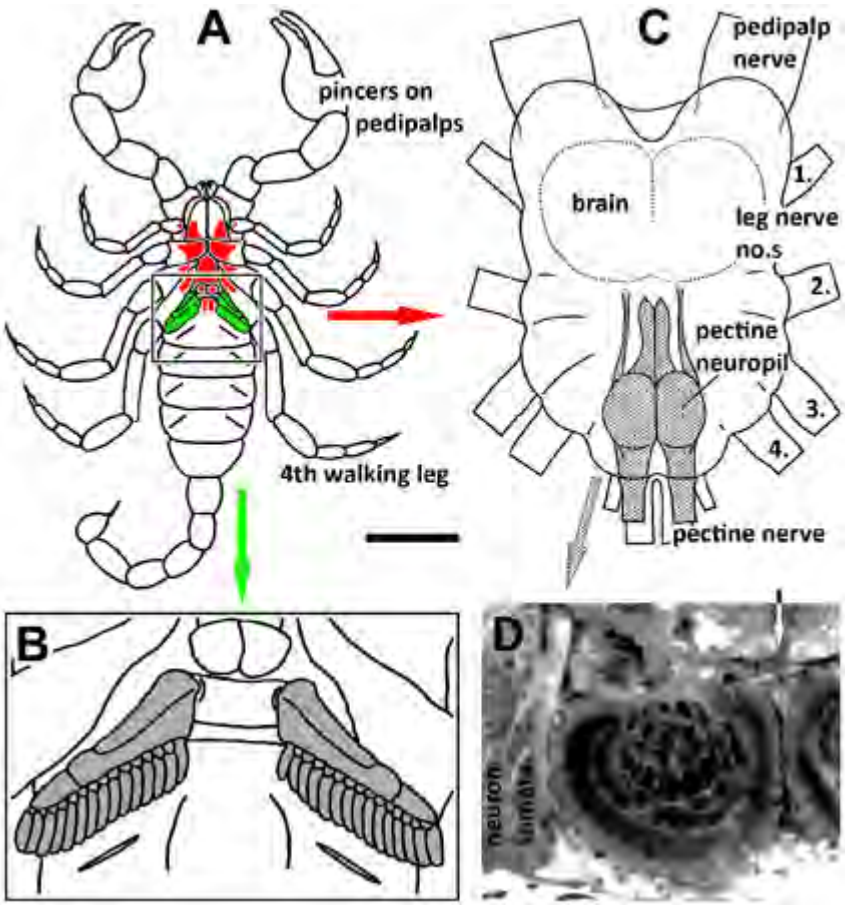
Figure legend

A, ventral view of a scorpion with pectines (green) and fused part of the central nervous system (red) indicated.

B, enlarged detail of the pectines (grey).

C, fused part of the central nervous system with pectine neuropils (grey) and major nerves indicated.

D, histological cross section through a posterior pectine neuropil; glomeruli are darkly stained (Wigglesworth staining). Scale bars: A, 22.5 mm; C, 0.1 mm; D, 0.475 mm


A
pincers on
pedipalps
4th walking leg
B
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pedipalp
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1.
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4.
pectine nerve
D
neuron somata

High conservation of family size and genomic structure of *ora* olfactory receptor genes in twelve teleost species

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Recently, a novel olfactory receptor gene family has been identified in teleost fishes (Saraiva and Korsching 2007). This family of *ora* genes forms a single clade together with the entire mammalian V1R family. All zebrafish *ora* genes are expressed in sparse cells in the sensory surface of the olfactory epithelium, which is consistent with the expectation of the expression pattern for olfactory receptors. Initial observations in 5 teleost species showed an unexpected high degree of conservation of ORA family size and genomic structure. However, recently gene expansions have been described for this family in another fish, coelacanth (Syed and Korsching 2014). Coelacanths are one of the very few extant fish species in the lobe-finned lineage, which gave rise to all tetrapods, whereas the previously analysed fish species belong to the ray-finned lineage. A further distinction between these two lineages is the presence of several introns in the *ora* genes, which was only found in teleost species.
Here we identified the complete *ora* gene families in five other teleost species *(Xiphophorus maculatus, Lepisosteus oculatus, Oreochromis niloticus, Astyanax mexicanus, Gadus morhua)*, and characterized their genomic organization. We report that in all species the *ora* gene family consists of six genes, which are direct orthologs of the respective zebrafish *ora* genes. Furthermore the exon/intron structure as well as the genomic arrangement in symmetric pairs for *ora* genes *ora1*,*2* and *ora3*,*4*, respectively, is faithfully preserved in all species analysed here, including the spotted gar *(Lepisosteus oculatus)*, a very early diverging species in the teleost lineage.

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High fat feeding affects the number of GPR120 cells and enteroendocrine cells in the mouse stomach

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Long-term intake of dietary fat is supposed to be associated with adaptive reactions of the organism and it is assumptive that this is particularly true for fat responsive epithelial cells in the mucosa of the gastrointestinal tract. Recent studies suggest that epithelial cells expressing the receptor for medium and long chain fatty acids, GPR120 (FFAR4), may operate as fat sensors. Changes in expression level and/or cell density are supposed to be accompanied with a consumption of high fat (HF) diet. To assess whether feeding a HF diet might impact on the expression of fatty acid receptors or the number of lipid sensing cells as well as enteroendocrine cell populations, gastric tissue samples of non-obese and obese mice were compared using an real time PCR and immunohistochemical approach. In this study, we have identified GPR120 cells in the corpus region of the mouse stomach which appeared to be brush cells. Monitoring the effect of HF diet on the expression of GPR120 revealed that after 3 weeks and 6 months the level of mRNA for GPR120 in the tissue was significantly increased which coincided with and probably reflected a significant increase in the number of GPR120 positive cells in the corpus region; in contrast, within the antrum region, the number of GPR120 cells decreased. Furthermore, dietary fat intake also led to changes in the number of enteroendocrine cells producing either ghrelin or gastrin. After 3 weeks and even more pronounced after 6 months the number of ghrelin cells and gastrin cells was significantly increased. These results imply that a HF diet leads to significant changes in the cellular repertoire of the stomach mucosa. Whether these changes are a consequence of the direct exposure to high fat in the luminal content or a physiological response to the high level of fat in the body remains elusive.

Identification of a natural source for the OR37B ligand

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The capacity of the mammalian olfactory system to detect an enormous array of different chemical compounds is based on a large repertoire of odorant receptors (ORs). A small group of these ORs, the OR37 subfamily, is unique due to a variety of special features. Members of the OR37 family are exclusively found in mammals, they share a high degree of sequence homology and are highly conserved during evolution. In order to identify ligands for these atypical receptors we exposed mice to odorant compounds and monitored the activation of OR37 glomeruli through the expression of the activity marker c-fos in juxtaglomerular cells. Stimulation with long-chain fatty aldehydes elicited strong activation of OR37A, B or OR37C glomeruli; each of them responding preferentially to an aldehyde with different chain length. In search for biological sources of these long-chain fatty aldehydes (penta-, hexa- and heptadecanal), the headspace of secretions and excretions from mice was analyzed by gas chromatography and mass spectrometry. In urine, skin swabs and saliva, these components were not detectable. However, in feces pellets a substantial amount of hexadecanal, the OR37B ligand, was found. Accordingly, exposure of mice to feces induced an activation of the OR37B glomerulus, whereas the OR37A- and the OR37C-glomerulus were not responsive. The amount of hexadecanal deposited with feces varied significantly, however, it was independent from the amount of feed. In many species, feces is covered with secretion from anal glands. Due to the size and the inaccessibility of these glands in mice, the headspace of anal gland secretion from dog was analyzed by GC/MS, which resulted in a prominent peak for hexadecanal. Exposure of mice to anal gland secretion from dog activated the OR37B glomerulus. Altogether, these data suggest that hexadecanal, a ligand for the receptor OR37B, is produced in anal glands and deposited with feces into the environment.

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Illuminating the function of inhibitory microcircuits in the zebrafish homologue of olfactory cortex

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The brain creates dynamic representations of the sensory environment by extracting stimulus features at early processing stages and synthesizing more abstract object representations in higher brain areas. We dissect the function of neuronal microcircuits in a higher olfactory brain area in order to identify elementary computations of basic cortical circuits and to analyze the underlying cellular mechanisms. To this end, we use a combination of genetic, electrophysiological and optical approaches to visualize and manipulate different types of interneurons (INs) in the posterior zone of the dorsal telencephalon (Dp) of adult zebrafish. This brain area is homologous to olfactory cortex in mammals and assumed to be involved in olfactory object representations and associative memory. We identified two types of INs that have similar electrophysiological properties but are differently connected to other neurons in Dp. Preliminary results suggest that optogenetic silencing of these IN types has different effects on odor-evoked activity patterns in Dp. Ongoing experiments further examine the influence of these interneurons on the processing of odor information in order to elucidate computations performed by different microcircuits in Dp. The results are expected to provide insights into canonical computations performed by basic cortical circuits.

Imaging molecular vibration sensing in honeybee olfactory circuit

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An odorant molecule interacts with an olfactory receptor triggering a signal cascade within the olfactory circuit, resulting in the perception of a frangrance. At present, no satisfactory theory exists that can fully explain the molecular mechanism which regulates the odorant-receptor interaction. According to the classical “lock-and-key” model, such interaction is based on chemical-physical properties such as shape, size and charge distribution. However, recent evidences have shown that insects are capable of distinguishing different isotopomers of the same olfacotry molecule (in which hydrogen atoms have been replaced with deuterium) and of learning them through differential conditioning paradigm. Such findings suggest the possibility that odors may be distinguished based on other biophysical properties such as their vibrational energy spectrum. To further investigate such hypothesis, *in vivo* calcium imaging analysis was performed on the primary olfactory processing centre (i.e. the antennal lobe) of the honeybee *Apis mellifera*. Four common scents and their relative deuterated counterparts were employed to test for differential neuronal activity in this animal model. The comparison between patterns of stimulus-induced neuronal activation strongly suggests the capability of the olfactory circuit to distinguish between two isotopomers. The findings hereby discussed, which will need to be further corroborated by a theoretical model, represent the first in vivo functional imaging data challenging the standard ‘lock-and-key’ model for ligand-receptor interaction.

Involvement of the GPCR, DopEcR, in the modulation of olfactory-guided behaviour in a moth

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In moths, males use female-emitted sex pheromones to find their mating partner and both males and females use plant-related odorants to detect food sources. The behaviour elicited by such odour sources is, however, submitted to modulation as a function of physiological state or experience. Both hormones and biogenic amines have been shown in the past to be involved in the modulatory mechanisms of olfactory-guided behaviour. In the migratory moth, *Agrotis ipsilon*, there is an age-dependent olfactory plasticity: the behavioural and central nervous responses to sex pheromone increase with age. The major insect hormones, juvenile hormone and 20 hydroxyecdysone (20E), as well as biogenic amines have previously been shown to play a role in the sexual maturation of this moth.
We investigated here the role of a membrane-bound G-protein coupled receptor, DopEcR, with a double affinity for 20E and dopamine in the maturation-dependent olfactory plasticity in the male moth *A. ipsilon*. The receptor, AipsDopEcR, was cloned and identified primarily in the brain and immunocytochemically detected in the primary olfactory centres, the antennal lobes, and in the mushroom bodies, which serve as higher integration centres. Expression of the AipsDopEcR transcript and the corresponding protein increased with age in adult males, in parallel with increasing behavioural responses to the sex pheromone. Inhibition of AipsDopEcR by injecting dsRNA into young males resulted in a strong reduction of behavioural pheromone responses in sexually mature males (Abrieux et al., 2013). In parallel, sensitivity of antennal lobe neurons to the sex pheromone decreased strongly. Behavioural experiments showed also that both ligands of AipsDopEcR, 20E and dopamine interact with this receptor when modulating responses to the sex pheromone (Abrieux et al., 2014). Whereas other dopamine receptors seem not to be involved in the age-dependent olfactory plasticity, the nuclear ecdysone receptor (EcR/USP) can also mediate 20E action to modulate sex pheromone responses at the behavioural and neuronal level. In conclusion, our results show that AipsDopEcR is an important element that modulates male sexual behaviour by controlling the central nervous processing of sex pheromone through the action of 20E and dopamine.

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Kenyon cell plasticity in adult red flour beetles

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With its fully sequenced genome and the susceptibility for reverse genetics based upon RNA interference (RNAi), the red flour beetle *Tribolium castaneum* is best suited to study the development and plasticity of the nervous system. While plasticity can be provided by various mechanisms, we focus on ongoing cell proliferation in the adult beetle brain. It is well established that neurogenesis persists in the mushroom bodies (MB) of adult insects (Cayre et al. 2002. Comp Biochem Physiol B Biochem Mol Biol. 132:1-15), including the red flour beetle *T. castaneum* where neuroblasts giving birth to MB Kenyon-cells remain active for more than one month after adult eclosion (Zhao et al.. 2008. Devel Neurobio, 68: 1487–1502). To label cell proliferation in the adult beetle we successfully adapted the 5-ethyl-2'-deoxyuridine (EdU) technique into living beetles. In combination with immunohistochemistry against various neuromediators - including e.g. neuropeptides and NO-synthase - and the glia-cell marker reversed-polarity, we labeled the progenies of adult persisting neuroblasts, determined their identity and counted the newborn Kenyon cells in the first days after adult eclosion.

In several studies it was proposed that newborn neurons of MBs may play a role during olfactory processing and learning. Currently we combine the EdU-staining with olfactory stimulation using different odors like the leaf alcohol cis-3-hexen-1-ol or the beetle's aggregation pheromone 4,8-dimeythydecanal (DMD), odor deprivation or knockdown of the ORCO receptor via systemic RNAi to investigate whether the rate of MB neurogenesis depends on olfactory input.

Magnetic field-driven induction of ZENK in the trigeminal system of pigeons (Columba livia).

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Birds can use the Earth´s magnetic field as a reference system for orientation. The upper beak has been suggested to contain a magnetosensor based on ferro-magnetic structures. Recently, the existence of such magnetosensitive structures in the upper beak of pigeons has been seriously challenged by recent studies suggesting that the previously described iron-accumulations are macrophages, not magnetosensitive nerve endings. Does that mean that there are no magnetic sensors in the upper beak of pigeons? The only nerve innervating the upper beak of birds is the ophthalmic branch of the trigeminal nerve (V1). V1 projects into the trigeminal brainstem complex, i.e. the principal and spinal sensory trigeminal nuclei (PrV and SpV) of the hindbrain. Thus, magnetic information transmitted by V1 in homing pigeons should lead to neuronal activation in PrV and SpV. We therefore exposed pigeons to either a constantly changing magnetic field (CMF), to a zero magnetic field (ZMF) providing no magnetic information, or to CMF conditions after V1 was cut bilaterally. Using an antibody against the neuronal activity marker ZENK, we observed strong neuronal activation in PrV and medial SpV, when pigeons with intact nerves experienced CMF conditions. This activation significantly dropped when birds experienced no magnetic field (ZMF) or after V1 sectioning. Neuronal tract tracing revealed close proximity between V1 terminations and ZENK immunosignal. Our data suggest that magnetic field information activates the trigeminal brainstem complex in pigeons. Thus, V1 does seem to innervate a region containing magnetic sensors

Metamorphotic remodeling of the olfactory organ of the African clawed frog *Xenopus laevis*

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The fully aquatic larvae of *Xenopus laevis* have an anatomically segregated main and accessory olfactory system. The larval main olfactory epithelium is located in the so-called principal cavity (PC), the epithelium of the accessory system is situated in the vomeronasal organ (VNO). During metamorphosis, a second cavity, the middle cavity (MC), also lined with sensory epithelium develops and a drastic reorganization of the epithelium of the PC takes place. In larvae, sensory neurons of the PC are used to detect waterborne odorants, but after metamorphosis the PC is thought to act as an 'air nose'. The MC very likely serves as a ‘water nose’. The VNO, like in the larval stages, is filled with water also in the adult frog.
Combining nerve backfills, in vivo electroporation, functional calcium imaging, immunohistochemistry, and a bromo-2'-deoxyuridine incorporation assay, we monitored the metamorphotic changes of the olfactory organ of *Xenopus laevis*. We first undertook a thorough examination of the anatomical changes of the olfactory cavity. We then set out to answer the question whether sensory neurons of the PC and the VNO are retained or replaced. We also investigated if sensory neurons are respecified and to which extent the sensory neurons of the MC are newly formed during metamorphosis. Together our findings illustrate the structural changes of the olfactory organ during larval development.

This work was supported by DFG Schwerpunktprogramm 1392 (project MA 4113/2-2), cluster of Excellence and DFG Research Center Nanoscale Microscopy and Molecular Physiology of the Brain (project B1-9), and the German Ministry of Research and Education (BMBF; project 1364480).

Molecular basis of olfactory receptor evolution in closely related *Anopheles* mosquitoes

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The closely related *Anopheles* sibling species *A. gambiae*, *A. arabiensis* and *A. quadriannulatus* exhibit great differences in their host preference for blood uptake. *Anopheles gambiae* as a major vector of the human malaria parasite *Plasmodium falciparum* shows a distinct host preference for humans, whereas the non-vector *A. quadriannulatus* blood-feed on animals and suggested to be zoophagic. An opportunistic and therefore intermediate blood-feeding behavior is shown for *A. arabiensis*. Besides CO_2 and the body temperature, most important for host seeking in mosquitoes are odorant molecules as long-range cues binding to specific olfactory receptors.
In this study, we identify structural differences among olfactory receptor genes that have accrued over evolutionary time in the three *Anopheles* sibling species that have lead to variations in the receptor protein structure and pharmacological profile. Thus, the modification of odour space at the molecular level among these closely related species may have lead different host preferences. Therefore, odorant receptors that have previously been demonstrate to be linked to host seeking were molecularly characterized. To investigate functional differences, native and mutated odorant receptor proteins were expressed in insect cells and their response to natural ligands was examined.

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Molecular elements in the detection of the minor sex pheromone component in *Heliothis virescens*

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Mate finding in many insects highly depends on female-released sex pheromone blends and the accurate and sensitive detection of the major and minor components by the males. In the moth *H. virescens* the females release a blend containing (Z)-11-hexadecenal (Z11-16:Ald) as the major and (Z)-9-tetradecenal (Z9-14:Ald) as principle minor sex pheromone component. Previously, we found that in males the detection of the major pheromone component involves three molecular elements: firstly, the pheromone receptor HR13, which is expressed by specialized olfactory sensory neurons (OSNs) projecting their dendrites into hair-like sensilla trichodea on the antenna; secondly, the pheromone binding protein type 2 (PBP2) supposed to capture pheromone molecules from the air and deliver them to the pheromone receptor in the dendritic membrane; and finally, the so-called “sensory neuron membrane proteins" type 1 (SNMP1) possibly operating as docking sites for PBP2/pheromone complexes and/or contributing to the release of pheromones to the pheromone receptor. Whereas for the main component Z11-16:Ald three key elements in the detection process are known, for the minor component only the pheromone receptor HR6 has been identified. In this study, we tried to illuminate which PBP-type is involved in the detection of Z9-14:Ald and whether SNMP1 may also play a role. Towards this goal we assessed the expression and colocalization of HR6, SNMP1 as well as of PBP1 and PBP2 on the male antenna by means of two-colour fluorescence *in situ* hybridization (FISH) with combinations of specific riboprobes and fluorescent immunohistochemistry (FIHC) using specific antisera. Because female trichoid sensilla also possess Z9-14:Ald responsive OSNs supposed to play a role in autodetection of the released pheromone component, we performed comparative studies with female antennae. In order to get first insight into a specific function of SNMP1 in the detection of Z9-14:Ald we have reconstituted the pheromone detection system in modified HEK293 cells and tested cell lines expressing HR6 and/or SNMP1 for their responses to the minor pheromone component.

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Morphological analysis of mitral cell populations in the mouse accessory olfactory bulb

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In most mammals, the vomeronasal / accessory olfactory system is crucial for inter- and intraspecific communication. The accessory olfactory bulb (AOB) represents the first stage of information processing within the vomeronasal pathway. Mitral cells (MCs) are the only excitatory AOB projection neurons. Two subpopulations of MCs display strikingly different spontaneous discharge patterns. Members of one population fire action potentials in a seemingly random fashion, while others intrinsically exhibit slow oscillatory discharge with subthreshold membrane potential fluctuations und superimposed trains of action potentials.

Here, we comparatively investigate the morphology of both MC subpopulations using an immunohistochemical approach. During patch-clamp experiments in acute mouse AOB slices, MCs were diffusion-loaded with biocytin for post-hoc staining and 3D reconstruction. Surprisingly, the morphological properties of both populations are strikingly similar. However, we observe one notable exception: primary dendrites of intrinsically oscillating MCs terminate in significantly larger glomeruli.

On-going multi-photon imaging experiments now aim to provide a more detailed characterization of the glomerular organization in the AOB, specifically focusing on correlations between MC physiology and glomerular morphology.

Multimodal coding in the gustatory system of the Drosophila larvae

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Multimodal coding in the gustatory system of the Drosophila larvae

Lena van Giesen and Simon G. Sprecher

Sensory Neurons are able to perceive direct input from the surrounding environment of an organism. Chemical molecules can be perceived by either the olfactory or the gustatory system. The gustatory system of the Drosophila larvae contains a small number of about 30-60 gustatory receptor neurons (GRNs), which in turn is functioning to decipher complex gustatory cues form the environment containing thousands of tastans. How this small number of neurons is able to achieve this astonishing function is of great interest to understand how sensory modalities can generally be encoded. Here we analyze the physiological responses of five different GRNs using genetically encoded Calcium sensors. Surprisingly our results show, that larval GRNs are able to sense more than one of the four classical taste modalities that can be perceived by the adult fly: bitter, sweet, salty and water/low salt per neuron. This form of multimodality might enable a broader perception regarding a relatively small number of receptor neurons. Investigating the behavioral impact of GRNs we found, that individual neurons play a role in the integration of different stimuli (e.g. bitter and sweet). In order to gain insight into the molecular nature of gustatory information coding, we performed RNAseq transcriptome analysis of the gustatory and olfactory organs of the larva.

NEUROCELLULAR BASIS OF OLFACTORY ACUMEN IN THE AFRICAN GIANT RAT (Cricetomys gambianus)

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The main olfactory bulb (MOB) is the initial processing site for volatile chemical stimuli and receives input from the olfactory receptor cells located in the olfactory epithelium. Olfaction, being critically important for food consumption, emotional responses, aggression, maternal and reproductive functions, neuroendocrine regulation, recognition of con-specifics, predators, and prey often times exceeds visual or auditory cues in importance in many species. One of such species is the African giant rat (AGR) - one of Africa's largest rodents with an important olfactory sensory system as evidenced by a relatively large olfactory bulb, large olfactory areas of the piriform cortex and hippocampus. It has been utilized to detect landmines and tuberculosis samples using olfactory cues. A striking gap however exists in the literature on the neurocellular components that govern this olfactory acumen. This study therefore, investigated and described the neurocellular organization, morphometric parameters and adult neurogenesis profile of the AGR MOB across age groups to identify structures that corroborate its olfactory behaviors. 15 AGR classed as neonates (n=5); juvenile (n=5) and adults (n=5) were used. Cresyl violet (for Nissl) and Klu¨ver–Barrera (for myelin sheath) histological staining of the olfactory bulb revealed a cytoarchitecture typical of most mammals with 6 cell layers, and 1–2-layered glomeruli measuring approximately 150 µm each in diameter with a mid-central glomeruli of up to 8 layers. Immunohistochemical staining with glial fibrillary acidic protein (GFAP) and 2',3'-cyclic nucleotide 3-phosphodiesterase (CNPase) showed soma and evidence of myelin sheath deposition at all layers of the bulb. GFAP immunohistochemistry also revealed cell bodies and processes within the periglomerular area which may potentiate signaling from the olfactory receptor cells. Adult neurogenesis was examined using the neurogenic markers Doublecortin (DCX) and Ki-67. Migration of newly generated cells was observed in all layers of the MOB with DCX in all ages and in most layers with Ki-67 with the juvenile AGR showing greatest potential for neuroplasticity. The cellular anatomy of the MOB is described in the AGR with features indicating olfactory importance including the presence of an accessory olfactory bulb; extensive myelination across all cell layers; the mid-central multi-glomerular layering; mitral cell and olfactory piriform cortex connections; large granule cell and periventricular layers with their glia associations and positive adult neurogenesis profile in the MOB and the somatosensory cortex of the brain of the juvenile AGR. Neurogenic cells are postulated to be from progenitors derived from the sub-ventricular germinal zone and or local parenchyma progenitor. This feature is proposed to be associated with the social lifestyle with its continuous burrowing which indicate a motor function of the brain and memory and indicate that juvenile AGR are best suited as a model for cognitive and olfactory training.

Neuropeptides and Blood Feeding Behavior – A Quantitative Analysis in *Aedes aegypti*

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Mosquitoes are among the most dangerous animals in world, as they transmit a wide range of viral and parasitic diseases. Two significant threats for human health are the viral-borne diseases Yellow fever and Dengue fever that are transmitted by the mosquito *Aedes aegypti*. A target for vector control is the mosquito reproductive cycle, which involves blood feeding as well as mating, both complex behaviors depending to a large extent on olfactory cues. These behaviors are not static, and the aim of our studies is to understand how these olfactory-guided behaviors are regulated in *Aedes aegypti*. Focusing on the primary olfactory centers – the antennal lobes – we performed semi-quantitative mass spectrometry to reveal changes in the concentration within five neuropeptide families (tachykinin, short neuropeptide F, allatostatin-A, neuropeptide-like precursor and SIFamide) depending on the feeding status of *Aedes aegypti*. Female *Aedes aegypti* were blood fed six days after adult eclosion and the concentration of neuropeptides between blood fed and sugar fed animals were compared 1h, 24h, 48h and 72h post blood meal, as well as 24h post oviposition. We show a significant decrease in the concentration of short neuropeptide F and allatostatin A in the antennal lobes of blood fed mosquitoes at 24h and 48h post blood meal. This corresponds to a period of inhibition of the host-seeking behavior of *Aedes aegypti*. Future behavioral studies are aimed at elucidating the significance of these neuropeptides in regulating this odor-mediated behavior.

Neuropeptides and Mating Behavior – A Quantitative Analysis in *Aedes aegypti*

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Mosquitoes are among the most dangerous animals in world, as they transmit a wide range of viral and parasitic diseases. Two significant threats for human health are the viral-borne diseases Yellow fever and Dengue fever that are transmitted by the mosquito *Aedes aegypti*. A target for vector control is the mosquito reproductive cycle, which involves blood feeding as well as mating, both complex behaviors depending to a large extent on olfactory cues. These behaviors are not static, and the aim of our studies is to understand how these olfactory-guided behaviors are regulated in *Aedes aegypti*. Focusing on the primary olfactory centers – the antennal lobes – we performed semi-quantitative mass spectrometry to reveal changes in the concentration within five neuropeptide families (tachykinin, short neuropeptide F, allatostatin-A, neuropeptide-like precursor and SIFamide) depending on the mating status of *Aedes aegypti*. Male and female *Aedes aegypti* were mated three days after adult eclosion, when their reproductive organs are fully developed. Semi-quantitative mass spectrometry analysis of the antennal lobes was then conducted at several time points after mating and compared to that of virgin animals of the same age. In both males and females we found a change in the concentration of SIFamide following mating. These changes were sex dependent.

Octopamine Modulates the Excitability of Identified Olfactory Interneurons in the Cockroach Antennal Lobe

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The insect antennal lobe (AL) is the first synaptic relay and processing center of olfactory information, received from olfactory sensory neurons located on the antennae. Complex synaptic connectivity between olfactory neurons of the AL ultimately determines the spatial and temporal tuning profile of (output) projection neurons to odors. The insect olfactory system as other sensory systems has to adapt its sensitivity and performance to changes in the environment.
Octopamine and its precursor tyramine are often considered as the invertebrate homologues to the vertebrate noradrenalin and adrenalin, respectively. Previous work in various insect preparations has shown that the monoamine octopamine can act as neurotransmitter, neurohormone and/or neuromodulator in the insect nervous system and is involved in the regulation of numerous physiological processes. Using biochemical and immunohistochemical approaches octopamine has been detected in the insect antennal lobe. In the honeybee antennal lobe Ca^{2+} imaging has been used to analyze the effect of octopamine on the network level, clearly showing a strong modulatory effect on odor responses (Rein et al. J. Comp. Physiol. Vol. 11:947 62).
Here, we investigate the modulatory effect of octopamine on identified antennal lobe neurons in an intact brain preparation of the American cockroach *Periplaneta americana*. Patch-clamp recordings of synaptically isolated inhibitory GABAergic local interneurons reveal that the general octopamine effect on these neurons is a concentration dependent increase in excitability. Currently, we are analyzing the cellular mechanisms that mediate the octopamine effects on the intrinsic electrophysiological properties.

Odor responses of *Drosophila* receptor neurons – Response profiles, mixtures and individual response dynamics

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Coding odors with patterns of differentially activated or inhibited olfactory receptor neurons (ORN) enables olfactory sensory systems to encode a huge number of different olfactory stimuli. These ensemble responses arise from classes of ORNs that are sensitive to different but overlapping sets of odorants. Tuning profiles range from broadly tuned generalists that may respond to hundreds or thousands of substances, to narrowly tuned specialist ORNs that respond to a handful or even single substances only. On a single cell level ORN responses vary in strength as well as in temporal dynamics, e.g. response polarity or response duration. Responses become even more complex when presenting odor mixtures. Two substances might interact, generating mixture responses with distinct strength or dynamics as compared to the individual component responses.

Here we present response profiles for eight classes of *Drosophila* ORNs. We recorded responses towards a set of ~100 mono-molecular odorants. We also analyzed the response dynamics of the resulting ~800 odorant-ORN combinations in detail. For a smaller set of odorants and ORNs we studied the responses elicited by binary mixtures compared to their components.

Within our set of eight ORNs we found breadth of tuning to be widely distributed, observing generalist- as well as specialist ORNs. For Or56a bearing neurons which are known to be narrowly tuned to geosmin, we found several active ligands besides that single odorant, even though these responses were much weaker. Most of the responses we analyzed were of an excitatory nature, fewer were inhibitory or showed initial excitation followed by a post-stimulus inhibitory phase. Dynamical response features were very diverse across odorant-ORN combinations: Some were strong and prolonged, meaning they continued beyond stimulus offset, others were short-lived (phasic) and still others were complex over time, including excitatory and inhibitory bouts. Binary mixtures generally produced weak or no interactions, though a few combinations lead to suppressive or synergistic mixture responses. Across ORNs about half of the mixture responses were dominated by one component, while the other half had distinct activity patterns. These measurements are relevant for understanding how odor information is coded in combinatorial activity patterns.

Olfactory imprinting and pERK related cellular activity in the zebrafish olfactory system

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Imprinting is a learning process during early development which leads to irreversible changes in behavior. Based on behavioral experiments, zebrafish larvae were shown to imprint on olfactory and visual kin cues during day 5 to day 6 post fertilization (Gerlach et al., 2008; Hinz et al., 2012). Furthermore, zebrafish imprinting depends on MHC class II related signals and only larvae that share MHC class II alleles can imprint on each other (Hinz et al., 2012, 2013a). Some zebrafish can also be imprinted on kin using a special synthetic MHC peptide mix (Hinz et al., 2013b). Using multiphoton imaging, MHC peptides evoked calcium signals in the olfactory bulb of transgenic GCaMP2 larvae at very low thresholds which overlapped spatially in the bulb with responses to kin water (Hinz et al., 2013b).

In contrast to most land vertebrates, the teleostean (e.g., zebrafish) olfactory system lacks a vomeronasal organ and consists only of a paired main olfactory epithelium which displays four types of olfactory sensory neurons (ONS). These perceive olfactory stimuli and mediate odor information into the olfactory bulb, the first central nervous station for odor processing. Similar to mammals, ciliated OSNs express olfactory receptors of the OR and TAAR families, whereas microvillous OSNs express receptors of the V1R and V2R gene families (Saraiva and Korsching, 2007; Hussain et al., 2009; Oka et al., 2011) Additionally, teleostean olfactory epithelia feature crypt cells, possessing microvilli and cilia. Crypt cells express apparently only a single olfactory receptor, the V1R-related ORA4 (Ahuja et al., 2013). A fourth type of OSNs was recently identified in the zebrafish, i.e. Kappe neurons, which only possess microvilli (Ahuja et al., 2014), but lie in similar superficial position like crypt OSNs.

Presently, the type of OSNs detecting kin specific odor are unknown. In order to investigate this, we differentially activated OSNs and showed neuronal activity reflected by an increase in pERK (phosphorylated extracellular signal regulated kinase) after odor stimulation. We stimulated larvae with food or conspecific odor with different stimuli exposure times and quantified activated OSNs. This work revealed that the numbers of all activated cell types did not show differences depending on stimuli duration (between 3 to 15 min). However, different odor stimuli activated various OSN types differently (for example, ciliated OSNs react strongly to food). We further present preliminary evidence that crypt OSNs and some subpopulations of microvillous OSNs respond strongly to synthetic MHC peptide mix. Further experiments will involve imprinted and non-imprinted larvae to see differences in OSN activation reflected by pERK with different olfactory stimulations.

Orco expression during larval and pupal development of the red flour beetle *Tribolium castaneum*

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Through the fully sequenced genome, the possibility to generate trans-genetic lines and its susceptibility to reverse genetics by RNA interference, the red flour beetle *Tribolium castaneum* has established as a model for neurobiological research.
We investigate the expression of the olfactory co-receptor Orco during the larval and pupal development of *T. castaneum*. Therefore we co-express Orco with two fluorescent proteins (tGFP and dsRed) in two different transgenic beetle lineages using the Gal4-UAS-system. We find expression of Orco in all developmental stages until adulthood except in the prepupae and early larval stage (P0 to P20). A strong fluorescent signal in distinct zones of the antennae and maxillary palps reappears in pupal stage P20, whereas the last antennal segment shows a weaker signal which gets stronger in the following stages.
The data suggest, similar to results found in *Drosophila melanogaster*, that larval OSNs expressing Orco undergo programmed cell death and are replaced with new OSNs between the pre-pupal stage and the first pupal stages of *T. castaneum* (Stocker 1994. Cell Tissue Res 275:3; Larsson et al. 2004. Neuron 43:703).

Organization of the blood–brain barrier in the olfactory nerve layer

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Olfactory ensheathing cells (OECs) are specialzed glia cells localized in the olfactory nerve layer (ONL). OECs ensheath bundles of axons of the olfactory receptor neurons (ORN) and support the axonal growth into the olfactory bulb. Recent studies show, that OECs are in contact to blood vessels in the ONL to regulate local blood flow (Thyssen et al. 2010 PNAS 107:15258-63). Astrocytes, as being a part of the blood-brain barrier (BBB) in the CNS, play a major role in providing nutrients and O_2 to neurons. Since only few astrocytes have been found in the ONL, we investigated the question whether OECs might fulfill these functions in the BBB in the ONL. To investigate the cellular components of the BBB in the ONL we performed immunostainings using four biomarkers: anti-GFAP (astrocytes), anti-S100b (OECs), anti-AQP4 (astrocytic endfeet), and anti-PDGFRß (pericytes on capillaries). Immunostainings for OECs and astrocytes suggest that OECs were found in the ONL only, whereas astrocytes project their endfeet in the inner ONL, but not the outer ONL, while their nuclei were localized in deeper layers. The immunhistochemical characterization of the BBB in the ONL showed that capillaries detected in the outer ONL (ONL_o) are ensheathed by OECs, whereas capillaries in the inner ONL (ONL_i) are surrounded by astrocytic endfeet. Electron microscopy studies confirmed that OECs contribute to the BBB. These oberservations indicate that both astrocytes and OECs contribute to the BBB in the ONL.

Origin of high variability in colony odor representation in the ants' brain

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Ants can discriminate nestmates (NM) from non-nestmates (nNM) and react aggressively against the latter. Discrimination is based on odors that consist of many different chemical components on the ants' body (cuticular hydrocarbons: CHCs). Although colony odors of a given species vary almost only in the ratios of the components between different colonies, ants are able to recognize amazingly fast and precise whether an encountered ant is a nestmate or a non-nestmate. Importantly, colony odors vary over time, depending on diet and environment.

Colony odors are represented, like general odors as well, in a spatio-temporal activation pattern of functional units in the antennal lobe (glomeruli). Repeated stimulations with NM colony odors revealed a significantly higher variability of spatial response patterns in the antennal lobes compared to repeated stimulations with nNM colony odors (Brandstaetter and Kleineidam 2011). The functional significance of this neuronal variability is unknown. We addressed two questions:

1) Are single glomeruli sufficient for classification of NM versus nNM odors?

2) What is the origin of the high variability in NM odor representation?

Using support vector machines (SVM), we obtained a mapped presentation of data points, which separate the two categories nestmate and non-nestmate odor stimulations. We found that the two most strongly loaded glomeruli per animal alone allow quite good separation.

In order to unravel the origin of high variability in colony odor representation, we analyzed the response behavior of single glomeruli over repeated NM and nNM colony odor stimulations. The total number of glomeruli that are only activated by NM odors and not by nNM odors (NM specific glomeruli) is not different to the total number of nNM specific glomeruli. Interestingly, only when NM colony odors are presented repeatedly, some glomeruli respond in addition to the previously as NM-specific identified glomeruli, and this was not the case for repeated nNM colony odor stimulations.

Such plasticity in representation of colony odors may promote discrimination of NM and nNM colony odors, particularly when colony odors change over time. However, the mechanism how this plasticity is generated within the neuronal network and how the glomeruli, which contribute most for classification, change as result of plasticity, remains to be investigated.

Brandstaetter A. S., and Kleineidam C. J. (2011) J. Neurophysiol. 106: 2437–2449.

Physiological characterization of formyl peptide receptor expressing cells in the mouse vomeronasal organ

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The mouse vomeronasal organ (VNO) is a chemosensory structure that detects both hetero- and conspecific social cues. Based on largely monogenic expression of either type 1 or 2 vomeronasal receptors (V1Rs / V2Rs) or members of the formyl peptide receptor (FPR) family, the vomeronasal sensory epithelium harbors at least three neuronal subpopulations. While various neurophysiological properties of both V1R- and V2R-expressing neurons have been described using genetically engineered mouse models, the basic biophysical characteristics of the more recently identified FPR-expressing vomeronasal neurons have not been studied. Here, we employ a transgenic mouse strain that coexpresses an enhanced variant of yellow fluorescent protein together with FPR-rs3 allowing to identify and analyze FPR-rs3-expressing neurons in acute VNO tissue slices. Single neuron electrophysiological recordings allow comparative characterization of the biophysical properties inherent to a prototypical member of the FPR-expressing subpopulation of VNO neurons. In this study, we provide an in-depth analysis of both passive and active membrane properties, including detailed characterization of several types of voltage-activated conductances and action potential discharge patterns, in fluorescently labeled versus unmarked vomeronasal neurons. Our results reveal striking similarities in the basic (electro)physiological architecture of both transgene-expressing and non-expressing neurons, confirming the suitability of this genetically engineered mouse model for future studies addressing more specialized issues in vomeronasal FPR neurobiology.

Postmetamorphic plasticity in the intrinsic neuropeptide repertoire of the *Tribolium castaneum* antennal lobes

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Neuropeptides are highly diverse neuronal signaling molecules. Among features such as the modulation of neuronal circuits, neuropeptides are thought to be involved in neuronal plasticity directly affecting processes connected to memory and learning. We examine neuropeptide plasticity in the antennal lobes (ALs) of the red flour beetle *Tribolium castaneum* during the time period after adult eclosion when the adult animals are for the first time confronted with odors from the environment. The paired ALs of insects are the first integration centers for the processing of olfactory information in the insect brain. To assess AL neuropeptides, isolated ALs were subjected to direct peptide profiling by MALDI-TOF mass spectrometry. To quantify relative differences of selected neuropeptides we use synthetic peptide analogues as stable isotope-incorporated internal standards. With this technique we revealed an increase in the relative amount of selected neuropeptides within the first seven days after adult eclosion (A7). In the ongoing project, we examine smaller time intervals between A0 (freshly eclosed) and A7 and a longer time period beyond A7 to better narrow the postmetamorphic phase in relationship to the adult phase.

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Post-stimulus activity in the olfactory pathway of *Drosophila*

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Animals are able to link immediate positive or negative experiences with stimuli that lie in the past. This time-bridging associative ability requires the existence of prolonged stimulus information (a so called stimulus trace), which can be investigated in trace conditioning experiments. In such experiments, animals learn to associate a conditioned stimulus with a reinforcing stimulus, which are separated by a temporal gap. *Drosophila* and other insects are able to solve an olfactory trace conditioning task, revealing an odor trace in insect olfactory systems which lasts for a few seconds. However, the neural substrate of this odor trace is still unknown. Searching for this substrate, we investigated whether olfactory information is kept after odor offset in the different stages of the olfactory pathway of *Drosophila*. We performed *in vivo* calcium imaging with Orco, GH146 and OK107 as driver lines and measured spatio-temporal response patterns in three consecutive processing stages: in olfactory receptor neurons and projection neurons in the glomeruli of the antennal lobe and in Kenyon cell somata of the mushroom body. Receptor neurons and projection neurons responded to odors with combinatorial response patterns of activated and inhibited glomeruli, as previously described (see http://neuro.uni.kn/DoOR). After odor offset, the activity patterns turned into prolonged post-odor response patterns, which were dissimilar to the initial odor response, but still odor specific. These post-odor activity patterns were invariant to changes in stimulus length. Kenyon cell somata also showed odor specific activation patterns during the odor stimulus and prolonged calcium activity after odor offset. However, in contrast to receptor neurons and projection neurons, post-odor patterns in Kenyon cells stayed similar to the initial odor response patterns for several seconds. These results show that both the antennal lobe and mushroom body exhibit ongoing odor specific calcium activity, which could serve as neural substrate for an odor trace.

Regeneration in the insect olfactory system

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We study regeneration processes in the olfactory system of locusts (Locusta migratoria) after damaging the axons of olfactory receptor neurons by crushing the base of one antenna.
On the neuroanatomical level, we observe regeneration of severed fibers into the antennal lobe by anterograde tracing with neurobiotin either through a cut nerve end or by labelling selected sensilla on single antennal segments. Olfactory afferents express the cell surface marker Fasciclin I, which enables us to measure the amount of regeneration within the antennal lobe by means of quantitative Fasciclin I immunofluorescence on vibratome sections. We find that olfactory sensory neurons reliably regenerate back into the brain within 1-2 weeks after damage, and that they re-innervate the microglomerular structures of the antennal lobe in a manner similar to the original innervation patterns. Both post injury degeneration and subsequent regeneration is quicker in subadult nymphs than in adult locusts.
Whether functional synapses are reestablished during regeneration is tested electrophysiologically. Directly after deafferentation, intracellular recordings from antennal projection neurons reveal complete absence of responses to various odor stimuli which elicit reliable responses in 40% of neurons on the untreated side. Re-establishment of synaptic connections is observed beginning seven days post injury, matching well to the time course of anatomical regeneration. In adult locusts the number of responding neurons increases between day seven and 21 after deafferentation until the normal response level is reached. Again, this process happens much faster in 5th instar nymphs where it takes only 10 days to regain the normal response level. We now extend studying functional regeneration in the olfactory system by including the mushroom bodies as the next station of odor information processing.

Representation of odor information in higher brain centers of the vinegar fly

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For insects, and especially for the vinegar fly *Drosophila melanogaster*, finding food sources, good mating partners as well as oviposition sites and avoiding danger is essential. This is mainly relying on the sense of smell. In the olfactory system of the vinegar fly odor information is perceived and trans-ferred from the antennae to the corresponding glomerulus in the primary olfactory neuropil, the antennal lobe (AL) by olfactory sensory neurons (OSN). Within the AL the OSNs synapse onto projection neurons (PN), which innervate higher brain centers, such as the mushroom body calyx and the lateral horn. PNs can be subdivided in different neuronal populations regarding their morphology and physiology. Most PNs are uniglomerular and excitatory, while one population is inhibitory and multiglomerular. The different PNs can be easily identified since they are projecting via different tracts from the AL to the higher brain centers.

In this project we are characterizing the different types of PNs at a morphological and functional level. In order to label individual PNs, we use transgenic fly lines to genetically express the photoactivatable green fluorescent protein (PA-GFP; Patterson & Lippincott-Schwartz, 2002), which is a codon-optimized version of the wild-type GFP. Using a MP-CLSM irradiation with 760 nm wavelength of PA-GFP molecules converts the chromophore population resulting in a 100-fold increase of the fluorescence signal. This enables us to label several PNs innervating the same glomerulus or even single PNs to follow their path from the AL to the higher brain centers. Single neurons were reconstructed and registered onto a reference brain to exert their dendritic and axonal fine arborizations within the different neuropils. This enables us to compare and analyze the innervation patterns in the higher brain centers such as the mushroom body calyx and the lateral horn.

For physiological analysis the calcium sensitive protein GCaMP was expressed in either of the different PN populations. A subset of odors with different hedonic valences (i.e. aversive or attractive) was used to compare the odor-evoked response patterns in the lateral horn for the different types of PNs.

Sulfated Steroids are Chemosensory Stimuli of Both the Main and Accessory Olfactory System of *Xenopus laevis*

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Sulfated steroids are negatively charged molecules with low molecular weight and low volatility that have been shown to be present in mouse urine. They trigger responses in a large number of sensory neurons in the mouse vomeronasal organ, where they are thought to act as pheromones. Using functional calcium imaging in acute slices of the olfactory organ, we found that some pregnanolone-derived (P-mix) and some estrogen-derived (E-mix) sulfated steroids elicit large calcium responses in sensory neurons of *Xenopus laevis*. Their non-sulfated analogues failed to activate the same subset of sensory neurons. Surprisingly, these compounds activate sensory neurons in both the main olfactory epithelium (MOE) and the vomeronasal organ (VNO). All steroid-induced responses were completely abolished in absence of extracellular calcium. However, pregnanolone- and estrogen-derived steroids showed a differential specificity in the two organs. Pregnanolone-derived steroids activated sensory neurons in both the MOE and VNO; estrogen-derived steroids only activated neurons of the MOE. In the response profile analysis, most P-mix-sensitive neurons responded to more than one steroid in the mixture, while most E-mix-sensitive neurons responded mainly to a single chemical in the mixture. This indicates that individual E-mix-sensitive neurons are more narrowly tuned than P-mix-sensitive neurons. Furthermore, many sulfated steroid-sensitive neurons in the MOE also responded upon application of frog and tadpole tank water, suggesting that these chemicals might be secreted by the animals themselves. To test this hypothesis we plan to perform solid-phase chromatography and mass spectrometry on tank water, in order to detect the presence of such chemicals in the natural environment of the frog. In sum, the results show that sulfated steroids can act as chemosensory stimuli, possibly pheromones, in amphibians. Our data provide the basis for further investigations of the physiological and behavioral meanings of these compounds.

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Temporal processing of odor stimuli from Olfactory Receptor Neurons to Projection Neurons

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Olfactory Receptor Neurons (ORNs) respond to odor stimuli with temporal patterns of activity that depend on odor identity, odor concentration and receptor type. We performed measurements of the activity of single ORNs in *Drosophila* and analyzed these temporal patterns taking in account a precise quantification of the time dependent stimulus. We found that the dynamics of the odor stimulus itself depends on odor identity and concentration and that it strongly modulates the ORN response. We were able to assign a single response function to a single ORN and to predict the response of this ORN to two different odors solely from measurements of the stimulus. We also found that ORN adaptation capabilities maintain response dynamics remarkably similar across a large range of stimulus and background intensities (Martelli, 2013).

These results suggest that ORN response properties enable the olfactory system to capture information about stimulus independently from its intensity. In order to investigate this hypothesis we developed a computational model of the *Drosophila* Antennal Lobe (AL), the first synaptic processing center of olfactory information. Our simulation includes three neuron populations: ORNs, inhibitory Local Neurons (LN) and output Projection Neurons (PNs). The model reproduces known static properties of the AL (the divisive normalization, Olsen 2010) while providing temporal resolution on the activity of the single neurons. We investigate how dynamical properties of the input ORN activity affect the encoding of single odorants and odor mixtures in the population of PNs.

The neuronal and molecular basis of caffeine taste signaling in *Drosophila* larvae

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Caffeine is a widely consumed substance, which strongly affects behaviour. Yet, the molecular and neuronal background underlying caffeine perception and processing is not entirely understood. Here, we use *Drosophila* larva as a simple system to investigate caffeine information processing. In larvae, caffeine induces avoidance behaviour, suppresses feeding and decreases survival, but does not reinforce aversive odour associations. Only 12 gustatory neuron pairs co-express the bitter receptors Gr33a and Gr66a. These neurons are necessary for caffeine avoidance. Individual receptor gene mutants for these two genes show partially reduced avoidance. Performing a neuronal screen to pinpoint total loss of caffeine avoidance to single gustatory bitter neurons out of the Gr33a/Gr66a set identifies the single Gr93a positive gustatory neuron pair of dorsal pharyngeal sensilla. This neuron pair responds physiologically to caffeine. Likewise, Gr93a receptor gene function is necessary for caffeine avoidance. In conclusion, our data suggests that caffeine is encoded using a dedicated sensory channel involving Gr93a. This finding opens new avenues to investigate how caffeine affects behaviour by separating stimulation of the sensory system from internal brain functions.

To go or not to go? - Behavioral responses to binary mixtures of attractive and aversive odors

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All animals are challenged by a huge amount of odors. Those can be of good (e.g. food related or sex pheromones), as well as of bad valence (e.g. predators or unhealthy food) for different organisms. In nature, these odorants of opposing valences can co-occur in mixtures and the animals have to decide for a compromise whether to tolerate the bad smell and go for the source or to refuse the odor source. In addition to the absolute valence of the odorants in a given mixture, relative concentration ratios of positive and negative compounds may also influence the behavioral decision. However, up to date it remains unclear how and where along the olfactory pathway this decision is made. Therefore we investigate the olfactory guided behavior of Drosophila melanogaster in the FlyWalk to get insight in flies` decision for or against different attractant:repellent ratios, presenting the attractant ethyl acetate in mixtures with the repellents benzaldehyde or geosmin. In addition we use calcium imaging to follow olfactory information along the pathway to highlight the spot of decision in the fly brain.

To go or not to go? Olfactory processing of odor features: Good vs. Bad

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Natural olfactory stimuli are often complex mixtures of volatiles, which might be mixtures of attractive and aversive odors, in which the identities and ratios of components are important for a fly to compromise and make a decision. Despite this importance, the mechanism by which the olfactory system processes and integrates this complex information remains unclear.
Combining behavioral experiments using the FlyWalk with neurophysiological experiments using optical imaging (from different orders of neurons), we sought to study how and where the information about odor valence, attractive and aversive odors, is encoded and ultimately integrated along the olfactory pathway.

Two octopaminergic VUM neurons bilaterally innervate muscles and sensory epithels of the honeybee antenna

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Actvity and sensitivity changes in muscles and sensory neurons are a well-documented phenomenon that has been related to factors such as satiation state, stress, and circadian rhythms. In insects, octopaminergic DUM neurons have long been known to innervate leg muscles and in the antennal system of the cricket, DUM neurons of the subesophageal ganglion innervate antennal muscles and modulate their activity. For sensory neurons, there is a large body of evidence for modulation by exogenous octopamine. The endogenous source of octopamine has yet to be identified, although the antennal hearts of cockroaches contain octopaminergic neurosecretory cells. Antennal VUM neurons are known in several hymenopteran species. In the honeybee, VUMmx3 and VUMmd3 have been identified individually by Ulrike Schröter (2007). These neurons appear to be the only efferent neurons other than motor neurons in the antennal system. Retrograde tracing combined with immunohistochemistry shows that both neurons are octopaminergic. These neurons are therefore the source of octopaminergic innervation of the antennal system. At the level of the dorsal lobe, collaterals of both neurons split off to innervate the antennal motor nerve supplying the scapus muscles. All four scapus muscles show octopaminergic innervation. In the antennal nerve, projections split into very thin collaterals. While tracers can be used to reveal the morphology of these neurons in the brain with incubation times of up to 2 days, even dextrans are released into and accumulated in extracellular space in the periphery, where varicosities abound. Thus, octopamine-like immunoreactivity has to be employed to display peripheral innervation. Octopaminergic innervation in the antenna is identified in the pedicel muscles but not in other parts of the scapus. In the pedicel, octopaminergic projections are seen near somata and neurites of sensory neurons of the Johnston organ. The flagellum contains very extensive octopaminergic projections that fill the sensory epithelium with a mesh of fibers. Since the number of sensory neurons in the honeybee flagellum is very high, the targets of octopaminergic fibers can only be identified when sensilla are clearly labeled by tracer accumulated following release from VUM neurons or by retrograde tracing of sensory neurons. With these methods, several types of olfactory sensilla (pore plates, basiconic sensilla, olfactory trichoid sensilla) and gustatory sensilla chaetica are shown to have close associations of their dendrites, somata, and axons with octopaminergic processes. Mechanosensory neurons of sensilla trichodea also appear to have octopaminergic innervation in their proximity.
While modulation of the electroantennogram in the honeybee and modulation of behavioural responsiveness to sucrose by octopamine have been shown in the past, it could also be demonstrated that single gustatory sensory neurons are modulated by octopamine and the octopamine antagonist epinastine. Due to the fact that octopaminergic innervation is widespread with many different targets supplied by just two neurons, this system is likely to be involved in state-dependent modulation. The time scale of modulation under natural circumstances is not yet clear, but one hypothesis is certainly the involvement in circadian activity changes.

Unique connectivity from OR37 expressing olfactory sensory neurons to higher brain centers

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Olfactory sensory neurons that express a member from the OR37 subfamily of mouse odorant receptor genes are wired to the main olfactory bulb in a unique monoglomerular fashion. To elucidate the wiring of projection neurons from OR37 glomeruli into higher brain centers, tracing experiments were performed. An application of the lipophilic tracer DiI onto the ventral area of the olfactory bulb, which harbors the OR37 glomeruli, led to the labeling of fibers not only in the typical olfactory cortical regions, but also in the medial amygdala and the paraventricular and supraoptic nucleus of the hypothalamus. No DiI-labeled cell somata were detectable in these nuclei, indicating that projection neurons originating in the OR37 region of the main olfactory bulb form direct connections into these nuclei. To visualize the projections from a defined OR37 glomerulus more precisely, transgenic mice were studied in which olfactory sensory neurons co-express the transsynaptic tracer Wheat Germ Agglutinin (WGA) together with the receptor subtype OR37C. Like in the DiI tracing experiments, WGA immunoreactivity was found in the medial amygdala and in the hypothalamus, however, not in the typical olfactory cortical areas. Double labeling experiments showed that WGA-labeled cells in the hypothalamus expressed vasopressin, but not the related neuropeptide oxytocin. Morphological analysis revealed that they comprise of magno- and parvocellular cells. Together, these results are indicative for a unique connectivity from OR37C sensory cells into higher brain centers.

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Vasopressin depresses excitatory synaptic transmission in mitral cells of the olfactory bulb

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Basic mammalian social interactions strongly rely on olfaction in order to recognize individual conspecifics in rodents, sheep, and even humans. The central arginine-vasopressin (AVP) system enhances social recognition in mammals, which so far has been investigated mostly in the hypothalamus. Within the olfactory bulb, AVP is released from local interneurons, and decreases the firing rate of mitral cells in vivo. This inhibiting action has been proposed to filter social odor information to facilitate odor-based social recognition (Tobin et al. 2010). Here we aim to elucidate the precise mechanisms of AVP release and action in the olfactory bulb. As a starting point, we investigated the effects of AVP on excitatory postsynaptic potentials (EPSP) generated by olfactory sensory neuron input as well as on spontaneous EPSPs in mitral cells. EPSPs were elicited in horizontal slice preparations of juvenile rats via extracellular stimulation of the olfactory nerve (20-300 µA, 100 µs, 30 s intervals). The average amplitude of these EPSPs was 8.1 ± 0.7 mV, and they showed a long-lasting plateau phase (n = 10, rise time: 10.2 ± 1.1 ms, half duration > 500 ms). Bath application of 2 µM AVP for 10 min produced a decrease in the evoked EPSP in 7 out of 10 of mitral cells tested, whereas application of 0.2 µM AVP did not alter EPSP amplitudes in separate experiments (n = 5). The average decrease in EPSP amplitude was to 82 ± 5 % of control (n = 7, $P < 0.05$). The action of AVP was fully reversed 5 min after washing out of the peptide (n = 7). For spontaneous EPSPs the average amplitude was 6.2 ± 0.7 mV, at a frequency of 42 ± 10 mHz (n = 6). Further, they showed a prolonged plateau phase (n = 6, rise time: 240 ± 50 ms, half duration > 300 ms) due to complex network activities described earlier by Carlson et al. (2000). In contrast to evoked EPSPs, 2 µM AVP did not cause consistent changes in spontaneous EPSP amplitude or frequency, but induced a reversible mean depolarization of the resting membrane potential by 2.3 ± 0.6 mV, which was also reversible (n = 6).
Thus, AVP reduces mitral cell excitability similar to previous findings in magnocellular vasopressinergic cells in the supraoptic nucleus of the hypothalamus (Kombian et al., 2000).

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Poster Topic

T20: Somatosensation: Touch, Temperature, Proprioception, Nociception

T20-1A A combined setup for neuronal recordings and behavioral measurements based on a thermal stimulation device for pain induction in mice
Achim Schilling, Konstantin Tziridis, Holger Schulze

T20-2A A giant descending interneuron in the stick insect conveying information about antennal movement and substrate vibration
Gaetan Lepreux, Jan Marek Ache, Stephan Shuichi Haupt, Volker Dürr

T20-3A A model for temperature-dependent locomotion in *Drosophila melanogaster*
Andrea Klara Adden, Martin C. Göpfert, Bart R. H. Geurten

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T20-5A Cold dog noses are sensitive to body heat radiation
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Christian Spalthoff, Alexandre Nesterov, Ramani Kandasamy, Radoslaw Katana, Vincent L. Salgado, Martin C. Göpfert

T20-1C Non-visual functions of visual opsins
Diego Giraldo , Damiano Zanini, Marta Andrés, Bart R. H. Geurten, Martin C. Göpfert

T20-2C Optophysiological investigation on the integral role of octopaminergic neurons in the motor neuron system of *Drosophila melanogaster*
Florian Bilz, Saskia Nagel, Hans-Joachim Pflüger, Marco Schubert

T20-3C Physiology of the femoral chordotonal organ of adult Drosophila melanogaster
Joscha Arne Alt, Reinhard Lakes-Harlan

T20-4C Representing infrared space using topographic microstimulation in the barrel cortex of the rat
Konstantin Hartmann, Eric E. Thomson, Miguel A. L. Nicolelis

T20-1D Setting the clock: Electrophysiological and optogenetic characterisation of light and temperature entrainment in *Drosophila*
Edgar Buhl, Maite Ogueta-Gutierrez, Chenghao Chen, Adam Bradlaugh, Ralf Stanewsky, James JL Hodge

T20-2D Stimulus preference profiles of whisker sensitive neurons in trigeminal nuclei
Shubo Chakrabarti, Andre Maia-Chagas, Cornelius Schwarz

T20-3D Thermosensation in the ant *Camponotus rufipes*
Manuel Nagel, Christoph J. Kleineidam

T20-4D Tight coupling of multiple desensitization mechanisms in mechanotransducer currents of DRG neurons
Janez Prešern, Aleš Škorjanc, Tomaž Rodic, Jan Benda

T20-5D VIP expressing interneurons in layer II/III of the barrel cortex
Alvar Prönneke, Bianca Scheuer, Mirko Witte, Martin Möck, Jochen F. Staiger

A combined setup for neuronal recordings and behavioral measurements based on a thermal stimulation device for pain induction in mice

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We here present a low-cost setup for the application of thermal stimuli to the hind limb of mice with simultaneous neuronal recordings and/or behavioral measurements. The setup is used to investigate pain perception, here, in an experiment inducing peripheral neuropathic pain by a sciatic nerve injury in mice. This approach is a common model in neuropathic pain studies, since hyperalgesia (the increased response to slightly noxious stimuli), and allodynia (the pain sensation induced by non-noxious stimuli) are two major indicators of neuropathic pain (Woolf & Mannion, 1999).
Our setup consists of a simple three-point control circuit controlling two peltier elements (RC 3-2.5 Marlow Industries) for the stimulation of both hind paws. The surface temperature is measured by two Pt100 thermo resistors (Heraeus M222) for each peltier element. The data is recorded by a microcontroller (Arduino Micro) and transformed into a low/high/zero signal. This signal is transferred to a motor driver integrated circuit (L298N STMicroelectronics) providing the current for cooling and heating of the peltier elements. The computer-microcontroller interaction is realized with a serial USB port allowing real-time data transfer from microcontroller to computer and controlling temperature stimulation protocol via a custom made computer program.
For neuronal recordings it is essential to provide a trigger signal allowing a proper synchronization of the recorded spikes and field potentials with the applied thermal stimulation. Therefore, the microcontroller sends a trigger pulse directly to the in-vivo neurophysiology acquisition system (MAP-System Plexon) allowing an offline synchronization of neuronal recordings and stimulation level. Additionally, the setup can also be used for behavioural measurements. The animal is kept in a mouse restrainer with its hind paws in direct contact with the peltier elements. A piezo force sensor (Honeywell FSG15N1A) simultaneously records the reaction of the mouse on the thermal stimuli. The behavioural and neurophysiological measurements can be combined to create a powerful tool for investigating pain perception in an animal model.

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A giant descending interneuron in the stick insect conveying information about antennal movement and substrate vibration

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Stick insects use their feelers (antennae) for active tactile exploration during walking. In Carausius morosus, the front legs and antennae are equally long, so that potentially anything the antenna touches is reachable by a front leg. Indeed, stick insects display reaching movements of the front legs targeted to an object previously touched by the antenna [1]. Thus, antennal information providing parameters such as touch position and joint position and movement is converted into aimed leg movements. This fast coordinate transfer is important for climbing. However, how the sensory inputs from the antennae lead to changes in leg motor control, such as retargeting an ongoing movement during the swing phase, is unclear. In crickets, descending interneurons (DINs) convey antennal mechanosensory information from the brain to thoracic motor networks [2]. In the stick insect, DINs that are sensitive to antennal contact, antennal joint position, joint movement, and joint velocity have been described, too [3]. A sub-group of three identified velocity-sensitive DINs has since been characterized in more detail, both anatomically and electrophysiologically. Together, they form a descending neural pathway that supplies the prothoracic ganglion with complementary and short-latency information about antennal movement. All of these DINs have their soma and large dendritic arborisations in the gnathal ganglia (GNG, formerly sub-oesophageal ganglion). Within the GNG, their dendrites ramify in close proximity to projections of antennal hair field afferent collaterals that encode joint position and movement. Therefore, it is likely that the DINs receive direct input from these afferents. From the GNG, the three DINs project to the thorax. One of them, cONv, also feeds this information back to the contralateral brain hemisphere via an ascending branch that arborizes in the tritocerebrum and the dorsal lobe. Within the dorsal lobe, cONv terminals are also found in the main projection area of antennal hair field afferents. Intriguingly, cONv also responds reliably to low-amplitude substrate vibration. Indeed, smooth taps on the substrate during recording elicit a response in a one to one correlation. Unlike antennal afferents that arborize in the brain and also descend to the GNG, afferent information from the vibration sensor does not reach the GNG via the brain. The GNG receive vibration sensor input directly. Nerve transection experiments imply that the vibration sensor is a bilaterally symmetrical system with the vibration-sensitive DINs on each side receiving bilateral input.

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A model for temperature-dependent locomotion in *Drosophila melanogaster*

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Temperature is one of the most important environmental factors for all animals, and it is crucial for an organism's survival to detect not only preferred temperatures, but most importantly to avoid noxious temperatures. This is especially true for small ectotherm animals such as the fruit fly *Drosophila melanogaster*, which therefore needs an efficient thermosensory system. Recently the field of insect thermosensation has been gaining momentum: Using genetically accessible organisms such as *Drosophila*, many new transduction molecules and sensory organs were identified in the last years. We investigated the temperature-dependent walking behaviour of *Drosophila* using neuroethological methods. In particular, we studied how the locomotion of fruit flies depends on the ambient temperature, and whether this is affected by different rearing temperatures.

A virtual tactile environment to study sensorimotor processing in mouse neocortex during locomotion

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Behavioral state can modulate neural responses to the same sensory stimuli resulting in different unified percepts. This modulation can be observed as early as in primary sensory cortices. Recent studies in mouse primary visual cortex (V1) reported that locomotion increases sensory response (Niell & Stryker, 2010), broadens size tuning (Ayaz et al, 2013), and is integrated with visual motion (Saleem et al., 2013). Further investigations of the underlying mechanism of locomotion effects in V1 reported increased depolarization of excitatory neurons (Bennett et al., 2013) as well as of PV- and SOM-positive inhibitory neurons (Pollack et al.,2013, but see Fu et al., 2014). Disinhibition of excitatory neurons via activation of VIP-positive interneurons, which are directly modulated by cholinergic inputs, has also been suggested to underly circuit effects of locomotion (Fu et al., 2014).

Mice are nocturnal animals, which navigate through dark corridors utilizing their whiskers. Thus navigational aspects such as locomotion might modulate activity in primary somatosensory cortex (S1) similar to the modulation observed in V1. To address this question, we established a naturalistic setup, in which head-restrained mice run on a ladder wheel while various textures are presented on rotating cylinders, in reach of whiskers. In the closed loop condition textures rotate with the same speed as the animal is running on the wheel, simulating the condition in which a mouse is running and whisking along a wall in darkness. We perform 2-photon calcium imaging of large numbers of neurons in S1 which were previously infected with the genetically encoded calcium indicator virus construct AAV1-EF1a-YC-Nano140. We also measure neuronal responses upon brief perturbations to the coupling between texture and animal speed as well as during the replay of texture speed from an earlier trial, in which texture speed and animal speed are decoupled. This behavioral setting allows us to investigate how various behavioral and sensory parameters (whisking, locomotion, texture contact and rotation) are integrated in barrel cortex.

Our preliminary results suggest that different neuronal subpopulations with distinct response profiles exist within the local circuitry of S1. A small subset of neurons showed increased activity during locomotion (and/or active whisking) but no or suppressed responses to texture contact. A larger subset of neurons was responsive to rotating texture stimuli. A small number of cells responded specifically to brief perturbations of texture/animal speed coupling. Subsequent immunostaining demonstrated that YC-Nano140 expression is almost exclusively restricted to excitatory neurons, indicating that our findings mainly report response properties of excitatory populations in superficial layers of barrel cortex. In future we aim to decipher how these differential response profiles arise in a local circuitry in S1, specifically by exploring effects on neuronal responses in deeper layers and in distinct inhibitory cell populations.

Cold dog noses are sensitive to body heat radiation

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It is common knowledge that dog noses are cold, but the function of the hairless nose-tip, called rhinarium, has long been elusive. Hypotheses such as a role in olfaction or a mainly mechanosensory function are incompatible with the innervation pattern and low tissue temperature, respectively. During the past two years, the Mammalian Rhinarium Group has gathered evidence supporting the hypothesis that the cold dog rhinarium is sensitive to radiating body heat.

The temperature dynamics of the dog rhinarium indicate that low skin temperature is critical for its sensory function. When a dog falls asleep, the rhinarium is warmed to almost body core temperature within minutes. When the dog wakes up again, the rhinarium is cooled down equally fast. The dog rhinarium can be sensitive to body heat radiation because it is colder than potential radiation sources in wide range of ambient temperatures. In wakeful dogs, rhinarium skin temperature is slightly below ambient temperature between 32 °C down to 15 °C and leveling off to values as low as 0.4 °C at freezing ambient temperatures. Such extremely low tissue temperatures are hard to reconcile with any sensory function other than heat sensing. In contrast, dogs maximizing olfactory sensitivity, e.g. while following a scent track, have warm rhinaria, indicating that the coldness of the rhinarium is unrelated to olfaction.

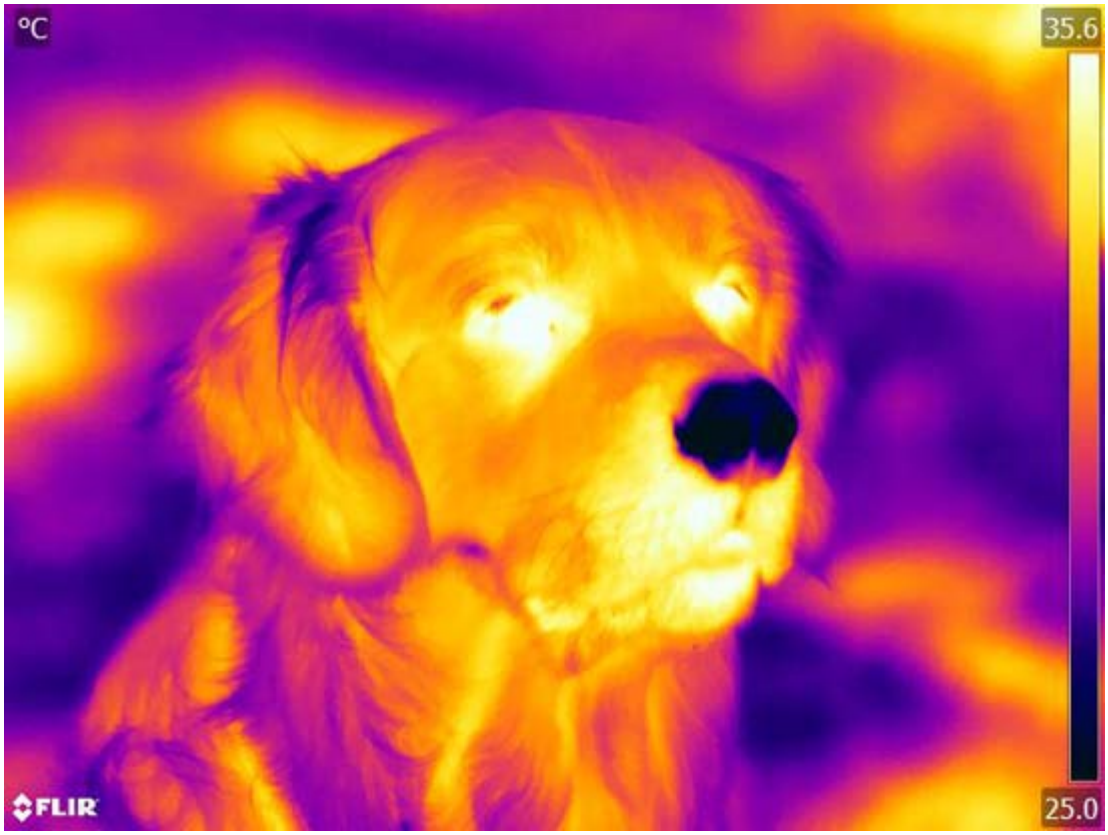
Dog rhinaria show tell-tale movements. Symmetric opening of the nasal wings is related to sniffing, i.e. olfaction. Lateral movements of both nostrils in parallel, on the other hand, are performed when wind conditions exclude the use of smell. This “nose-scanning” behavior of dogs is reminiscent of the scanning head movements performed by snakes while using their infrared-sensitive pit organs. The rhinarium is also in a premium location for a heat sensor, at the very front end of the body, pointing away from the own warm body.

Dogs are born with the ability to cool their rhinaria. Newborn pubs, still blind and deaf, cool their rhinaria on waking up and warm them again when going to rest. The teats of the mother dog are prominent heat signals and an infrared sense can provide more reliable directional information than olfaction, since odorant gradients are dependent on wind direction and may be distorted by turbulences.

One dog has been trained to discriminate between objects differing in temperature by 10 °C. The dog makes its choices from a distance of about 1 m. Another dog finds a person hidden behind one of two screens that are opaque for light, but transparent for heat. Fans generating an air flow of about 1 m/s exclude the use of air-borne smell and scent tracks on the ground are carefully avoided. This dog makes its choices from a distance of 10 m. Both dogs perform nose-scanning and do not sniff before making their choices. Recently, we have taken into use a training apparatus delivering carefully controlled heat signals and we have also switched to innate warm targets hidden behind the screens. We have furthermore increased the number of dogs being trained.

Comparative studies have shown that all studied terrestrial species in the order of Carnivora (N = 29, covering all extant families) are cold-nosed, while herbivorous Artio- and Perissodactyla are warm-nosed. The ability to sense weak heat signals may be widespread among mammalian predators.

The thermograph below shows the cold nose (black, 26 °C skin temperature) of a dog on a warm summer day (27 °C ambient temperature).


°C
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Electrophysiological investigation of anesthesia in lobsters

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To our knowledge there are no peer reviewed publications investigating the neuronal aspects of anesthesia in crustaceans. Therefore data about the information processing and transmission in the CNS during anesthesia are scarce. The animals are superficially anesthetized e.g. paralyzed but it is unknown if the central nervous system is still responsive. This paralysis may affect neuronal motor pathways but may not act on sensory pathways. Therefore we implanted hook electrodes in the CNS and measured the activity of the nervous system to stimuli prior and after different methods of anesthesia.

Young lobsters *Homarus gammarus* and adult lobsters *Homarus americanus* were exposed to following anesthetization methods: (1) cooling in ice water (2) CO_2 bubbled to the aquarium water (3) 10% $MgCl_2$ in the aquarium water (4) stunning with electrical current. The animals were stimulated either by mechanical brushing, electrical stimulation, or heat treatment. The recorded signals were analyzed and processed by Fast Fourier Transformation FFT to prepare power spectra.

Cooling of the animals is known to reduce the metabolic rate. We observed a reduced mobility of the animals visually (after up to 60 min at -1,8°C), but there was no complete inhibition of the sensory response in the CNS to external stimuli. CO_2 exposure shows a high variability of time of incubation (15-60 min) until a state of paralysis was reached which was accompanied by a significant reduction of the neuronal transmission into the CNS. Incubation of the animals in high Mg^{2+} concentrations showed no effects after 60 min. Anesthesia by electrical stunning renders the animals superficially anesthetized. But it can also cause an epileptiform activity in the nervous system similarly to reports from fish after electrical stunning.

Extraction of spatial information from infrared-input in the primary infrared sensitive nucleus (LTTD) of rattlesnakes

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Rattlesnakes, like all members of the familiy of pitvipers (Crotalinae), possess a specialized sensory system which enables them to sense infrared (IR) radiation. Their bilateral pit organs are highly sensitive towards IR stimulation. Rattlesnakes use their IR-sense for various tasks like thermoregulation, predator avoidance or prey capture. The IR-system seems to provide a high amount of spatial information given the fact that rattlesnakes are able to use its input very precisely (e.g. by striking towards warm-blooded prey even in complete darkness). Since the physics of the pit organ are those of a simple pinhole camera the question rises where and how spatial information is extracted from the sensory input.

The pit organ consists of a 10-15 µm thick membrane, which is innervated by two branches of the trigeminal nerve. Prior to entering the pit membrane each main branch furcates into several subbranches. These innervate certain areas of the membrane and give rise to a dense sheet of terminal nerve masses. The membrane-innervating nerves project to the exclusively infrared sensitive nucleus of the lateral descending trigeminal tract (LTTD) in the hindbrain. Neurons in the LTTD show background discharges and are highly active during infrared stimulation of the pit organ. Excitatory receptive fields of some LTTD-neurons are flanked by inhibitory receptive fields. Also a high amount of axo-axonic synapses (1/3 of the synaptic contacts) has been demonstrated in the LTTD. These and other characteristics of the IR-system led to the hypothesis that the pit organ and the LTTD play an important role in extracting spatial information from the IR-input.

An in-vitro whole brain preparation was used to investigate anatomical and physiological properties of the LTTD. We found that the LTTD shows a topographic innervation by the primary afferents that reflects the innervation pattern of the pit membrane. In addition we measured local field potentials (LFPs) in the LTTD while stimulating different membrane-innervating subbranches of the trigeminal nerve. Our recordings showed activity-maxima at various positions in the nucleus that reflect its topographic innervation. Pharmacological blocking of glutamate receptors by CNQX suppresses all postsynaptic LFP-activity. Strychnin and bicucullin as blockers of glycin- and GABA-receptors, respectively, also seem to affect the postsynaptic LFPs. However, their effect needs to be further clarified in ongoing pharmacological studies. We now started to record the global LTTD-activity during IR-stimulation using multiarray-electrodes. We will use these data together with our single-electrode LFP and intracellular recordings to further unveil the physiological mechanisms by which spatial information is extracted from sensory input in the LTTD.

Integration of visual and tactile inputs in a cricket antennal giant fibre

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Understanding how the nervous system integrates information of different sensory modalities is of fundamental importance. A giant fibre descending from the brain of the cricket (*Gryllus bimaculatus*) responds to both visual and tactile stimulation, allowing the integration of visual and tactile information to be studied at the level of a single neuron. By combining calcium imaging with intra- and extra-cellular recordings, I have analysed the extent and mechanism of the interaction between sight and touch. A spatially-restricted calcium increase in the dendrite is observed to the different modalities, apparently at the site of synaptic input. The calcium seems to impart differing degrees of neuronal adaptation to the stimuli, with the time-course of the calcium signal being of key importance. In essence, this system represents a simple model of decision-making within a single neuron which may shed light on how complex multi-modal stimuli are processed.

New agonists for insect stretch receptors

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[2]BASF Corporation, Research Triangle Park, NC 27709, USA

Insect mechanosensation both in hearing and proprioception often relies on chordotonal organs, which contain arrays of ciliated sensory neurons that act as stretch receptors. While several models for mechanotransduction and putative receptor proteins exist, the exact action and interaction of the necessary proteins and their mode of activation has not been fully resolved.
We found that in *Drosophila*, a class of substances specifically acts on these neurons by activation of a heteromultimeric protein whose two subunits co-occur exclusively within these cells. In our experiments, compound induced activation of ciliated neurons leads to tonic calcium influx *in vivo* and causes loss of hearing and gravitaxis in treated flies, an effect that is abolished in mutants deficit for either of the subunits.

Heterologous *in vitro* protein expression in chinese hamster ovary cells confirms that only expression of both subunits together induces sensitivity for this class of substances in vertebrate cells, and that these units assemble in form of a heteromeric complex.

Non-visual functions of visual opsins

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Evidence is accumulating that opsins can sense more than light. In *Drosophila*, opsins were recently implicated in larval temperature preference behaviours and in hearing in adult flies. Here, we report that *Drosophila* larvae also require visual opsins for locomotion, and show that the proprioceptors that control locomotion do express opsins. Opsin mutant larvae crawling in darkness displayed locomotion defects, including reduced peristaltic movements and reduced locomotion speeds. When we genetically rescued the function of the respective opsin gene, normal locomotion was restored. Opsin-dependent locomotion defects associated with altered temperature preference behaviours and closely resembled the locomotion deficits of mutants whose chordotonal neurons are impaired. Promoter-fusions revealed that opsins are expressed in the serially arranged, proprioceptive chordotonal neurons in the larval body wall. Opsin expression was confirmed with antibodies, and chordotonal neurons seemed to be the only neurons that express opsins outside the larval eye. This suggests that larval locomotion and temperature preferences might converge on chordotonal neurons. It also strongly supports the idea that light-independent opsin functions evolutionarily predated their use as photoreceptor proteins.

Optophysiological investigation on the integral role of octopaminergic neurons in the motor neuron system of *Drosophila melanogaster*

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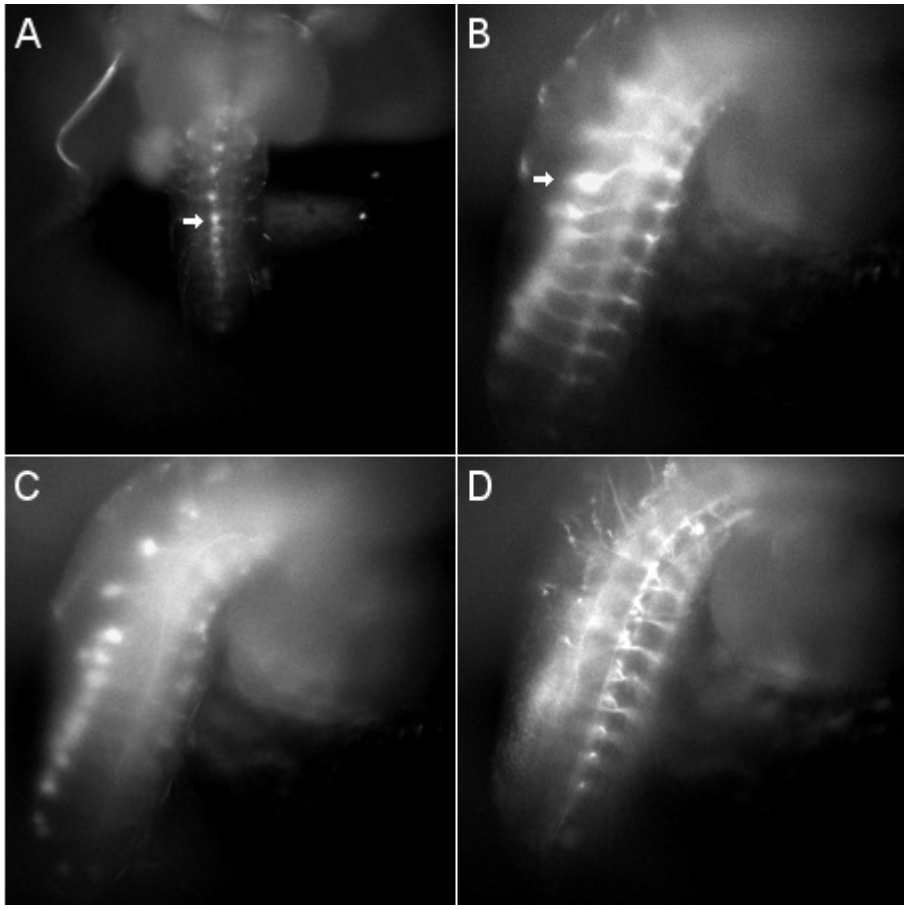
Insects execute exploratory behavior guided by environmental sensory information. Such multisensory information is processed and integrated by the brain to let the insects perform goal oriented behaviors. Locomotion and the underlying neuronal networks innervating responsible muscles are the basis for such coordinated sensory-driven orientation. In the fruit fly *Drosophila melanogaster* larvae the physiological interphase between motor neurons and the body muscles, the neuro muscular junctions (NMJ), stands under the modulatory influence of the biogenic amine octopamine. The monoamines octopamine and tyramine constitute the invertebrate counterpart of the vertebrate's adrenaline and noradrenaline system. In a small-sized network parallel to the motor neuron network octopaminergic neurons release (when activated) octopamine onto NMJs and modulate synaptic transmission and catabolism. Octopamine is released under stress conditions and, in general, preparing the whole organism for soon to come "energy demanding dynamic behavior". In our study we investigated if, when and how the octopaminergic system is integrated in the neuromuscular network during locomotory crawling behaviors. *Drosophila* 3rd instar larvae offer a simple behavioral repertoire including peristalsis (forward and backward crawling), bending and turning. When the larvae is crawling forward it does peristaltic body wall contractions traveling from posterior to anterior. Each of the 11 body segments incorporate 60 muscles (30 per hemi-segment) innervated by 80 motor neurons originating from the ventral nerve cord projecting their axons in a stereotypic pattern towards their muscles targets. The GAL4/UAS system allowed us to use the transgenic fly lines Tdc2-GAL4 and UAS-G-CaMP3 to establish animals expressing GCaMP in octopaminergic neurons in the brain, the suboesophagial ganglion and the ventral nerve cord (VNC). Using conventional calcium imaging techniques we investigated simultaneously the activity pattern of the whole ensemble of octopaminergic cell body clusters in the brain lobes and the ventral unilateral median (VUM) neurons found in the suboesophageal ganglion and the VNC. Thus, we measured neuronal *in vivo* activity in octopaminergic neurons while the animals performed peristaltic muscle contractions typical for crawling behavior. By a vibrating needle we induced different kind of stimulations to individual body wall segments of the larvae (varying in their number, frequency and intensity, ranging from 'gentle' to 'very strong' touches) and measured the activity in octopaminergic neurons and motor neurons. Body wall contractions were monitored by video camera and electrophysiological techniques measuring motor neurons in individual peripheral neurons connecting the VNC with the body wall muscles. The simultaneously measured imaging data allowed the analysis of neuromodulatory effects on the motor system and gave insights into the underlying control and coordination processes.

Fig. 1: VNC preparation of *Drosophila melanogaster* larvae expressing GCaMP3.0 in tyraminergic and octopaminergic neurons (TDC2-Gal4 – UAS-GCaMP3.0) and their corresponding nerves. **A** dorsal view on the VNC on a micro-platform, 100x magnification. **B-D** lateral view on the VNC at different focal planes, 200x magnification. White arrows indicate TDC2-positive VUM-cell bodies.


A
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Physiology of the femoral chordotonal organ of adult Drosophila melanogaster

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Chordotonal organs are mechanoreceptors composed of scolopidial units. They are located in and between different joints of insect body to detect position and movement. In the legs of adult Drosophila melanogaster the femoral chordotonal organ is a large sensory organ consisting of three groups or scolopidia. One large group terminates at the cuticular surface of the distally femur whereas the other two groups are distally associated with the femoral muscle 1 . A passively flexion of the tibio-femoral joint activates the femoral chordotonal organ and this in turn causes a resistance reflex which can be displayed via electrophysiological recordings 2 .

In our study the origin and composition of this resistant reflex is investigated. Therefor the forelegs of adult Drosophila melanogaster were stimulated and extracelluar recordings were made from the leg nerve and muscles. As proprioceptive stimuli 5 Hz sine waves were used, for vibratory stimuli pulses with 500 Hz carrier frequency were used. The responses of different neuronal units were extracellularly recorded by use of sharpened tungsten electrodes (Fig.1). In order to verify the origin of the responses pharmacological experiments were performed. Cholinergic synapses of the reflex pathway were blocked with scolopolamine and mecamylamin. The extracellular recordings were analysed for threshold, intensity-response curve and latency. The results confirm that the responses origin in primary sensory units.

This enables a new possibility to investigate the physiology of the Drosophila melanogaster femoral chordotonal organ. The physiology will be analysed further, especially in respect to the function of the different groups in the femoral chordotonal organ and their function in behaviour.

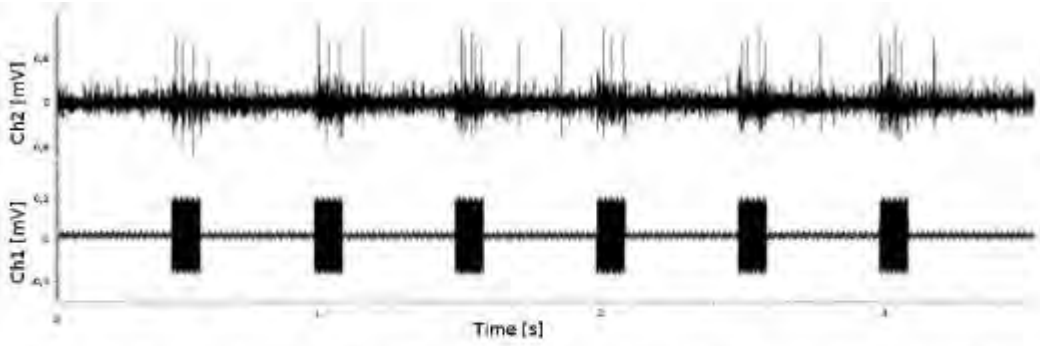

Fig.1 Electrophysical recording of the leg nerve from an adult Drosophila melanogaster. Channel 1 (Ch1) shows the applied vibrational pulses with 500 Hz carrier frequency and a duration of 100 ms. There is a 400 ms interval between individual pulses. Channel 2 (Ch2) shows the electrophysiological response of the leg nerve due to the stimulation.

Representing infrared space using topographic microstimulation in the barrel cortex of the rat

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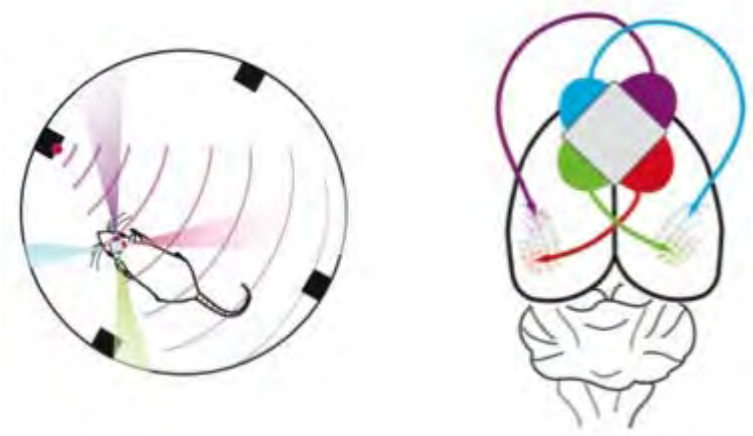
Sensory neuroprostheses show the potential to liberate subjects from major sensory impairments. Recently, we trained rats to discriminate among infrared (IR) sources using IR-sensor coupled cortical microstimulation. This demonstrated that sensory prosthetic systems could augment the subject's native range of perceptual abilities, even in adults. In this study we expanded the IR-sense of rats by training them to use four IR sensors that were coupled to four topographically organized microstimulators in somatosensory cortex (S1). The sensors were mounted orthogonally to each other, providing a 360° spatial map of the animal's IR environment. The rats not only learned to use this artificial topographic map of IR space in a four-choice discrimination task, but also performed better than rats with fewer IR sensors. In one set of experiments, we "ablated" certain sensors, observing a clear drop in IR-discrimination performance. In a second set of experiments, we disrupted the topographic representation of IR space by remapping the connections between sensors and stimulators. The rats can adapt to the remapped condition within a short time at a high performance. We also recorded the change of the local response to the micro stimulation while the rats learned to use the prosthetic system. Preliminary data suggests that repeated stimulation leads to significant changes in the brain's response to stimulation during the process of learning.



Setting the clock: Electrophysiological and optogenetic characterisation of light and temperature entrainment in *Drosophila*

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Circadian clocks regulate changes in behaviour, physiology and metabolism to ensure they occur at certain times during each day allowing adaption to the organism's environment. It is important that internal time is synchronised with external time via daily natural cycles of light intensity and quality as well as temperature. In *Drosophila* the clock is comprised of ~150 neurons grouped into identifiable clusters that sub-serve different circadian functions. The clock neurons express clock genes such as *period* (*per*) and *timeless* (*tim*) whose gene products negatively feedback to switch off their own expression forming the molecular clock. In the absence of environmental cycles, the clock free-runs with a period of approximately 24h and remarkably, this period length is maintained at different ambient temperatures, i.e. it is temperature compensated. Nevertheless, the same clock is extremely sensitive to daily temperature changes, because temperature cycles (TC) with an amplitude of only 2-3°C can entrain it. Synchronisation of the clock to environmental light:dark cycles is achieved by visual and non-visual pathways including *cryptochrome* (*cry*) and *quasimodo* (*qsm*). These are known to promote the degradation of TIM within clock neurons. However, the mechanism of *qsm* function and how rhythmic temperature changes reach and synchronise the molecular clock is currently unknown.

By using behavioural essays, whole-cell recordings and genetically encoded calcium, chloride and membrane potential reporter imaging from clock neurons in whole adult brains we have characterised circadian changes in physiological properties as well as the effect of light and temperature. Our results show that while wild type flies are arrhythmic in constant light (LL), flies lacking QSM have rhythmic patterns of behaviour as if they were in constant darkness (DD). Additionally, certain potassium channel and ion transporter mutants are also rhythmic in LL suggesting a role in light entrainment. As electrical activity has been shown to be important for the co-ordination of the clock network and neurons are known to be in a depolarised state during the day, we are focusing on channels and transporters that help controlling resting membrane potential in the clock. Initial results show that in control flies the large ventral lateral neurons (l-LNv) respond differentially to blue light depending on the time of day. In early night, exposure to intense blue light leads to a depolarisation and an increase in firing whereas during morning these cells become more hyperpolarised and less excitable. In order to determine the mechanism by which light causes these switches in excitability, we are characterising molecules known to interact with QSM. We find that reduction in the sodium potassium chloride cotransporter (NKCC) in the clock neurons makes them more hyperpolarised and unable to respond to blue light while reducing QSM or the potassium channel Shaw seems to activate them.

Finally, internal stretch receptors have been implicated in temperature synchronisation and we show that the Ionotropic Receptor IR25a is expressed in these sensory organs and is required for synchronisation to low-amplitude TC. Ectopic IR25a expression in clock neurons results in temperature-dependent firing of action potentials. In summary we show that IR25a is a temperature sensor in central and peripheral clocks, and that it is responsible for the high sensitivity of the circadian clock to TC.

Stimulus preference profiles of whisker sensitive neurons in trigeminal nuclei

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The rat brainstem trigeminal nuclei principalis (Pr5) and interpolaris (Sp5i) contain whisker sensitive neurons that represent the first synaptic station on the ascending somatosensory pathway originating from the whisker primary afferents and terminating in the primary somatosensory cortex. From a previous study, we know that primary afferents (TG) convey enormous amounts of information about broad-spectrum dynamic whisker deflections (Chagas et al., 2013). In the present study we were interested to quantify, how much of this information is preserved in Pr5 and Sp5i. We recorded well isolated single units from the Pr5 and Sp5i of anesthetized rats during single whisker stimulation using a white noise stimulus. We computed instantaneous information transfer from spike triggered ensembles of the kinematic parameters position, velocity and acceleration). The time interval between stimulus time series and response at which instantaneous information rate reached a maximum indicated the neuron's response latency. Further using a novel neuronal encoding model (Theis et al., 2013) we calculated the mutual information between the spike train and the different stimulus kinematic parameters to determine which parameter or parameter combination yielded the highest information transfer. Our results obtained so far showed Pr5 and Sp5i neurons convey information about the three tested kinematic parameters and show an optimal latency above 2 ms expected from the synaptic delay. Importantly information rate about the stimulus drops considerably from TG to both Pr5 and Sp5i neurons, possibly reflecting considerable neuronal computation in the brainstem network and/or integration of non-sensory signals.

Thermosensation in the ant *Camponotus rufipes*

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As ants are small, ectothermal animals with their body temperature close to environmental condition, precise detection of thermal fluctuations and steady state temperature is crucial for their own survival. Additionally, as social insects they take care of their immobile brood, translocating it inside their nests to meet favorable climatic conditions. The successful brood-care increases colony growth and finally leads to an increase of their inclusive fitness.
In behavioral tests, Camponotus ants can discriminate temperatures by differences of only 0.2°C (Roces & Núñez, 1995). Moreover, the temperature preference of a *Camponotus rufipes* worker during brood-care is influenced by its own circadian rhythm, preferring temperatures around 28°C in the morning and 32°C in the evening (Weidenmüller et al., 2009).
It is unknown, how the sensory system receives thermal information that allows such fine-tuned behavior.

In this study, we identified two thermo-sensitive receptor-neurons associated with the sensillum coelocapitulum in the carpenter ant *Camponotus rufipes*. Our results, based on extracellular recordings of the receptor-neurons, are dose-response-curves for each neuron during temperature stimulation.
One of the antennal cold-sensitive neurons is only sensitive to temperature in a limited temperature range (27-33°C) that matches the preferred brood temperature. We speculate that the neuron acts like a temperature switch (TS-neuron) and triggers the behavioral response during brood-care. The second cold-sensitive neuron is a flux-detector, transiently coding for temperature changes (TT-neuron) and provides information for orientation in a thermal environment.
We currently investigate the morphology of the sensillum coelocapitulum in *Camponotus rufipes*, and we describe the ultrastructure of the sensillum in order to unravel possible transduction mechanisms. In addition, we conduct single sensillum stainings to indentify the brain neuropile where processing of the received temperature information takes place.

Tight coupling of multiple desensitization mechanisms in mechanotransducer currents of DRG neurons

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Three (four) types of mechanically activated ion currents in somatosensory neurons of dorsal root ganglia (DRG) have been characetrized based on their kinetics of desensitization. In rapidly-adapting currents, which are considered important for mechanoreception *per se*, the inactivation time-constant is in the range of a few milliseconds (Rugiero2010). Hao et al. (2010) observed that by preadapting mechanical stimuli activation curves are shifted along the stimulus axis in addition to a multiplicative inactivation process. They conclude that two mechanisms are needed to explain the data: (i) an adaptation process relaxing the force acting on the channel, thus shifting the activation curve, and (ii) inactivation of the channel explaining the multiplicative scaling of the activation functions. The similar time scales of both processes depend almost identically on membrane voltage and calcium did not effect any process.

Because of the high similarity of the dynamics of the two phenomena we hypothesize that adaptation and inactivation are not independent but tightly coupled. We tested this hypothesis by means of a Hodgkin-Huxley type model of the mechanotransducer current that we fit to the published data. In the model, both the adaptation and the inactivation are driven by the very same process. In fact, we modeled the adaptive shift simply proportional to the inactivation variable. Responses of the model to ramp-and-hold stimuli matched the traces obtained in vitro experiments well. The model correctly predicted a rapid rising phase and a quick decay of the current while the stimulus amplitude was still in the plateau phase. Also, recovery from mechanotransducer desensitization is reproduced by the model. We conclude that a single process that drives both adaptation and inactivation is sufficient to explain all the experimental data. This raises the interesting question how this is mechanistically implemented in the machinery of the mechanotransducer ion channel.

Hao, J., Delmas, P., Oct 2010. Multiple desensitization mechanisms of mechanotransducer channels shape firing of mechanosensory neurons. J Neurosci 30(40), 13384-13395.

Rugiero, F., Drew, L. J., Wood, J. N., Jan 2010. Kinetic properties of mechanically activated currents in spinal sensory neurons. J Physiol 588 (Pt 2),301-314.

VIP expressing interneurons in layer II/III of the barrel cortex

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GABAergic interneurons in primary somatosensory cortex of mice are highly diverse but can be grouped by molecular markers. The least well characterized group expresses vasoactive intestinal polypeptide (VIP). Therefore, we characterized VIP expressing interneurons (VIP-IN) morphologically and electrophysiologically in brain slices of young adult transgenic VIPcre tdTomato labeled mice. We performed whole cell patch clamp recordings, treatment with serotonin (5HT) or acetylcholine (ACh), biocytin staining, and subsequent quantitative reconstructions.

Somatodendritic morphologies were diverse, including bipolar, modified bipolar (i.e. tripolar) and tufted configurations. Axonal arborizations formed by layer II/III VIP-IN were usually found in layer II/III and descended further towards layer VI. Membrane properties and firing patterns of VIP-IN varied greatly. The most abundant firing pattern observed was regular spiking with different adaption ratios in response to stronger depolarizing current pulses. Other firing patterns included irregular spiking, high threshold bursting, and bursting. The enormous variability of the aforementioned parameters does not suggest a classification scheme as of yet.

The neuromodulators ACh (via nicotinic ACh receptors) and 5HT depolarized all VIP-IN in layer II/III, where a subgroup elicited a switch from bursting to tonic firing mode contingent on membrane potential. The neuromodulator-induced depolarization was sufficient to trigger this switch. This suggests a brain state dependency of firing patterns of a subset of VIP-IN that, by virtue of their extensive translaminar axonal arbor, will have a profound effect on columnar sensory processing.

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T21-8D Walking and running in desert ants - Gait parameters at different walking speeds in the ant, *Cataglyphis fortis*
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3D reach cage for movement planning in extrapersonal space

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Sensorimotor neuroscience is typically conducted in highly controlled and constraint experimental environments. Depending on the brain area, researchers investigate spatial parameters which influence neuronal sensorimotor processing such as head position, gaze direction, body posture, and more. Especially in neuroscience with non-human primates, these requirements lead to highly specialized and controlled experimental setups. Typically, monkeys are seated in a primate chair and respond to visual, auditory or tactile cues by operating for instance a handle or a touchscreen.

Recent advances in wireless neural recording technology, in combination with behavioral motion tracking devices, open the field for working with freely moving primates and investigating less restrained behavior[1,2]. Such approach provides new opportunities for sensorimotor neuroscience, since it allows investigating movement parameters which influence sensorimotor integration or motor planning more comprehensively. Here, we introduce a neuroscientific experimental setting which will allow comparing motor planning to targets in peripersonal and extrapersonal space.

We developed a cage-based experimental setup ("reach cage") for conducting controlled sensorimotor tasks in physically unrestrained rhesus monkeys. The cage is equipped with computer controlled capacitive touch sensors and LEDs serving as visually cued reach targets. Like in a conventional chair-based setup, animals can be engaged in a controlled behavioral task. Yet, the reach cage enlarges the potential workspace significantly and allows adding new modes of movement to the tasks, like walking and whole-body posture. As an intermediate step and for better comparison, we trained a rhesus macaque in a conventional reach task on a touchscreen but mounted this inside the reach cage rather than the conventional chair-based setting[3]. In contrast to a chair-based setup, the monkey could move away from the workspace and was exposed to more sources of distraction.

As a result, it was possible to train the monkey on a memory-guided center-out reach task with a performance similar to monkeys trained in conventional setups. The comparable training progress suggests that training monkeys on advanced sensorimotor tasks is possible in extended and less constraint settings, like the reach cage, and will allow conducting controlled sensorimotor neuroscience experiments in more complex environments.

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A comprehensive approach for the automated measurement of kinematic parameters during walking in *Drosophila*

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Optical motion capture consists of a set of well-established techniques that allow for the contact-free measurement of body motions in behaving animals. The most precise approaches thereby require the placement of markers on the animal's body. Marker positions, and thus the orientation of body parts, can then be extracted optically from camera pictures, making a reconstruction of movements possible. In larger animals markers can be attached relatively easily, but typically have a minimum size of a few mm. Compared to other organisms used in the study of behavior Drosophila is very small; its total length is approx. 2 mm. In that light, marker-based motion capture in Drosophila, especially of its legs, is difficult to realize. A marker-based system for Drosophila has been developed recently; this approach, however, uses special markers and depends on illumination with a HeNe laser, thus increasing complexity and cost.

Marker-less approaches can overcome this limitation, but have to rely on more sophisticated image processing algorithms for the extraction of movement data. Here, we describe an integrated setup for marker-less and automated extraction of behavioral data from Drosophila during walking, with a focus on walking trajectories, leg kinematics, and inter-leg coordination. We combine several previously established techniques and extend those with multi-view, high-speed video recordings and image processing algorithms for the continuous extraction of tarsus positions.

The system allows for the automated measurement of several important parameters of leg kinematics in a walking fly. More specifically, it can automatically infer lift-off and touch-down events in all six legs simultaneously. We achieve this by detecting the tarsi of all six legs continuously, using relatively simple and computationally efficient image processing algorithms. Individual lift-off and touch-down events (i.e. the start of swing and stance phases, respectively) are then determined by comparing tarsus movement vectors with local movement of the ball surface. In order to demonstrate the capabilities of the system we characterized the walking behavior of fruit flies in two paradigms: open-loop, stimulus-induced curve walking behavior and closed-loop control of walking speed. The system is particularly well suited for the examination of curve walking. During this behavior the kinematics of all legs change in a curvature dependent fashion; a comprehensive description of this behavior is only possible if walking speed and direction, as well as the movement of all six legs can be monitored simultaneously. In a second set of experiments we recorded leg kinematics during closed-loop operation while we modulated expanding and contracting stimuli thus inducing changes in walking speed.

In conclusion, the setup described here provides a detailed description of many relevant kinematic parameters of walking in Drosophila. The preparatory complexity with regard to the individual fly is low; the animals only have to be tethered, application of markers is not necessary. In principle, the length of a recorded behavioral sequence is only limited by the fly's willingness to walk and we are able to record walking bouts with up to one hundred consecutive steps. The automated tracking of legs and the extraction of walking parameters from these sequences takes several to a few tens of minutes; previously, analysis of such long sequences required many hours of manual annotation.

A descending interneuron that underlies turning behavior in flying *Drosophila*

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To navigate through the world safely, a flying animal must be able to execute precisely controlled steering maneuvers including turns around different axes. One of the most widely studied behaviors of flies is turning around their vertical body axis (yaw). The reasons for this are that intended yaw turns can be measured in tethered flies as torque or as changes in the difference between the left and the right wing stroke amplitude and that this behavior can reliably be elicited by visual motion stimuli. In addition, large-field visual interneurons in the lobula plate of the fly that are relatively easily accessible to electrophysiological recordings have been shown to respond to these visual motion stimuli and are thought to underlie steering maneuvers on the sensory side. However, very little is known about how this information is relayed to the motor system in the thoracic ganglion. The identities and signaling properties of descending interneurons that control either stimulus-triggered or spontaneous turns remain largely unknown. A major reason for this lack of knowledge is that finding neurons that are involved in the execution of a behavior, requires recording their responses in behaving animals, a technical feet that in flies has only been achieved very recently.

We set out to find descending interneurons involved in steering responses in *Drosophila melanogaster* by performing 2-photon calcium imaging and patch-clamp recordings from candidate neurons in tethered flying flies. At the same time we measured yaw turning responses by monitoring the left and right wing stroke amplitude. We discovered a descending interneuron whose responses are strongly correlated with changes in the difference in stroke amplitude between the two wings independent of visual stimulation. To analyze under which conditions this correlation persists, we presented different visual stimuli that should elicit turning responses, as well as compared responses during open and closed loop conditions. In the latter case the flies' behavior controls the motion of the visual stimulus with a certain gain factor that can be varied. We also try to analyze whether activation of this neuron using genetic tools is sufficient to trigger turning behavior of the fly, to find out whether these response are indeed causal of the behavior.

Our results provide insights into how steering responses in flying flies are controlled on a neuronal level. The signals of descending interneurons conveyed to the motor system should be the result of the integration of information from different sensory systems and the internal state of the fly. We thus hope that this work represents a first step towards understanding the computations in the central brain that result in a specific behavioral reaction.

A model of inter-segmental coordination producing tripod and tetrapod coordination patterns of insects

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Inter-segmental coordination is crucial for normal locomotion, in particular, for walking of insects. During the years, a large amount of studies has been devoted to this topic. Behavioural (e.g. Graham, 1972, Cruse, 1990) and electrophysiological investigations (e.g. Borgmann, 2007,2009) have contributed a lot to a better understanding of the details of (normal) locomotion and the role inter-segmental coordination plays in it. There also exists a number of modelling studies in this field (e.g. Cruse et al., 1998). Despite the progress
the aforementioned diverse studies have achieved, we are in want of a deeper understanding of the details of the neuro-muscular processes, especially inter-leg coordination, during locomotion.
In this paper, we should like to contribute to this by using a modelling approach. Our model consists of the neuro-muscular systems of three ipsilateral legs. Each leg model comprises the three main antagonistic muscle pairs (m. pro- and retractor coxae, m. levator, depressor trochanteris, and m. flexor, extensor tibiae) and their controlling local neuronal networks. Each of them has a central pattern generator (CPG) as its central functional unit. Due to intra-segmental coupling between the local networks, regular stepping movement can emerge in each of the legs. To produce coordinated movement between the ipsilateral legs, we found, by means of simulations with the model, a number of conditions, which, when fulfilled, guarantee the existence of the tripod or tetrapod coordination patterns. Moreover, additional conditions built in the model enable it to mimic transitions between these coordination patterns. The main finding of the model is that position (angular) information suffices to produce and maintain the tripod coordination pattern, while the tetrapod one requires additional sensory signals that encode (angular) velocity of the leg. In physiological terms, the tetrapod coordination pattern depends more on peripheral sensory input than the tripod one. This is in agreement with many experimental findings (e.g. Zill, 2009, and Zill & Keller, 2010). The model also suggests that, in some sense, it is easier to switch to tetrapod from tripod than the other way around.

Audio-vocal interaction in the monkey prefrontal cortex

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The ability to recognize vocalizations of others and to produce vocal utterances in return has enabled primates to develop sophisticated audio-vocal communication systems. To that aim, auditory feedback is required to adjust and guide vocal motor output. At the neuronal level, interactions between the auditory system and the vocal motor system are therefore needed and exist on several brain levels such as the lower brainstem, midbrain and the auditory cortex. The ventrolateral prefrontal cortex (vlPFC), consisting of Brodmann areas (BA) 44 and 45, is a key sensory-motor area operating at the apex of the cortical hierarchy. Recently, we have demonstrated that rhesus monkeys are able to cognitively control their innate vocalizations indicating that they possess the minimum requirement for goal-directed instrumentalization of their vocal output. Moreover, single-neuron activity in BA 44 and 45 specifically predicted the preparation of volitionally initiated vocal output. Auditory activations in the vlPFC have also been reported, but it is virtually unknown, whether single PFC neurons reflect audio-vocal interactions, i.e. response modulations to both external calls and self-produced vocalizations.

Here, we performed single-unit recordings from about 500 neurons in BA 44 and 45 of the vlPFC of two rhesus monkeys (Macaca mulatta) during external acoustical stimulation and self-initiated vocalizations. The monkeys were trained to vocalize in response to the presentation of arbitrary stimuli in a computer-controlled Go/NoGo detection task to receive a reward. In addition, monkeys were acoustically stimulated with species-specific vocalizations.

In agreement with earlier reports, we find a small fraction (12%) of auditory neurons in BA 45, but also in BA 44, that show changes in firing rates in response to auditory stimulation with species-specific calls. The discharge rates of most auditory neurons (59%) was increased during auditory stimulation, while 41% of auditory neurons were suppressed. Almost three fourth of the auditory neurons (72%) showed a significant modulation of their discharge rates either prior to vocal onset (15%), during vocal output (22%) or in both cases (35%). In these auditory neurons, discharge rates were, on average, stronger modulated before and during vocal output than they were during auditory stimulation. Our results show that audio-vocal integration processes are taking already place on cognitive level in monkey vlPFC, an obligate precursor for the evolution of complex audio-vocal communication systems in the primate lineage such as human speech.

Body Side-specific Modification of Load Processing during Turning in the Stick Insect

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The neural control of straight forward walking in invertebrates and vertebrates alike is well studied. However, the mechanisms underlying the flexibility of motor programs between the segmental neural networks that control the individual leg during behaviors such as curved or backward walking remains largely unknown. Here, we investigated the origin of differences between the leg movements of the two body sides observed during optomotor-induced turning . When tethered, intact insects walked on a slippery surface, steps of the outside leg (oL) of the intact animal showed a significantly greater period than those of inside legs (iL). The activity and timing of all muscles in the middle leg, was virtually the same between oL and iL, however, the phasing of the protractor and retractor muscles as stance phase muscles was reversed regularly in iL, causing iL to reverse stepping direction. Lesioning and deafferentation of the front leg ipsilateral to the mesothoracic recording site demonstrated that the previously found bias in mesothoracic motor activity during optomotor-induced turning of the front legs is only partly dependent on the stepping in the front leg. We show that an additional mechanism by which the nervous system generates inside and outside turning is the differential processing of local load feedback. Whereas load stimuli cause a reinforcement of retractor activity during outside stepping of the front leg, three types of influences are observed during inside steps: increase in retractor activity, reduction of retractor activity, even to no response, and, finally, reversal of influence, i.e. activation of protractor MN. These responses are reminiscent of the three types of stepping pattern observed in inside stepping, namely, forward, sideward and backward steps (Gruhn et al. 2009). Together with previous findings on the task-dependent processing of position signals during curve walking (Hellekes et al. 2012), our findings suggest that task-dependent, descending, and unilateral influences on the processing of mesothoracic load signals during oL and iL walking play a pivotal role in generating turning kinematics in the stick insect. Supported by DFG grant Bu857/14.

Cellular mechanisms underlying behavioural state-dependent modulation of motor cortex output *in vivo*

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Cortical signal processing critically depends on behavioural states. However, the cellular mechanisms underpinning behavioural state-dependent changes in motor cortex output remain largely unknown. Here we combined *in vivo* intracellular recordings, selective pharmacology, projection target mapping and computational modelling to investigate the effect of behavioural state on membrane potential (V_m) dynamics of layer 5B (L5B) pyramidal neurons in the primary motor cortex (M1) of awake, head-restrained mice.

Our intracellular recordings revealed two functionally distinct subpopulations of L5B pyramidal neurons during self-paced, voluntary forelimb movement. $L5B_{suppressed}$ neurons experienced a decrease in slow membrane potential fluctuations that reduced V_m variability, input sensitivity and average firing rates (quiet wakefulness: 6.3±3.9 Hz, movement: 2.8±2.5 Hz, $p<0.001$, n=17). In contrast, a subpopulation of L5B pyramidal neurons ($L5B_{enhanced}$, 53 %) received elevated excitatory input during movement that increased mean V_m, input sensitivity and firing rates (quiet: 5.7±3.8 Hz, movement: 12.9±7.4 Hz, $p<0.001$, n=24). By employing post hoc immunohistochemical analysis of the transcription factors Ctip2 and Satb2 – selectively expressed in intratelencephalic (IT) and pyramidal-tract (PT) M1 projection neurons, respectively – we show that the functional classification of individually recorded $L5B_{supp}$ and $L5B_{enh}$ neurons is not dependent on projection target.

Given that bidirectional modulation of M1 output occurs via two opposing mechanisms: 1) a global reduction in network-driven V_m variability and 2) a coincident, targeted increase in excitatory input to $L5B_{enh}$ neurons, we sought to identify the source of this increased excitatory drive during movement. L5B neurons receive strong top-down input from L2/3 and ascending input from the ventrolateral 'motor' thalamus (MTh). However, we found that L2/3 firing rates were not affected during motor activity (quiet: 0.6±0.7 Hz, movement: 0.6±1.1 Hz, $p=1.0$, n=8), and that pharmacological inactivation of MTh still resulted in significant V_m depolarisation in L5B pyramidal neurons during movement.

Changes in behavioural state have been associated with altered levels of arousal and release of neuromodulators such as noradrenaline (NA). We next tested whether noradrenergic neuromodulation could be the source of movement-related V_m depolarisation in $L5B_{enh}$ neurons. Selective blockade of a and ß NA receptors resulted in a significantly higher proportion of neurons being suppressed during movement ($L5B_{supp}$ control n=24/45 or 53 %; $L5B_{supp}$ NA blockade: n=14/16 or 88 %, $p=0.0049$). This suppression in movement-related spiking resulted from a subtle hyperpolarisation of mean V_m and a significant reduction in the distribution of ΔV_m values across the population of L5B pyramidal neurons.

Thus, we have demonstrated that changes in behavioural state – from quiet wakefulness to movement – bidirectionally modulate output from M1 via two opposing mechanisms: 1) a global reduction in network-

driven V_m variability and 2) a coincident, noradrenaline-mediated increase in excitatory input to a subpopulation of L5B neurons. Together, our data provide the first detailed description of the cellular mechanisms underpinning behavioural state-dependent changes in motor cortex output during self-paced, voluntary movement.

Changing Codes - Modulation of axonal spiking in a sensory neuron by descending projection neurons

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Neuromodulators like amines and peptides are ubiquitously released in the nervous system. Their short- and long-term effects on synapses and ion channel properties are major sources of plasticity. Malfunctions of the neuromodulatory system can cause severe pathological conditions. Typically, neuromodulators are thought to affect dendritic and synaptic regions of neurons. In contrast, axons are assumed to faithfully conduct action potentials from their site of initiation towards the output synapse. Recent studies, however, have shown that axons are not mere cables and that action potential initiation and propagation is subject to neuromodulation. As a consequence of such modulation, information encoding on the axon might change as action potentials are transferred towards the postsynaptic circuits. Very little is known about the origin of axonal modulators and about the consequences of axonal modulation on the postsynaptic circuits.

We use the anterior gastric receptor neuron (AGR) to study axonal modulation of sensory pathways. AGR is a single-cell muscle tendon organ in the well-characterized stomatogastric nervous system of the crab. Its soma is located in the stomatogastric ganglion (STG). Our previous data have shown that AGR protrudes two long axons: a peripheral one that innervates muscles and a central one that innervates a set of identified modulatory projection neurons. At rest, AGR produces tonic ectopic spike activity of 2-5 Hz initiated at a spike-initiation zone in the central axon. Even small changes in ectopic activity cause prominent changes in the motor pattern generated by the postsynaptic STG circuits, indicating that AGR determines the state of the motor system and its response to sensory stimuli. Optical imaging and fluorescent dye injections revealed that AGR's central spike initiation zone is located close to the anterior neuropil of the STG and that AGR possesses 1 to 3 neurites in this region (average 1.85± 0.89, N=13).

Here, we show for the first time that axonal spike initiation of AGR is directly modulated by neuromodulators released from modulatory projection neurons. We recorded ectopic spike activity in the AGR axon during activation of the inferior ventricular (IV) neurons - olfactory projections neurons which descend from the brain and exert their actions via the release of Histamine and a FLRFamide-like peptide. AGR's ectopic firing frequency diminished by 26.72 ± 7.87% (N=8) during IV neuron stimulation. This diminished activity level was retained for several minutes beyond the stimulation after which AGR firing frequency returned to its pre-stimulation value. The diminishment of AGR firing frequency was absent when chemical neurotransmitter release near the central spike-initiation zone was blocked. Blocking had no direct effect on the STG motor neurons, indicating a direct modulation of the AGR axon via chemical release of neuromodulators from the IV neurons. The drop in AGR firing frequency was mimicked by focal application of Histamine to the central AGR axon (29.53± 8.89% decrease, N=10) and was diminished when Histamine receptors were blocked (N=5). Axonal modulation had no obvious effect on action potential conduction dynamics, however. We are currently testing whether axonal modulation affects the reliability of action potential propagation and which functional consequences result a direct modulation of a sensory axon by a sensory pathway (the IV neurons) of a different modality.

Command and control – nonspiking interneurons modify specific parameters of searching movements

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Animals that move through the environment constantly need to adapt their behavior to the surrounding conditions. For this purpose, many animals use their tactile senses to gain information about their surroundings, e.g. by palpating objects with their hands (primates [1]), whiskers (rodents [2]), or antennae (insects [3]). Similar, stick insects, when losing ground contact perform rhythmic searching movements with the respective leg in order to find a new foothold. Once stick insects touch an object with their leg the movements are modified in order to grasp the object [4] or –if the object disappears before it can be grasped- to center the searching movements on the former position of the object [5]. Thus, stick insect searching movements constitute an experimentally accessible behavior that can serve to investigate neuronal mechanisms underlying rhythmic motor behavior and its modification.
The motoneuronal activity underlying rhythmic searching movements has been investigated [6,7]. However, neither the activity of premotor interneurons during rhythmic searching movements, nor their potential contribution to a modification of leg movements has been the objective of research. Therefore, we analyzed the activity of nonspiking interneurons (NSIs) –a subpopulation of local thoracic interneurons- during searching movements. To this end, we intracellularly recorded NSI activity in the semi-intact behaving stick insect simultaneously with the muscle activity of four main leg muscles and leg movement trajectories.
We found that the membrane potential of several identified premotor NSIs was rhythmically modulated during leg searching movements. NSIs received alternating synaptic excitation and inhibition. When we artificially changed the membrane potential of single NSIs by tonic current injection throughout ongoing searching movements, we could induce changes in specific searching movement parameters. These parameters were movement amplitude, velocity, position and interjoint coordination. One NSI, I4 [8], provided general drive for the generation of searching movements. Tonic depolarization of NSI I4 started rhythmic coordinated searching movements, hyperpolarization stopped spontaneous searching. Also, when NSI I4 was hyperpolarized, animals could not be activated in other ways to perform searching movements (e.g. by tactile stimulation). Therefore, NSI I4 is both sufficient and necessary for the generation of searching. Another interneuron, NSI E4 that bears similarities to I4 in several ways, does not have such a driving function. These differences are interesting because both NSIs, I4 and E4, are shown to be part of central-rhythm-generating networks for leg joint control [9]. Overall, our results show that NSIs are an important element for the control of searching movements. One NSI even is essential for the generation of the whole behavior.
In addition, all NSIs that were active during searching also were active during walking behavior. Therefore, our results on the role of NSIs may have implications for motor control in general.

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Contribution of RGMa inhibition to recovery of motor functions after spinal cord injury in macaques

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Impairments in dexterous manual movements after spinal cord injury (SCI) are often caused by disruption of the corticospinal tract (CST). Axonal sprouting and/or regeneration after SCI are considered to be critical to the recovery of motor functions. However, such changes in the adult mammalian central nervous system are prevented by various obstacles such as myelin, glial scar, and so on. The repulsive guidance molecule a (RGMa) was originally identified as an axon guidance molecule in the visual system. We previously reported in adult rats that RGMa expression was increased around the lesion site after SCI and, therefore, this molecule might be involved in the suppression of axonal plasticity. In the present study, we showed the expression pattern of RGMa in the cervical cord after SCI in adult macaques. Moreover, we tested whether RGMa inhibition could enhance the recovery of dexterous manual movements after SCI. We made a moderate extent of unilateral SCI at the C7 level. After the injury, RGMa was inhibited with anti-RGMa antibody. Multiple behavioral tests were then performed to assess the recovery of dexterous manual movements. Next, CST fibers were examined in the cervical cord by anterograde tract-tracing with biotinylated dextran amine. Immunohistochemical analysis revealed that RGMa was expressed in microglias/macrophages around the lesion site at day 10 after SCI, compared with a remote cervical cord region in the same monkey and a normal control. According to the behavioral assessment, the recovery of dexterous manual movements after SCI was enhanced in the anti-RGMa group, compared with the control group. The anterogradely labeled CST fibers were extended by application of the anti-RGMa antibody beyond the lesion site. The present results indicate that inhibition of RGMa promotes CST fiber extension around the lesion site after SCI and enhances the recovery of motor functions in macaques.

Cortical control of language-related muscles in speech production

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Introduction: Human language is a unique form of communication among primates. It consists of lexical items expressed following phonological, semantic and syntactic rules by means of a sensory-motor system able to connect internal word to the external one. The aim of the study is to investigate the role exerted by three cortical areas, i.e. Broca's area, ventral PreMotor (vPM) and Primary Motor (M1) cortices in the motor control of speech. Candidates for this study were patients with gliomas undergoing surgery performed with the brain mapping technique. During resection, M1, vPM and Broca's area are exposed and the patient is awakened and asked to perform language tests. During language tests, Direct Electrical Stimulation (DES) is applied over the exposed areas, in order to identify the cortical sites where it interferes with the language tests, the so-called "eloquent areas", and to preserve them. By recording the muscular activity during resection, it is possible to study, offline, the order of motor units voluntarily recruited during the language tests, and which specific recruitment alterations occur when DES applied over M1, vPM and Broca's area during the language tests. The analysis of the EMG signal here proposed aimed at describing the specific alterations occurring in phono-articulatory muscles when DES interferes with speech production in order to disclose the role of the three areas in motor control of phono-articulatory production.

Methods: Eligible for the study were 14 subjects affected by gliomas located in the left frontal lobe, not infiltrating the three areas of interest. During intraoperative resection, the patients performed a counting test and a naming test based on images presented on a computer screen. During tests, DES was applied over M1, vPM and Broca's area through a bipolar probe delivering trains (3-4 seconds) of pulses (1ms duration) at 60 Hz. ElectroMyoGraphic (EMG) activity of lip, mylohyoid, mentalis, anterior neck and masseter (contro- ipsi-lateral) muscles was recorded using by pairs of subdermal hook needle electrodes. The EMG signals were analyzed offline: i) in the frequency domain, by using the short-time Fast Fourier Transform and the Power Spectrum, PS; ii) in the time domain by using the Root Mean Square, RMS.

Results: DES applied over M1, vPM and Broca' area induces the so called "speech arrest", with a different pattern of interference in the three areas: i) stimulation on M1 induces a tonic recruitment, over the spontaneous activity, of all phono-articulatory muscles with an increase of all parameters of RMS; ii) stimulation on vPM induces dysarthria, with a variable pattern of interference including excitation of muscles not involved in speech, inhibition of those normally recruited or an overall inhibition; iii) the effect of stimulation on Broca's area is intensity dependent: at low intensity DES does not affect speech production, nor it alters EMG activity, while at high intensity prevents motor output.

Conclusions: The preliminary data confirm a direct role of M1 and vPM in motor unit recruitment in speech production. The lack any direct effect of Broca's area on phono-articulatory muscles suggests that the role of this area in the naming process is exerted at a cognitive rather than motor level.

CPG Neurons for Species-Specific Singing in Cricket Species

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Cricket acoustic signalling represents one of the most interesting invertebrate communication systems, as species-specific song patterns (chirp or trills) evolved from a common ancestor. Driven by sexual selection males of extant species use very different species-specific temporal patterns of sound pulses (or syllables) for mate attraction. This makes cricket singing behaviour an excellent model to study the neural circuit design and function that underlies species-specific motor activity and to trace the progressive specialization of traits. My study aims to analyse the changes at the level of the singing CPG that are instrumental for the generation of the species-specific patterns and thus aims to shed light on the course of singing network evolution.

Male crickets sing by rhythmic opening and closing movements of the front wings. Singing motor activity is driven by a central pattern generator (CPG) network, composed of opener- and closer-interneurons, localized in the abdominal ganglion (A3). In different species (with different song patterns). I elicit fictive singing by pharmacological brain stimulation and intracellularly record, label and manipulate singing interneurons in A3 to functionally analyse the network.

For example in *G. bimaculatus*, a single opener interneuron in A3 controls the chirp pattern of singing. I identified in two different North American species of field crickets (*G. assimilis* and *G. rubens*) a corresponding opener-interneuron with a similar general arborization pattern to the one in *G. bimaculatus*. The neuron shows rhythmic activity in phase and spikes strictly before the opener motoneuron burst. Like in *G. bimaculatus* transient perturbations of the opener–interneuron activity reset and considerably alter the singing chirp pattern. In all three species, a post-inhibitory rebound mechanism is present and this could be involved in the generation of species-specific syllable rhythm. This syllable generation appears to be an intrinsic property of the network, related to interaction between the opener- and closer interneurons. Besides these similarities the opener-interneuron in the three species reveals different species-specific patterns of membrane potential changes. Interestingly, in *G. bimaculatus*, when the animals were not singing, it was possible to reproduce the chirp and the trill pattern of the three different species studied by applying depolarizing current pulses with different rates and durations. This data indicates that the chirp generator mechanism is an extrinsic property to the singing CPG network housed in A3.

The current data indicate the importance of the opener interneuron as a key interneuron for singing in all three species. I now aim to analyse how the singing CPG network changed at the level of this interneuron to allow the production of species specific song patterns.

Descending tyraminergic/octopaminergic locust brain neurons

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In insects, octopamine and its precursor tyramine are both known to act as neurotransmitters and neuromodulators. Most cells are either purely tyraminergic or tyraminergic/octopaminergic. However, in locusts, Kononenko et al. (2009) identified descending interneurons, synthesizing octopamine from tyramine under stressful conditions. These cells which we labeled as OA3/TA cluster, are part of a bilateral cell population in the brain, which shows similarities to the dorsal or ventral unpaired median (DUM or VUM) neurons in the thoracic ganglia of locust with respect to receiving bilateral mechanosensory input and showing dense arborisations in SOG and thoracic ganglia, in addition to having similar shapes, duration and frequency of action potentials. We observed that these neurons descend at least to the fifth abdominal ganglion.

Considering the projection pattern, the similarities in spike shape and frequency to thoracic DUM cells, at least some of these cells are in a prominent position to provide neuromodulatory output to thoracic and most likely, abdominal ganglia, for example after stressful conditions.

Encoding of volitional initiation of vocalization in the macaque's anterior cingulated cortex.

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In a recent study we have showed that rhesus monkeys can be trained to perform a computer-controlled go/nogo detection task by using their vocalizations as a response. This indicates that non-human primates are able to instrumentalize their vocalizations in a goal directed way. As a neuronal correlate for goal-directed production of vocalizations, we found that the activity of call-related neurons in the ventro-lateral prefrontal cortex (vl-PFC) predicted the preparation of instructed vocalizations. However, the vl-PFC may only be part of a larger frontal-lobe network encoding cognitively-controlled vocalizations. Anatomical and lesion studies indicate that the anterior cingulated cortex (ACC) might play an important role in the initiation of monkey's vocal utterances. We therefore investigated the role of the ACC in the cortical vocalization network.

We trained two rhesus monkeys to vocalize within a given time period in response to a visual go cue (red/blue square). If the go stimulus did not appear the monkey had to withhold the vocalization. According to the applying signal detection theory, go trial in which the monkey uttered a vocalization were defined as 'Hits', whereas catch trials in which the monkey vocalized were defined as 'False Alarms'. As a measure of sensitivity, the d'-values were calculated.

We collected data for monkey C over 34 recording sessions and 27 sessions for monkey T. On average, monkey C and monkey T produced 62±18 and 88±48 vocalizations per session. The mean d'-values of both animals were 2.6±0.3 and 2.4±0.2, respectively, across all sessions. We recorded single-unit activity in the ACC while the monkeys were performing the task. Neuronal responses were analysed in the task period between go-cue onset and vocalization onset. After excluding all neurons that showed a significant eye-movement or fixation-related activity, we compared the neuronal activity in cued vocalization trials (go trials) with the same neurons' response during silent trials (miss trials). A large proportion of recorded neurons showed significant differences in activity during go trials compared to miss trials ($p < 0.05$, the Mann-Whitney U test). Such neurons predict the preparation of cued vocalizations and were defined as vocalization correlated neurons. Our findings support the hypothesis that ACC is a potential key structure operating at the interface between cognitive control and motor output for vocalization.

Frequency-selective functional cortico-cortical interactions between monkey parietal reach region and dorsal premotor cortex in memory guided reaching movements

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During reach planning we need to encode and integrate sensory and motor-related information. The frontoparietal network in the primate cerebral cortex plays a major role in this process, in particular, the parietal reach region (PRR) and the dorsal pre-motor cortex (PMd). Previous studies suggested that these anatomically connected areas play different roles during sensorimotor integration, PRR being predominantly involved in the representation of spatial target information, and PMd being primarily involved in processing more rule-based stimulus-response associations. Yet, neural tuning in both areas can be quite similar and the functional interaction between these two areas during sensorimotor integration is not understood. In this study, we analyze functional interactions between PRR and PMd in a behavioral task that required integration of visual spatial information with learned transformation rules (context), to test if such interaction supports space-context integration.

We recorded local field potentials (LFP) simultaneously in PRR and PMd from two rhesus monkeys that conducted a memory-guided center-out reach task. Space and context information, which had to be integrated for proper reach goal selection, were presented at different times during the trial, either before or after an instructed reach planning phase. Reach goals were either directly indicated by cue position (pro-reach), or had to be inferred by a spatial transformation rule (anti-reach). We quantified the functional connectivity between PMd and PRR in different frequency ranges during different phases of the trial using a Granger causality measure (directed transfer function, DTF). Our hypothesis was that phasic functional interaction between PMd and PRR would occur at the time of space-context integration. Our results revealed a functional interaction pattern between PRR and PMd, in which PRR-to-PMd interaction involved signals in the beta-band (12-32Hz) during the trial periods which required holding a current state (fixation, memory, or target-hold period). The strength of this interaction was independent of the space and context information. In addition, we found a transient PMd-to-PRR interaction carried by low-frequency signals (1-10 Hz) around the time of the go-instruction. The strength of this interaction was modulated by the task-relevant information available to the animals during motor planning.

The results contradict our expectation that functional interaction from PMd to PRR would occur during space-context integration. At least this is true as long as the integration only results in a preliminary motor goal. Based on our finding, instead, we suggest that the frontal-lobe area PMd exerts a brief functional impact on the parietal processing in PRR at the time when a motor goal is confirmed, and an update of the movement status will be initiated.

From vision to action: a comparative population study of hand grasping areas AIP, F5, and M1

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Hand grasping requires the transformation of visual object information into corresponding hand actions. In the primate brain these processes are linked to area AIP (anterior intraparietal cortex), F5 (ventral premotor cortex) and M1 (primary motor cortex). Although these areas demonstrate selective responses when hand movements are planned or executed, it is up to now unclear how visual and motor information is encoded on the neuronal population level.

To address this question, we trained two macaques to grasp up to 50 different objects in a delayed reach-to-hold task. In this, we measured the kinematics of hand and arm together with spiking activity recorded from up to 300 single- and multi-units using microelectrode arrays. The high variation of visual stimuli and motor responses in this task allowed us separating visual attributes of objects from motor features of the hand. Canonical variant and hierarchical cluster analysis demonstrated a dominant visual role of AIP during both the planning and execution epoch. The neural population separated the objects primarily based on their shape and secondarily on their size. Furthermore, we found indicators for the processing of object affordances relevant for grasping. In contrast to AIP, we could identify in F5 a distinct motor representation that encoded the objects in motor terms. However, the highest correspondence to the recorded hand kinematics was observed in the M1 population activity that closely matched the multi-joint space of the hand and arm.

Together our results demonstrate distinct roles of AIP, F5, and M1 at the population level that are highly relevant for understanding how visumotor transformations are processed in the brain.

Graded neural selectivity with graded preference for rule-based reach goals in monkey sensorimotor cortex

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In natural action selection and planning, visual stimuli often determine movement targets, such as chasing prey. However, motor goals sometimes need to be inferred from visual stimuli based on spatial transformation rules, such as fleeing from a predator. Neurons in dorsal premotor cortex (PMd) and parietal reach region (PRR) can encode two alternative motor goals when monkeys have equal preference to choose either goal. This is true even if goals are selected based on two competing spatial transformation rules, i.e. without spatial target stimuli marking each potential motor goal location (Klaes et al, 2011). Here, we test whether a graded preference for rule-based reach goals is reflected in graded spatial selectivity of individual neurons in PMd and PRR during decision making.
A rhesus monkey had to determine the correct reach goal from two instructive cues. A pre-cue consisted of two differently colored triangles which appeared at one of the four cardinal directions from the center (e.g. top) and which pointed to two opposite directions (e.g. left and right). The pre-cue indicated, first, the two possible goals in a given trial, located at 90^o clockwise and 90^o counterclockwise relative to its own position (rules). Second, the triangle sizes could differ and represented the probability with which the rule corresponding to each triangle would be instructed later. After a delay, a spatially neutral cue was presented. In instructed trials the cue was equal-colored to one of the pre-cue triangles and indicated the valid rule. In interspersed free-choice trials the cue color was neutral and both potential goals were rewarded with a random schedule (50% of the trials more reward, other half of the trials less reward than instructed trials), providing the monkey always with equal reward expectancy for both options.
Behavioral results show that the graded rule pre-cueing successfully induced a graded bias in the monkey. The response alternative which was more likely to be instructed at the end of the trial according to the size of the pre-cue was chosen more often and faster even in free-choice trials when there was no objective advantage from freely selecting this alternative. Preliminary neural results show that, first, the spatial selectivity of individual PMd and PRR neurons during motor planning was modulated in accordance with the degree of later choice bias, i.e. showed weak spatial selectivity in balanced trials and increasingly stronger selectivity with increasing bias of the animal. Second, potential motor goal representation emerged earlier in PRR than in PMd, but once the final reach goal is selected, PMd neurons, in turn, represent it earlier than PRR counterparts.
We conclude that in a rule-based motor goal selection task, the probability with which a rule is instructed induces a reward-independent subjective preference in free-choice behavior. The degree of behavioral preference is reflected in the degree of spatial selectivity of individual neurons in PRR and PMd, suggesting that graded decision variables result in graded motor planning activity. The early encoding of potential motor goals in PRR supports its role in competitive selection among rule-based and mutually exclusive action alternatives.

Klaes et al. (2011) *Neuron* 70:536.

Grasp force coding in F5 and AIP in a delayed grasping task

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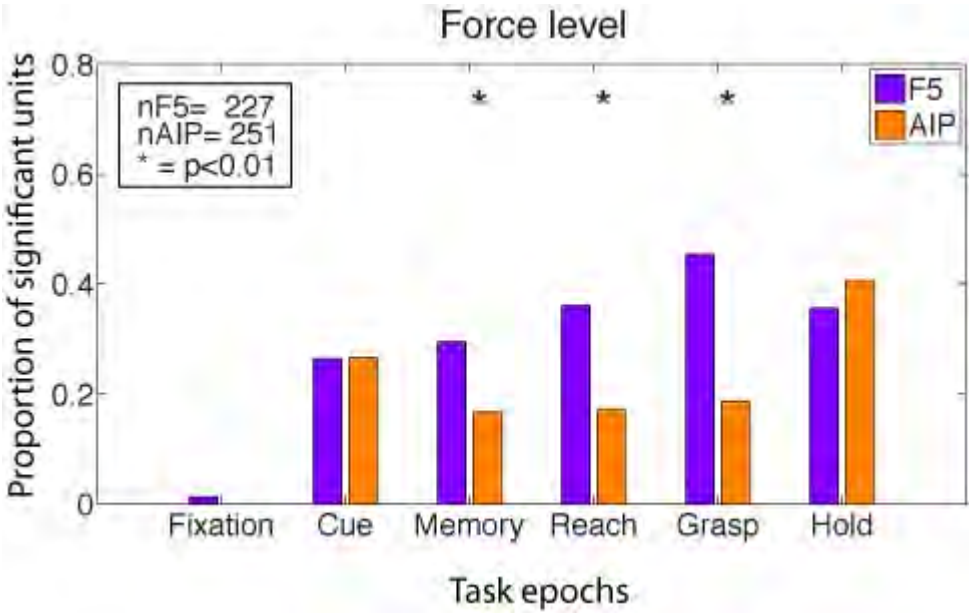
Studies focusing on the neural representation of hand forces have traditionally targeted the primary motor cortex (M1) due to its direct connections to the corticospinal tract. Few studies have also looked at premotor areas, where a stronger representation of movement planning is found. In this study we focused on the macaque ventral premotor cortex, also known as area F5, because of its strong relation to grasping movements, and on the anterior intraparietal area (AIP) that is directly connected to F5. AIP is highly active during the planning and execution of grasping movements, but its role in the control of grasp force is virtually unknown.

We trained a macaque monkey on a delayed grasping task, in which a manipulandum (handle) was grasped with the right hand with one of two grip types, either a power grip or a precision grip. Every grip had to be held for 1 second at one out of three force levels. Both the particular grip type and the required amount of force were cued to the monkey in the beginning of each trial. We then recorded neural activity from F5 and AIP in the left hemisphere, contralateral to the moving arm, with chronically implanted floating microelectrode arrays (FMAs; MicroProbes for Life Sciences). Two 32-channel FMAs were implanted in each area (total of 128 electrodes).

We found that single unit activity in F5 and AIP was strongly modulated by both grip type and grasping force. Response to grip type was similar in both areas. However, grasping force was more strongly coded in F5 than in AIP during most epochs of the task. Only during the cue presentation and the holding phase, when the monkey was actively maintaining the force level, similar proportions of AIP neurons showed force modulation as in F5. Neural modulation was also different in both areas, with F5 neurons showing more often an increase in activity with increased hand force, whereas AIP neurons were modulated to similar degree toward an increasing and decreasing force level.

These preliminary results demonstrate a clear, but potentially different involvement of AIP and F5 in the planning and execution of grasp forces.


Force level
nF5= 227
nAIP= 251
* = p<0.01
F5
AIP
Proportion of significant units
0.8
0.6
0.4
0.2
0
*
*
*
Fixation
Cue
Memory
Reach
Grasp
Hold
Task epochs

Habenula Circuitry Controlling the Dopaminergic System in Anuran Amphibians

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With all vertebrate groups anuran amphibians share the basic components of the basal ganglia circuitry, including dopaminergic midbrain neurons in the posterior tuberculum (ntp), the homologue of the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) of higher vertebrates (Maier et al. 2010). It remains unresolved how exactly the activity of these dopaminergic neurons is controlled. Based on anatomical and functional data in lampreys (Stephenson-Jones et al. 2012, companion poster Twickel at al. 2015) and in mammals (Hikosaka 2010) the lateral habenula (LHb), a small phylogenetically conserved nucleus, has been shown to take part in regulating the activity of these dopaminergic neurons via direct glutamatergic and indirect GABAergic pathways. The habenula has shown to be excited after negative reward and inhibited by reward itself, implying it's involvement in control of reward-seeking and punishment-avoidance behaviors (Hikosaka 2010). Here the afferent and efferent connectivity of the habenula in anuran amphibians is investigated using (double) retrograde and anterograde fluorescence tracings with focus on the lateral habenula and the striatum, together with tyrosine hydroxylase (TH) and GABA immune-labeling. It could be shown, that the habenula afferent and efferent connectivity is conserved: afferents arise from sensory, limbic and basal ganglia systems and efferents project to VTA/SNc and raphe nuclei. Efferents from the lateral habenula terminate in close apposition to neurons in the ntp, which project in turn to the striatum. Furthermore terminations of efferents from the lateral habenula in close apposition to dopaminergic neurons could be discovered. These results indicate a possible further operating principle of the habenula circuitry besides signaling aversive situations. The presented work is seen as a basis for future functional in vitro and in vivo studies.

Habenular Glutamatergic Excitation of VTA/SNc Neurons in Lamprey

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In vertebrates, dopaminergic midbrain neurons are crucial for the operation of the basal ganglia with regard to action selection and motor control. The lateral habenula takes part in the control of dopamine neurons. In mammals and in lampreys the lateral habenula (Stephenson-Jones et al 2012, 2013) is thought to play a role in the phasic modulation of the dopamine neurons and in aversive behaviour by triggering a phasic decline in the dopamine discharge. In lamprey, dopaminergic neurons are located in the nucleus tuberculi posterior (ntp), a homologue of the mammalian substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA). The organization of the dopamine innervation of different structures within the CNS is conserved to a remarkable degree (Fernandez-Perez et al 2014). Dopamine neurons in ntp were recorded (patch clamp recordings) in brain slices, which also included the major efferent habenular pathway, the fasciculus retroflexus (fr). Neurons recorded in ntp were reconstructed and stained immunohistochemically for tyrosine-hydroxylase (TH) to identify whether they could be classified as dopaminergic (n=21) or non-dopaminergic (n=24). The two groups differed with regard to input resistance (higher in dopamine cells) and half width of the action potential membrane potential, and both groups displayed spontaneuous spike activity. Using extracellular stimulation of the fr, the synaptic responses in ntp cells were characterized. The synaptic inputs from the lateral habenula to the dopamine neurons were mainly excitatory and primarily AMPA receptor-mediated, which could be demonstrated by bath application of the receptor antagonist NBQX. Inhibitory effects were investigated by applying gabazine. This suggests a wider role for the lateral habenula in value-based decision making in lampreys, which, due to the strong evolutionary conservation of the basal ganglia circuitry, is likely to apply also to higher vertebrates.

How to find home backwards? Reverse walking in *Cataglyphis fortis* desert ants

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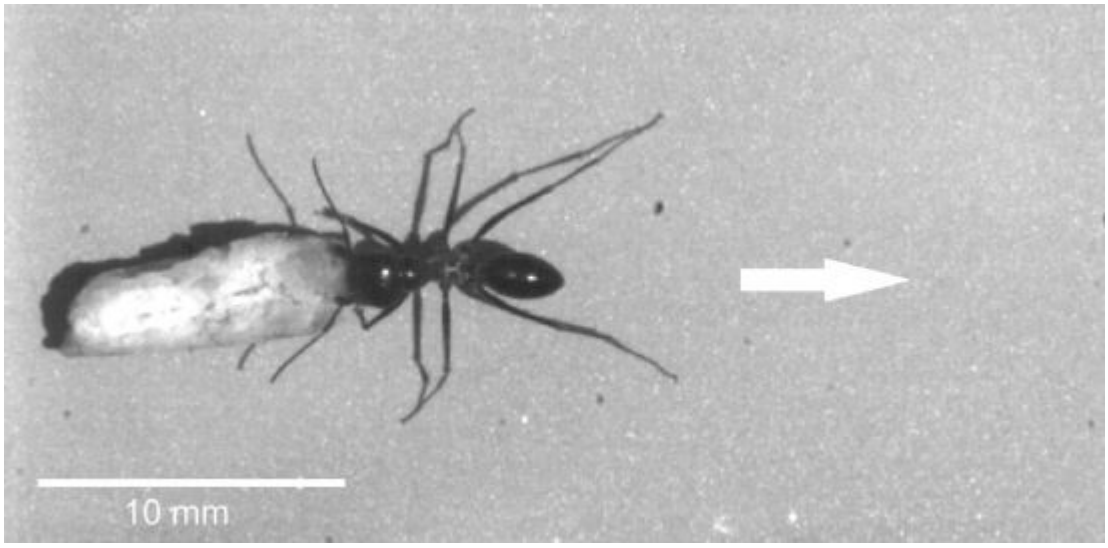
Desert ants of the genus *Cataglyphis* have been a model organism for insect navigation for decades. Due to their extreme desert habitat, they often have to accomplish meandering and long foraging trips. During these excursions, they continuously update their angle steered and distance travelled and combine this information to a global home vector. This process of path integration has been studied since the beginning of *Cataglyphis* navigation research. Nevertheless, the focus was exclusively on forward walking ants. The characteristics of how *Cataglyphis* ants actually walk backwards has not been investigated yet. Here we present a study of walking kinematics and odometric abilities of homing in backward walking ants.

We first trained the ants to forage at a feeder with little food crumbs. The feeder was placed at a distance of 10 m within a linear aluminium channel. To make the animals take their homebound trip back to the nest in backward locomotion, we first caught them right at the feeder after they had taken the booty. Then we replaced the little food crumb with a much larger food item which the ants were not able to carry. Thus, the ants were prevented from lifting up the food and transport it in regular forward-faced body orientation. Instead, the bulky load was hauled backwards. We tested the food dragging ants in an identical channel that was aligned in parallel to the training channel. On their way home, we recorded the homing and nest search behaviour for further odometric analyses. In addition we performed high speed video recordings to characterize the ants' backward walking kinematics.

In our first approach, we asked how legs are coordinated during backward locomotion. Surprisingly, our results show irregular stepping patterns with some coordinated sequences that can be assigned to wave gait. Normal forward moving ants at walking speeds comparable to the backward walks (27 mms^{-1} - 83 mms^{-1}) exclusively employ regular tripod gait.

In our second approach we asked, if backwards moving ants - despite their irregular leg coordination pattern - are still capable of estimating their homing distance. We could show that this is indeed the case. The search behaviour is, statistically speaking, not discernible from normal homing ants. Interestingly, when the ventral parts of the compound eyes were occluded, backward homing capabilities were not as good – accuracy and search certainty deteriorate significantly. This is, however, not true for forward moving. This may indicate that optic flow input plays a major role for odometry in irregular backward locomotion.

Figure: Backward walking in *Cataglyphis fortis*. An ant was offered a food item too heavy to be carried between mandibles in normal forward-faced orientation. Therefore the ant is hauling its load backwards in homing direction. Still image extracted from video sequence.


10 mm

Joint moments in the limbs of an insect walking freely on stable and unstable ground

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Coordinating forces in a multi-jointed limb challenges biological and artificial walking systems alike. All joints of a limb have to act together to propel the body, stabilize it against gravity, and adjust locomotion to variations in the environment. Studies on insects have provided many insights into the neuromechanics underlying joint control. However, little is known about joint control in freely behaving insects, which have to carry their own body weight. How do individual leg joints contribute to locomotion when movements are unrestrained and under body load? How do they respond to sudden changes in the environment, such as unstable ground? To further our understanding, we analyzed net joint moments in intact, freely walking stick insects. Joint moments were derived from rigid link models of all limbs by combining high-speed motion capture with single leg force recordings. Three-dimensional forces were recorded with strain gauge based force transducers of varying compliance, which served the legs as either stable (>20 N/m) or unstable (0.3 N/m) footholds during walking. On stable ground, we unexpectedly found that the coxa-trochanter joint (CT, part of the “hip”) not only provided support but also substantially modulated propulsion. As the legs switched from a supinated to a pronated orientation, large supporting moments at this joint concurrently reduced propulsion at the beginning of stance and increased it toward the end. In the other leg joints, stabilizing forces dominated propulsive forces. In hind legs, for instance, flexion and protraction moments about the femur-tibia (FT, “knee”) and thorax-coxa joint (TC, part of the “hip”) during the second half of stance assisted in stabilizing the leg against gravity-induced collapse. On unstable ground, individual leg joints maintained their ‘locomotor functions’, although the foot was considerably moved away from the body. Basic step parameters as well as supportive and propulsive forces from the CT joint were essentially unaffected. Moments were adjusted in the other two joints, which further stabilized the leg. These adjustments may reflect the actions of a highly flexible motor program, which may be under feedback control involving dedicated force sensors (campaniform sensilla) for each joint. Future studies with manipulated sensory input may help us unravel their importance for walking control and inspire control algorithms for compensatory motor reactions of multi-jointed robotic legs.

Laterality of grasp-related activity in macaque areas AIP and F5

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In primates, the anterior intra-parietal area (AIP) and area F5 of the ventral premotor cortex (PMv) play key roles for the transformation of visual target information into appropriate motor plans for object manipulation. While much research has focused on how these areas represent grasp planning and execution signals for contra-lateral movements, very little is known about grasp planning for the ipsi-lateral hand.

To study this, we trained a female macaque monkey to perform a delayed grasping task, in which a handle was grasped in one of 5 distinct orientations with the contra- or ipsi-lateral hand with a power or precision grip. On any given trial, cue lights and an auditory cue indicated the appropriate grip type and the hand to be used, respectively. During the task we recorded single- and multi-unit activity simultaneously from AIP (n = 201) and F5 (n = 173) in the right hemisphere.

In order to quantify the laterality of neural tuning, we compared the activity of each unit during contra- and ipsi-lateral grasps. During the instruction and movement epochs, firing rates were on average 25% higher during contra-lateral trials in both areas. However, most units were modulated by grasps of either hand, suggesting task dependency regardless of hand used. Interestingly, grip type tuning was 1.5 times more prevalent during contra-lateral trials, suggesting a more functionally significant role.

The hand used for grasping was significantly encoded during the instruction epoch in 38% of units in AIP and 56% of units in F5 (3-Way ANOVA). During movement execution this tuning increased in AIP (50%), but decreased in F5 (36%). Grip type and handle orientation tuning was present in 16% of units in AIP during instruction, while grip type tuning was present in 25% of F5 units. Grip tuning was always maximal during grasp execution. Interestingly, many units switched their preferred grip or hand during memory or movement, suggesting a flexible encoding of task parameters during grasp preparation and execution.

To further elucidate the functional role of contra- and ipsi-lateral activity during grasping preparation we calculated the functional correlation between AIP and F5 during the instruction epoch (i.e. how similarly task conditions were encoded in the neural state spaces of AIP and F5). We found that task conditions were encoded similarly in AIP and F5 during contra-lateral grasps (r-value: 0.74), but not during ipsi-lateral grasps (r-value: 0.07).

Taken together, our results suggest that AIP and F5 are very active during grasp movements of either hand, but functional communication between AIP and F5 might take place primarily during contra-lateral grasping.

Loss of the calcium channel ß$_4$ subunit reduces the pacemaker frequency of cerebellar Purkinje neurons in ataxic mice

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Mutations in the major cerebellar subunits of the PQ-type calcium channels, $Ca_V2.1$, ß4 and a2d-2, cause ataxia in humans and mice. Mutants for $Ca_V2.1$ and a2d-2 are well characterized but little is known about the cerebellar pathophysiology of ataxic ß4-null mice (*lethargic*); thus the critical role of ß4 in the neuronal circuit presiding motor coordination is still elusive. ß4 comes in three splice variants (ß4$_a$ ß4$_b$ and ß4$_e$) with different subcellular targeting and functions. All three splice variants are part of the channel complexes in the membrane; in addition ß4$_b$ and to a lesser extent ß4$_a$ also target to the nucleus where they regulate expression of genes involved in excitability and synaptic function (Etemad et al., J. Neurosci. 2014). Our current data show that ß4$_a$ and ß4$_b$ are expressed in juvenile and adult cerebellum. The third isoform, ß4$_e$, is only expressed in adult. In light of these findings we investigated the electrophysiological and morphological properties of juvenile and adult Purkinje neurons in acute cerebellar slices from healthy and *lethargic* mice. Current clamp recordings of the spontaneous activity of Purkinje cells demonstrated that the juvenile pacemaker was irregular in *lethargic mice*. Otherwise, juvenile Purkinje cells had the same pacemaker frequency, excitatory and inhibitory synaptic inputs and dendritic size as healthy age-matched controls. In contrast, profound differences were found in the morphology of adult Purkinje cells, where *lethargic* neurons had strikingly sparser and shorter dendritic arbors than age-matched controls. However the synaptic spine density in adult *lethargic* dendrites was equal to that in controls. Notably the unchanged spine density combined with a smaller dendritic size implies a loss of synaptic input in the adult *lethargic* molecular layer. Adult *lethargic* Purkinje neurons also failed to develop similarly high pacemaker frequency as healthy age-matched controls. Tetanus toxin (10 µg / ml) reduced the firing frequency of healthy adult neurons. In *lethargic* neurons it synchronized the pacemaker activity but did not affect its frequency. This implies that synaptic or extra-synaptic vesicular release in adult cerebellum promotes the development of a high pacemaker frequency in Purkinje neurons. Together our morphological and physiological data suggest that this mechanism is reduced or absent in the *lethargic* mouse. Because effective inhibition of the deep cerebellar nuclei depends on the Purkinje pacemaker frequency and regularity, the cerebellar phenotype observed in the absence of ß$_4$ can significantly contribute to the lethargic motor impairment.

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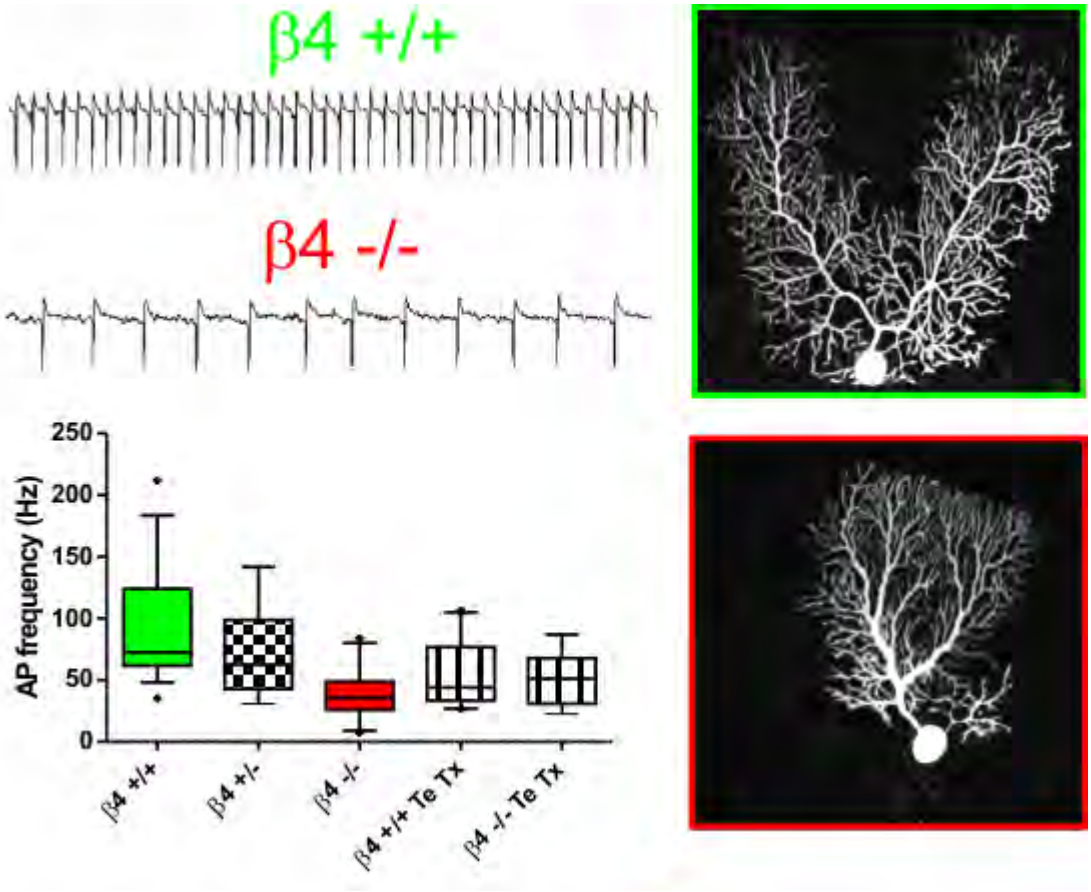
β4 +/+
β4 -/-
AP frequency (Hz)
250
200
150
100
50
0
β4 +/+
β4 +/-
β4 -/-
β4 +/+ Te Tx
β4 -/- Te Tx

Modulation of reach adaptation through transcranial direct current stimulation (tDCS) in patients with cerebellar degeneration

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Treatment of ataxia in patients with cerebellar degeneration remains a major challenge. Because of the general lack of medication, the mainstays of therapy remain physiotherapy, occupational therapy and speech therapy. Recent findings showed that motor learning was facilitated by cerebellar tDCS in young and healthy subjects in a visuomotor reach adaptation task (Galea et al. Cerebral Cortex 2011). It has therefore been proposed that cerebellar tDCS may be beneficial in patients with cerebellar degeneration by improving their known motor learning deficits.
In the present study reach adaptation in a force field was tested in 20 patients with pure cerebellar degeneration (mean age 54.1 ± 10.7 years; range 30–74 years) and 20 age and gender matched controls (mean age 55.3 ± 11.3 years; range 28–74 years). Three sessions were performed which were one week apart. During each session subjects received either anodal tDCS of the cerebellum, anodal tDCS of the primary motor cortex (M1) or sham tDCS in a randomized order. As expected, reach adaption was significantly reduced in cerebellar patients compared to controls. However, there was no significant effect of stimulation neither in controls nor in cerebellar patients. A control experiment was performed in three groups of ten young controls who received either cathodal, anodal or sham tDCS of the cerebellum. Again, there were no significant effects of tDCS on force field adaptation. In conclusion, neither tDCS of the cerebellum nor tDCS of M1 were able to improve reach adaptation deficits in patients with cerebellar degeneration. tDCS effects, however, may be task-depended given that anodal tDCS did not facilitate learning in controls.
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Motor activity in the three major leg joints of the turning stick insect is modified in a context-specific way

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Locomotion like swimming, flying or walking, depends on neural control, muscle contraction, and sensory feedback. The generation of straight walking has already been well studied, but the neuronal mechanisms underlying motor flexibility, are still largely unresolved. In the stick insect, for example, much is known about the neural activity underlying basic locomotor patterns (Büschges 2005), but the knowledge on local segmental activity during adaptive locomotion and the role of descending influences on it and on the processing of sensory feedback in this context is scarce (Rosenbaum et al. 2010, Hellekes et al. 2012).

In this study we analyze the neural mechanisms underlying curve walking in stick insects (*Carausius morosus*). Turning of the tethered animals on the slippery surface was induced, using an optical stimulus, and walking sequences were monitored by video from above. To study the influence of descending signals from rostral segments, we performed extracellular recordings from leg nerves nl2 (containing protractor motoneurons), nl5 (containing retractor motoneurons), C1 (containing levator motoneurons), and nl3 (containing extensor motoneurons) of the otherwise deafferented mesothoracic ganglion of a reduced preparation with all except for the two front legs cut off. In addition, *Flexor tibiae* muscle activity of the front legs was monitored by EMG to obtain the beginning of stance phase. During inside curve walking, mesothoracic protractor motoneurons showed an activity that was stronger compared to outside turn movements. In contrast, mesothoracic retractor motoneuron activity was generally stronger during outside compared to inside turning movements. In most cases the mesothoracic extensor motor activity (consisting of FETi, SETi and CI) was strongly tonic during outside turns, while, during inside turns, its FETi activity was reduced, and showed patterning that was dependent on front leg stepping in many cases. In contrast to these direction dependent changes, the levator motoneurons in nerve C1 did not show changes depending on the turning direction. The mesothoracic levator motoneuron activity increased as soon as the front legs started stepping in either direction.

Our results indicate that changes in mesothoracic motor activity during curve walking of the front legs occur separately on the level of the thorax-coxa and femur-tibia joints, and depending on the function of the leg as either inside or outside leg, but not on the coxa-trochanter joint. In addition to previously described task-dependent changes in local sensory processing, this suggests a unilaterally descending, and highly specific behavior-dependent control of the single joint motor networks.

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Probing the Function of Motoneuron Dendrites in Drosophila

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Dendrites form the blueprint for wiring the brain and give neurons their unique morphological characteristics. Accordingly, dendritic abnormalities are consistent correlates of mental retardation as occurring in numerous neurological disorders. However, in many cases it remains unclear whether dendritic defects are the cause or the consequence of a disease, as it remains a challenging task to relate structural defects of dendrites directly to neuronal function.

This study determines the functional consequences of dendritic defects in Drosophila motoneurons. The strategy is to selectively manipulate dendritic structure without affecting other properties of these motoneurons or other network components. We have recently found that targeted RNAi knock-down of Down syndrome cell adhesion molecule (Dscam) prevents Drosophila wing motoneuron dendrite formation (Hutchinson et al., JN, 2014). We now demonstrate that motoneuron membrane currents, axon arbors, and neuromuscular transmission are not affected by Dscam knock-down. Similarly, other parts of the network are not affected. This now allows for testing motoneuron firing output and behavioral performance in flies with dendrite-less wing motoneurons.

Even in the absence of 90 % of all dendrites flight motoneurons receive synaptic input and display adequate firing activity during flight and courtship song, thus allowing the execution of basic motor tasks. By contrast, dendritic defects negatively impact intricate functions of motoneurons, such as modulations of firing frequency during the integration of optomotor information, or during switching between different courtship song elements. Therefore, the function of complex dendrite structure is eminent only in challenging computations but not during basic function.

Speed-dependent interplay between CPG activity and sensory feedback during walking in Drosophila

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The rhythmic and coordinated motor output required for terrestrial legged locomotion is a result of interactions between sensory signals from the legs and the activity of central pattern generators (CPGs). In that notion, CPGs provide basic rhythmic output that is modulated on a cycle-by-cycle basis by sensory input mediating the current state of the motor system. While this holds in general for walking in insects, the specific role of CPGs and particularly the walking speed-dependent modulation of their activity in this context is understood only insufficiently.

Amputation experiments are one of the oldest methods to gain insights into the neural control mechanisms underlying walking. Amputation of individual legs removes load signals and mechanical coupling between legs: in principle, the stump is still able to move but is unimpeded by contact with the ground. Previous work in cockroaches showed that the lack of sensory input created by the amputation of single legs affects the timing of motor activity in ipsilateral legs in a speed-dependent manner.

Previous experiments indicated that Drosophila is still able to walk coordinately with only five legs. Therefore, we investigated the respective roles of inter-leg sensory feedback and CPGs during walking in Drosophila in a systematic manner. We observed intact flies and single-leg amputees walking on an air-suspended ball, while we recorded the animals' behavior with a high-speed camera and monitored walking speed and the position of the leg tarsi or stumps. We compared oscillation periods, phases, and absolute inter-segmental leg movement intervals during walking in intact flies and front, middle, and hind leg amputees, respectively.

We found that leg stumps of front, middle, and hind leg were still rhythmically active during walking. While the movement period of intact legs was clearly dependent on speed, the period of the leg stumps was largely independent. This was particularly evident in front and middle leg stumps, but also hind leg stumps showed a clear tendency towards independent movement.

Interestingly, the temporal coordination between legs and stumps became stricter with increased walking speed. While at low walking speeds there was no coordination between stumps and intact legs, this changed markedly at approx. 6 body lengths per second: at this speed coordination of the stump with respect to the remaining intact legs increased to the point where it became undistinguishable from non-amputees. Interestingly, the transition range to strong coordination is the point at which the stepping period in intact legs becomes very similar to the base frequency of the stump oscillations.

Our findings suggest a strong influence of sensory feedback on the modulation of single leg stepping. The reduced sensory feedback reveals a probably CPG-generated movement of the individual stump, generating several stump oscillations during a single step of the remaining intact legs at slow walking speeds. At higher speeds stump oscillations seem to become more strongly coupled to the intact legs. Importantly, the largely constant stump period indicates that descending signals controlling walking speed do not access the segmental CPGs directly. They rather seem to mediate their influence via other pathways; this includes the possibility that they act via modulation of sensory signals.

Subesophageal modulation of thoracic motor activity in the stick insect

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The neuromodulatory substance octopamine (OA) plays a major role in insect motor control. In inactive stick insects, for example, OA alters the response properties of a proprioceptive feedback system towards those that characterize the active state of animals (Büschges et al. 1993). Furthermore, OA increases the tonic depolarization in mesothoracic crural motoneurons during front leg stepping (Westmark et al. 2009). OA is released from dorsal unpaired median (DUM) neurons. Until now, the identity of DUM neurons mediating the afore mentioned effects has remained elusive. Six DUM neurons with somata located in the posterior part of the locust subesophageal ganglion have axons that are bilaterally descending (abbreviated DUM-SD) to thoracic ganglia (Bräunig and Burrows, 2004). We hypothesize that presumably homologous neurons in the stick insect *Carausius morosus* might be candidates for the modulation of thoracic motor activity. Using semi-intact preparations and intracellular recordings, we observed phasic depolarization of DUM-SD neuron membrane potential and the generation of action potentials during stance phases of a single stepping middle leg and during six-legged walking. Mechanical stimulation by passive movement of legs was excitatory to DUM-SD neurons. In contrast, pharmacologically evoked activity of central pattern generating neurons (CPGs) had no effect on DUM-SD neuron activity. Thus, the excitatory input to DUM-SD neurons during walking most likely arises from leg sensory organs rather than from coupling to CPG activity. In order to test a possible role of DUM-SD neurons in the modulation of sensory-motor interaction, we studied the effect of DUM-SD neuron activity on reflex responses evoked by the stimulation of the mesothoracic femoral chordotonal organ (fCO). Two major effects were observed: 1. Stimulation of some DUM-SD neurons decreased resistance reflex responses in middle leg *extensor tibiae* motoneurons (N=5). 2. Spike activity in other DUM-SD neurons induced an increase in *extensor tibiae* motoneuron activity and the occurrence of assistance reflex responses during fCO stimulation (N=10).

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Task- and Segment-Specificity of Movement Feedback Signal Processing in a Curve Stepping Insect

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Depending on the environment and the motor task, walking animals steadily adjust their leg movements, e.g. to surpass obstacles, or to change velocity and direction of walking. Changes during curve walking are generated by specific modifications in the kinematics of each leg on both sides of the animal (Jander, 1982; Dürr & Ebeling, 2005), also detectable in experimental conditions with reduced mechanical coupling (Gruhn et al. 2009): on the “slippery surface”, a middle outside leg generates large amplitude longitudinally directed stance movements, supported by *Retractor coxae* activity. At the same time, the middle inside leg generates small amplitude stance movements with marked tibial flexion, supported by *Flexor tibiae* activity (Mu and Ritzmann, 2005; Gruhn et al. 2009).

In a previous study (Hellekes et al. 2012), we have shown that, in the stick insect *Carausius morosus*, such task-specificity in leg stepping kinematics are accompanied by differences in the processing of movement-related feedback on both sides of the curve walking animal. On the inside, flexion signals from the middle leg Femur-Tibia (FTi-) joint, reported by the femoral chordotonal organ (fCO) induce reinforcement of *Flexor tibiae*, and inhibition of *Extensor tibiae* activity, reminiscent of the “reflex reversal” reported originally by Bässler (1988). The same flexion signals only rarely produce similar modifications in tibial motoneuron activity during middle leg outside steps.

In the present study, we asked two questions resulting from this finding: 1) Are different parameters of tibial movement processed differently during a given directional stepping, and, 2) are the same parameters of tibial movement processed differently between inside or outside stepping to cause this changed reflex response in the stick insect.

To answer these questions, we stimulated the middle leg fCO with a large range of stimulus velocities (150-750deg/s), varying amplitudes of FTi-joint movement (40-100deg) and varying starting angles (70-150deg) while recording tibial motoneuron and muscle activity in curve walking animals.

The frequency of occurrence of reflex reversal in tibial motoneuron activity was increasing from smaller to larger starting angles and from faster to slower stimulus velocities (cf. Bässler,1988) for both the inside and the outside curve stepping leg, while the amplitude of the FTi-joint movement caused only minor differences. For inside steps we found that the likelihood for the generation of a reflex reversal for all three modalities was significantly higher compared to outside steps, except for flexion stimuli starting at very extended positions (150deg) and a very large stimulation amplitude (100deg). The highest probability for the generation of a reflex reversal was found to be 70% for the inside leg condition with an amplitude of 100deg FTi-joint movement, a velocity of 150deg/s and a starting of 150deg.

Our results show that the occurrence of reflex reversal during inside and outside stepping caused by fCO flexion signaling is in both cases mostly dependent on starting angle and the velocity of the angular movement. However, the thresholds for eliciting the response are drastically lower for the inside vs. the outside leg.

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The effect of hunger on muscle innervation by octopaminergic neurons in larvae of *Drosophila melanogaster*

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Adrenergic signaling plays an important role in the modulation of behavior in vertebrates by affecting synaptic plasticity according to environmental conditions. In invertebrates this role is fulfilled by the biogenic amines octopamine (OA) and tyramine (TA). These monoamines are synthesized from the amino acid tyrosine. The enzyme tyrosine decarboxylase catalyzes the decarboxylation of tyrosine, converting it into TA and OA is synthesized by the enzyme tyramine-ß-hydroxylase. In large insects like locusts OA not only modulates synaptic transmission but also regulates energy metabolism by increasing rate of glycolysis. Corresponding to this mutant fruit flies, *Drosophila melanogaster*, cannot fly for long periods[1], and starvation induces the formation of synaptopods (axon sprouting) in octopaminergic neurons in Drosophila larvae[2]. In this study we used transgenic larvae in which all octopaminergic neurons were labeled by green fluorescent protein (GFP) and quantitatively measured the extend of innervation of identified muscle fibers after various times of starvation and its time course. In addition we studied the normal time course of innervation patterns during metamorphosis. It turns out that the most crucial pupal stages are those between P4 and P7.

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The Role of Leg Touchdown for the Control of Locomotor Activity in the Stepping Stick Insect Leg

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For the coordinated movement of a leg, it is essential that the different leg muscles are activated in clear phase relationships to one another, and with the right intensity to allow correct kinematics. Much is known on how select sensory feedback contributes to the activation of different motor neuron pools in the locomotor control system of stick insects (Büschges & Gruhn, 2008). However, even though activation of stance phase muscles *depressor trochanteris, retractor unguis, flexor tibiae* and *retractor coxae* are correlated with the touchdown of the leg (Gruhn et al. 2006, Rosenbaum et al. 2010, Berendes et al. 2013), the sensory basis of this coupling or its connection to burst intensity is by now only poorly understood (Büschges et al. 2008). In our experiments, we are using a trap door setup to investigate how and which afferent input signaling leg load and ground contact contribute to stance phase muscle activation and burst intensity in the middle leg of three stick insects species (*Carausius morosus, Cuniculina impigra and Aretaon asperrimus*; Berendes et al. 2013).

Of all the above stance phase muscles, only the timing and magnitude of activation of the *flexor tibiae* muscle in the middle leg were changed if the leg unexpectedly stepped into a hole. In this case the latency between the fictive touchdown and the activity of the first *flexor tibiae* unit was significantly increased over control steps and the total activity of the *flexor tibiae* was reduced. The only other effect on one of the muscles observed was a significant increase in the magnitude of depressor activation during steps into the hole. We henceforth focussed our further analysis on the *flexor tibiae* muscle activation. Individual and combined ablation of different force sensors on the leg (trochanteral, femoral and tibial campaniform sensilla) demonstrated the influence from femoral campaniform sensilla on *flexor tibiae* muscle activation: it lead to a significant increase in latency of both, control and air steps. *Retractor unguis* tendon ablation, on the other hand, caused a strong increase in the variability but otherwise no change of the flexor activation latency or the magnitude of its activation between steps on ground and steps into the hole. Our results show that load feedback signals determine primarily the timing of *flexor tibiae* activation at the swing-to-stance transition in the stepping stick insect middle leg, but that additional yet unidentified feedback contributes as well. With respect to timing all other investigated stance phase muscles appear to be under neural control other than that elicited through leg touchdown at the swing-to-stance transition during stepping.

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Time-dependent effects of pulvinar microstimulation on visually-guided saccades and target selection

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The thalamic dorsal pulvinar (dPULV) has greatly expanded during primate evolution and is strongly interconnected with fronto-parietal cortical areas (Asanuma et al., 1985; Gutierrez et al., 2000). Consistent with its anatomical connectivity, inactivation studies have suggested that the dorsal pulvinar plays a critical role in visuomotor transformations and spatial decision making (Wilke et al., 2010, 2013). We used electrical microstimulation of the dPULV to investigate the functional contribution of the pulvinar to saccade generation and spatial target selection. We employed MRI-guided electrical microstimulation (biphasic 50-300 µA pulses in 200 ms trains at 300 Hz) in the dPULV in two macaque monkeys performing a visually-guided direct saccade task with instructed (single target) or choice (two targets) trials. To investigate effects of microstimulation on visually-guided saccades and spatial choices, we varied the timing of stimulation onset, which started prior to, at or after onset of saccade target(s). Stimulation modified the reaction time of the saccades, and target selection, in a time-dependent manner. Stimulation prior to target onset reduced the reaction time for ipsiversive (relative to the stimulation site) target positions; stimulation at and after target onset caused a systematic increase in reaction times for both, ipsiversive and contraversive positions. Similarly, stimulation prior to target onset led to increased ipsiversive target choices, while the stimulation after target onset increased contraversive target choices. These results point towards a time-specific role of the dorsal pulvinar in target selection and call for further electrophysiological investigations of pulvinar contribution to goal-directed behavior.

Walking and running in desert ants - Gait parameters at different walking speeds in the ant, *Cataglyphis fortis*

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Path integration is a form of dead reckoning and thus inherently error-prone. It is nonetheless a common navigation strategy in animals, particularly where environmental orientation cues are rare or absent. The desert ant *Cataglyphis* is a prominent example, covering well above 10,000 body lengths on foraging excursions, primarily guided by path integration. Sun and polarization compasses provide reliable external direction cues for navigation, making *Cataglyphis'* distance gauge, a stride integrator (Ronacher and Wehner, 1995; Wittlinger et al., 2006, 2007), the probably major source of path integration errors. A detailed analysis of walking behaviour in *Cataglyphis* is thus of primary importance for assessing possible sources of navigation errors and potential compensation strategies. Zollikofer (1994) demonstrated consistent use of the tripod gate in desert ants, and suggested an unexpectedly constant stride length as a possible means of reducing navigation errors. Here we extend these studies by more detailed analyses of walking behaviour across a large range of walking speeds. Most notably, stride length (leg movement in relation to substrate coordinates) increases linearly with walking speed. Stride amplitude (leg movement in relation to body coordinates) is not related to walking speed for front and hind legs, while the stride amplitude of the middle legs slightly increases linearly with walking speed. With increasing walking speed an initial decrease of swing phase duration is observed at lower velocities. After that, it stays constant across the behaviourally relevant range of walking speeds of 40-600 mms^{-1}. Walking speed is increased by shortening of the stance phase duration, of the stance phase overlap, and by increasing stride frequency. However, at speeds larger than 370 mms^{-1} stride frequency levels off, and the ants reach aerial phases.

Poster Topic

T22: Homeostatic and Neuriendocrine Systems, Stress Response

T22-1A Ependymal Cilia generate Complex Flow Patterns of Cerebrospinal Fluid
Regina Johanna Faubel, Christian Westendorf, Eberhard Bodenschatz, Gregor Eichele

T22-2A Excitation-inhibition balance of hypothalamic orexinergic neurons in the obese (*ob/ob*) mouse
Thorsten Becker, Luigia Cristino, Vincenzo Di Marzo, Giuseppe Busetto

T22-1B Mapping of Fos expression in mesencephalic periaqueductal gray and deep tectal nuclei of pigeons (*Columba livia*) after tonic immobility.
Fernando Falkenburger Melleu, Cilene Lino de Oliveira, José Marino Neto

T22-1C Modulation of Identified Paraventricular Nucleus Neurons by Fuel Sensing Signals
Andreas C. Klein, Simon Hess, Jens C. Brüning, Peter Kloppenburg

T22-2C Molecular effects of oxytocin on stress: Oxytocin delays CRF gene expression through CRTC3
Benjamin Jurek, David A Slattery, Ying Liu Liu, Greti Aguilera, Erwin H van den Burg, Inga D Neumann

T22-1D Sexually dimorphic effects of fluoxetine in the repeated forced swimming test: the contribution of the serotoninergic metabolism in the brain.
Cilene Lino de Oliveira, Anicleto Poli, Áurea Elizabeth Linder, José Marino Neto, Fernanda B. Lima Christian, Inae Spezia, Lais Cristina Theindl, Fernando Melleu, Karolina Domingues

T22-2D Similarities within arthropod neuroendocrine systems
Anja Dünnebeil, Andrea Wirmer

Ependymal Cilia generate Complex Flow Patterns of Cerebrospinal Fluid

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Constituents of the cerebrospinal fluid (CSF) play a crucial role for the homeostatic balance of the central nervous system and need to be delivered at specific times to exert their signaling function. Propagation of CSF depends on motile cilia that cover the ependymal walls of the ventricles. Using a combination of microscopy and particle tracking with explants of the third ventricle, we discovered that beating cilia create complex flow patterns. We found that cilia bundles are arranged in micro domains in which cilia are beating in a coordinate fashion. Collectively, these cilia micro domains give rise to a characteristic flow pattern, and in this way cause a spatial separation of CSF flow. As consequence, constituents of the CSF are transported with high precision along well-defined cilia pathways at the ventricular wall. Rates of translocation are in the range of 1 mm/sec and considerable faster than free diffusion. The flow patterns and the cilia beat organization are identical between animals. The patterns on the left and the right wall of the third ventricle are mirror-symmetrical. Additionally, we observe daily recurrent changes in the flow patterns and characteristic changes during healthy aging. We also provide evidence that cilia beat direction and consequently the flow of CSF is autonomously controlled within the ependyma.
We discuss these findings in the light of a novel signaling mechanism in which CSF constituents are rapidly and vectorially transported by cilia with the aim to target compounds with high temporal and spatial precision to areas located in the periventricular zone of the hypothalamus. This cilia-mediated signal transduction system may complement the well-documented interneuronal synaptic signal transmission.

Excitation-inhibition balance of hypothalamic orexinergic neurons in the obese (*ob/ob*) mouse

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The leptin-deficient *ob/ob* mouse - a renown murine model for obesity- was shown to exhibit a switch in the innervation of hypothalamic orexin-expressing neurons (OX-N) from predominantly excitatory to largely inhibitory inputs, compared to wild type (wt) mice. This switch of innervation of OX-N appeared to be functionally relevant as the frequency of miniature inhibitory post-synaptic currents (mIPSCs) was more than 2-fold increased in weaned, but not in pre-weaned *ob/ob* mice. The frequency of spontaneous IPSCs (sIPSCs) was strongly reduced after activating the pre-synaptically located cannabinoid receptor type 1 (CB1R) with its agonist WIN 55,212-2. The reduction of sIPSC frequency could be prevented by using the CB1R antagonist AM251. Brief artificial depolarization of the post-synaptic OX-N yielded a similar effect as WIN and was abolished by AM251. This suggests the involvement of the endocannabinoid system, probably acting by releasing 2-arachidonoyl glycerol (2-AG) from the OX-N and activating the pre-synaptically located CB1R, a phenomenon known as “depolarization-induced suppression of inhibition“ (DSI). As a consequence of this strong dis-inhibition, *ob/ob* mice express higher levels of orexin in the brain areas innervated by OX-N compared to wt. All these effects are reversed within 24 hours by in vivo leptin administration.

Currently, we are investigating the functional organization of the excitatory inputs on OX-N and their modulation by endocannabinoids. Furthermore, we are assessing the final outcome of the altered excitation-inhibition balance and its modulation by the endocannabinoid system in terms of resting membrane potential and firing activity. Due to a CB1R-mediated inhibition of the inhibitory tone of the OX-N innervation, meaning a dis-inhibition of the OX-N in *ob/ob* mice, we expect to see stronger firing activity and a more depolarized resting membrane potential in *ob/ob* mice, compared to wt mice.

For the time being, we have not observed any differences of the frequency and amplitude of miniature excitatory post-synaptic currents (mEPSCs) between pre-weaned *ob/ob* and wt mice, which is in concordance with our anatomical observations and our mIPSC results. Furthermore, we have found the resting membrane potential of pre-weaned wt mice to be -53.1 ± 2.54 mV and a corresponding firing activity of 3.4 ± 0.84 Hz.

Mapping of Fos expression in mesencephalic periaqueductal gray and deep tectal nuclei of pigeons (*Columba livia*) after tonic immobility.

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Tonic immobility (TI) is an innate defensive behavior characterized by immobility, analgesia and lack of responsiveness to external stimuli, which is observed in several vertebrate species in response to an inescapable attack of a predator. In birds, the TI response was extensively studied in Galliformes (chickens and quails), but little is known about the central circuitry involved in the control of this behavior. Periaqueductal gray (PAG) and deep tectal regions of the mammalian midbrain are implicated on the expression of TI as well as of other defensive behaviors. Here, the expression of Fos protein in the mesencephalic central gray (GCt) and adjacent n. intercollicularis (ICo, or GCt-ICo complex, anatomically comparable to the mammalian PAG) associated to TI in pigeons was investigated. All experimental procedures were approved by the local ethical committee in animal research (CEUA/UFSC). Twelve adult pigeons (8 males and 4 females, 420-500g bw) were randomly divided into three groups (n=4 each): 1-IT: transferred from the home cage to a separate room where TI was induced; 2- Handling (H): transferred from the home cage to a separate room where handling for 5 minutes was performed; 3- Control (HC): remained non-handled in the home cage. For brain dissection, pigeons were transcordially perfused under anesthesia (ketamine+xylazine) 90 min after the experimental procedures. Formalin-fixed brains were sectioned (40 um) and processed for DAB-based immunohistochemistry to detect Fos protein in cellular nuclei (Fos-ir). Boundaries of ICo-GCt complex were determined using NADPH-Diaphorase staining (NADPHd, marker for the dorsolateral and lateral mammalian PAG). In TI group, the number of Fos-ir cells in the lateral part of the ICo (ICo-L) and in the dorsal part of the stratum griseum periventriculare (SGPd) of the optic tectum increased significantly, as compared to H and HC groups. A similar pattern could be observed at intermediate midbrain sections. In the rostral midbrain, IT was found to increase Fos-ir cells only SGPd. The differences in Fos expression were found in NADPHd-enriched regions of the ICo. TI also induced Fos changes in the n. mesencephalicuslateralis pars dorsalis (MLd), part of ascending auditory pathway.These data indicate that TI may be associated to an intense activation of auditory circuits. Moreover, the complex ICo-L and the contiguous deep tectal SCPd, but not GCt or medial ICo, may be comparable to the mammalian dorsolateral PAG.**Supported by Capes and CNPq.**

Modulation of Identified Paraventricular Nucleus Neurons by Fuel Sensing Signals

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Energy homeostasis is tightly controlled by neuronal networks that are mainly located in the hypothalamus. Within these circuits information about the metabolic state of the organism is processed generating a neurosecretory output that regulates homeostasis. Proopiomelanocortin (POMC)- and agouti-related peptide (AgRP)- expressing neurons in the arcuate nucleus of the hypothalamus are key components of this homeostatic system integrating peripheral fuel sensing signals to neuronal activity regulating food intake and energy expenditure. Second order neurons to AgRP and POMC neurons express the melanocortin-4 receptor (MC4R). These neurons are thought to be activated by the POMC product a-melanocyte-stimulating hormone (a-MSH) and being inhibited by AgRP.

Amongst the neuropiles that are innervated by POMC neurons is the paraventricular nucleus (PVN) of the hypothalamus. The PVN is an important hypothalamic autonomic control center that comprises several subdivisions with a heterogeneous neuronal population (Expert Opin. Ther. Targets. 2008; 12(6): 717-2; J Neuroendocrin 2002; 14: 929-932). As such, it plays an important role in regulating autonomic renal and cardiovascular functions and stress responses. Lesioning the PVN causes hyperphagic obesity (Physiol & Behav 1981; 27(6): 1031-1040) and injection of MC4R agonist melanotan-2 (MT2) into the PVN reduces food intake while the antagonist AgRP (and neuropeptide Y (NPY)) increases food intake (Neuron 1999; 24: 155-163). While electrophysiological recordings showed that MC4R activation (and inactivation) modulates PVN neuron activity the intracellular pathways and the identity of the responsive neurons remained unknown (J Neurosci. 2003; 23(18): 7143-7154; J Neurosci. 2011; 31: 8271–8279; PNAS 2011; 108(1): 355-360).

Here we aim to define cell type specific cellular and molecular processes that are induced by metabolic modulation of identified PVN neuron sub-types under different physiological conditions. Elucidating the molecular mechanisms involved in the activation and inactivation of PVN neurons is crucial to the understanding of the neuronal regulation of food intake and energy expenditure.

Acknowledgments

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Molecular effects of oxytocin on stress: Oxytocin delays CRF gene expression through CRTC3

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Acute threatening stimuli evoke fear and physiological stress responses, which, however, become pathological, when exaggerated or prolonged, and can lead to severe psychological and somatic disorders including depression and anxiety. The hypothalamic corticotropin-releasing factor (CRF) is a key regulator of fear, anxiety and stress responses and controls the hypothalamo-pituitary-adrenal (HPA) axis. Neuronal CRF expression is regulated by the transcription factor CREB and its cofactor CRTC (cofactor of regulated CREB activity, also known as TORC) that, upon an appropriate stimulus, translocates from the cytoplasm to the nucleus to form a complex with CREB and activate gene transcription by binding to the promoter region.

We have shown that the neuropeptide oxytocin exerts anxiolytic [1] and anti-stress [2] effects, for example within the hypothalamic paraventricular nucleus (PVN). However, the underlying molecular mechanisms of these effects are unknown, yet are important for the quest for pharmacological intervention that may dampen (exaggerated) stress responses. We hypothesized that oxytocin inhibits HPA axis activity via altering CRF gene expression and prevention of CRTC translocation, and assessed this using in vivo and in vitro assays.

In male Wistar rats, oxytocin (1 nmol, icv) delayed the restraint stress-induced increase in CRF hnRNA levels in the PVN as assessed by qPCR, but was without effect under non-stress conditions. CRF hnRNA levels were parallel to hypothalamic nuclear CRTC3 levels, and could be modulated by icv TGOT in rats as well as in C57Bl/6 mice. Stress-induced pCREB levels were unaffected by oxytocin or the specific oxytocin receptor agonist TGOT. In vitro studies performed in primary hypothalamic Wistar rat neurons, a Sprague Dawley rat cell line and in a human neuroblastoma cell line revealed that TGOT (10 nM) delayed forskolin- (1 or 50µM) induced CRF transcription,, comparable to oxytocin effects on stress-induced CRF expression in vivo. Selective knock-down of CRTC3 by siRNA prevented these effects of TGOT on CRF, whereas CRTC2 siRNA did not interfere with the actions of TGOT. ChIP analyses revealed that forskolin induced binding of both CRTC2 and CRTC3 to the CRF promoter, and that TGOT specifically prevented CRTC3, but not CRTC2 binding to the CRF promoter.

These results indicate that oxytocin controls CRF gene expression by modulating nuclear CRTC3 levels, thus attenuating/delaying CRF synthesis during the initial phase of an acute stress response. In support, we found a delay in the peak plasma ACTH response from 10 min in the vehicle-treated group to 15 min in the oxytocin-treated group. Taken together, we provide a molecular mechanism underlying the inhibitory effects of oxytocin on the CRF gene and on the initial response to an acute stressor.

The author declares no conflict of interest.

Sexually dimorphic effects of fluoxetine in the repeated forced swimming test: the contribution of the serotoninergic metabolism in the brain.

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Sex differences were reported to influence behavior of rats in the forced swimming test (FST). The repeated FST is a test planned to evaluate short and long term effects of antidepressants in the same group of rats. Repeated FST consisted of a 15 min- swimming test (pretest) followed by a 5 min-swimming test in the first (test), seventh (retest 1) and fourteenth (retest 2) day after pretest [1]. In male rats, the immobility increased over retesting and it is counteracted by antidepressant treatment [1] [2]. In the first part of the present study we aimed to compare the effects of fluoxetine (a selective serotonin reuptake inhibitor) on behaviors of male and female rats in the repeated FST. For that, male or female rats (70 days old, n=5/group) were trained for 7 days to ingest sucrose 5% and then treated orally with vehicle (sucrose 10%) or fluoxetine (2.5 mg/kg) during the period of repeated FST. In females, the estrous cycle was verified by the analysis of vaginal smears before every session of swimming. Independent of the oral treatment, males and females rats presented similar immobility time in the test (Mann-Whitney, U=44, p=0.7), retest 1 (Mann-Whitney, U=48, p=0.9), and retest 2 (Mann-Whitney, U=45, p=0.7). Contrary to expected, retesting failed to affect significantly the immobility time in males (Friedman ANOVA (N = 5, df = 2) = 0.4, p =0.8) and females (Friedman ANOVA (N = 5, df = 2) = 2.8, p = 0.25). The ingestion of sucrose, as a vehicle, over the course of this experiment could explain the lack of effect of retesting in the present study. Similar to the literature [1], treatment with fluoxetine (2.5 mg/kg) decreased immobility time in male rats in retest 2, as compared to the test (Friedman ANOVA (N =5, df= 2) = 8.4, p = 0.015). However, chronic treatment with fluoxetine failed to affect immobility in female rats (Friedman ANOVA (N = 5, df = 2) = 2.8, p < 0.25), independent of the phase of estrous cycle. Together, data indicate that behavior of female Wistar rats were more resistant to fluoxetine than behavior of males in repeated FST. Therefore, we hypothesized that could exist a sexual dimorphism in the metabolism of serotonin (5-HT) in the brain of Wistar rats. To investigate that, male and female rats were decapitated under anesthesia for brain dissection after retest 2. Brain regions were incubated with 5-HT for posterior quantification of 5-HT and its metabolite 5-hydroxy-indol-acetic acid (5-HIAA) in the tissues (index of 5-HT turnover) using High Performance Liquid Chromatography with electrochemical detection. The ratio 5-HIAA/5-HT was significantly higher in the hippocampus of females as compared to males (Mann-Whitney, p=0,049). In addition, the ratio 5-HIAA/5-HT was significantly higher in the cerebellum of females rats treated with fluoxetine as compared to vehicle (Mann-Whitney, p=0,049). No significant differences in 5-HT metabolism were observed in the frontal cortex or brainstem. The higher degradation of 5-HT in the hippocampus and cerebellum of female rats as compared to males may help to explain the lack of effect of the fluoxetine in the female rats submitted to repeated FST. References: [1] Mezadri et al., 2011, J Neurosci Meth 195: 200–205, [2] Gutiérrez-García and Contreras 2009, Pharmacol Biochem Behav v91: 542–8. Financial Support: Capes, CNPq.

Similarities within arthropod neuroendocrine systems

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The neuroendocrine systems of arthropods resemble each other in morphology and innervation pattern. RFamide peptides are released by brain neurons into the neuroendocrine organs, those neurons are located at comparable sites and the glands themselves contain neurons. In insects, the retrocerebral complex contains two different neuroendocrine glands, the corpora cardiaca (CC) and the corpora allata (CA). While the CC have been compared to the hypophysis in vertebrates, the position of the CA is still unclear. We performed stainings against peptides known to occur in both the hypophysis of vertebrates and the insect retrocerebral complex in scorpions and centipedes. In addition, we used antibodies against adiopokinetic hormone which is expressed exclusively in the CC of insects to separate between parts resembling the CC and those resembling the CA in other arthropod species.

Poster Topic

T23: Neural Networks and Rhythm Generators

T23-1A Analysis of light entrainment pathways from the compound eye to the circadian clock of the Madeira cockroach *Rhyparobia maderae*
Julia Yvonne Gestrich, Azar Massah, Andreas Arendt, Monika Stengl

T23-2A BMAL1-deficiency affects neural progenitor proliferation and fate
Beryl Schwarz-Herzke, Amira Ali, Anna Stahr, Timour Prozorowski, Orhan Aktash, Charlotte von Gall

T23-3A Convergence of morphological and physiological properties in a hub neuron in a coordinating network
Felix Blumenthal, Henriette A. Seichter, Carmen Smarandache-Wellmann

T23-4A Cross-modal modulation of spiking patterns in the primary somatosensory cortex of Brown Norway rats
Malte Bieler, Nicole Cichon, Kay Sieben, Ileana Hanganu-Opatz

T23-5A Cyclic nucleotide oscillations in the circadian pacemaker of the Madeira cockroach *Rhyparobia maderae*
Andreas Arendt, Julia Schendzielorz, Thomas Schendzielorz, Monika Stengl

T23-6A Different spatial and temporal activation patterns in the perirhinal and entorhinal cortex in response to afferent stimulation of the neocortex and amygdala
Janske Willems, Pascal Chameau, Taco Werkman, Wytse Wadman, Natalie Cappaert

T23-7A Dissecting the balance between excitation and inhibition in the CA1 and DG regions of the hippocampus
Sicco de Knecht, Wytse Wadman, Pascal Chameau

T23-8A Firing patterns of identified neurons in the medial septum/diagonal band nucleus and their relationship to behaviour and hippocampal network oscillations
Tim James Viney, Abhilasha Joshi, Peter Somogyi

T23-9A Functional analysis of the *Rhyparobia maderae* molecular clock
Achim Werckenthin, Monika Stengl

T23-10A Glutamatergic system controls synchronization of spontaneous neuronal activity in the murine neonatal entorhinal cortex
Petr Unichenko, Jenq-Wei Yang, Heiko J. Luhmann, Sergei Kirischuk

T23-1B Influence of LFP-independent spikes on in-vivo LFPs

Stephan Waldert, Roger N. Lemon, Alexander Kraskov

T23-2B Intra- and Inter-segmental Coordination of Rhythmic Motor Activity in the Walking System of the Stick Insect
Charalampos Mantziaris, Ansgar Büschges, Anke Borgmann

T23-3B Intracellular calcium responses to the neurotransmitter GABA in circadian pacemaker neurons of the Madeira cockroach *Rhyparobia maderae*
Maria Giese, Hongying Wei, Monika Stengl

T23-4B Intrinsic coupling modes in the ferret cortex at different levels of isoflurane
Florian Pieper, Felix Fischer, Gerhard Engler, Andreas K. Engel

T23-5B Intrinsic Electrophysiological Properties of Locus Coeruleus Neurons
Stephan Bremser, Lars Paeger, Peter Kloppenburg

T23-6B Investigating the molecular clock of the carpenter ant *C. floridanus*: genes, neurons and behavior
Janina Kay, Pamela Menegazzi, Stephanie Mildner, Flavio Roces, Charlotte Förster

T23-7B Living at different latitudes: the role of *Drosophila* l-LNv in setting the phase of the evening activity peak.
Pamela Menegazzi, Elena Dalla Benetta, Marta Beauchamp, Christiane Hermann-Luibl, Charlotte Helfrich-Förster

T23-8B Local neuronal phase coupling in monkey's area V4 is modulated by selective attention as if only the attended stimulus is present
Eric Drebitz, Marcus Haag, Iris Grothe, Sunita Mandon, Andreas K. Kreiter

T23-9B Modulation of firing patterns in midbrain dopaminergic neurons
Ursel Collienne, Svitlana Popovych, Simon Hess, Martin E. Hess, Jens C Brüning, Silvia Daun-Gruhn, Peter Kloppenburg

T23-10B Molecular and Functional Characterization of Lateral Horn neurons in *Drosophila melanogaster*
Sudeshna Das, Silke Sachse, Bill Hansson

T23-11B Long-range synchronization by intermingled delta and theta oscillations.
Jonatan Biskamp, Jonas-Frederic Sauer, Marlene Bartos

T23-1C Movement detection systems are generally colour-blind, the startle response circuitry in goldfish is not.
Peter Machnik, Nathalie Peper, Wolfram Schulze, Stefan Schuster

T23-2C New anatomical insight into the circadian clock network of *Drosophila melanogaster* using Flybow
Frank K. Schubert, Dirk Rieger

T23-3C Optimal feature integration in critical, balanced networks
Nergis Tömen, Udo Ernst

T23-4C Optogenetic dissection of cellular interactions in the prefrontal cortex of neonatal mice
Joachim Ahlbeck, Sebastian H. Bitzenhofer, Amy Wolff, Ileana L. Hanganu-Opatz

T23-5C Origin and function of depolarizing afterpotentials in stellate cells in the medial entorhinal cortex
Franziska Kümpfbeck, Martin Stemmler, Andreas Herz, Felix Felmy

T23-6C Photo-periodic diapause in *Drosophila ezoana*: what does it tell about circadian involvement in photo-periodic time measurement?
Charlotte Helfrich-Förster, Saskia Eck, Koustubh Vaze

T23-7C Rapid temperature adaptation of the life-saving escape C-start is mediated by NMDA receptors at the level of the Mauthner cells in larval zebrafish
Alexander Hecker, Wolfram Schulze, Stefan Schuster

T23-8C Representation of visual information in the goldfish Mauthner cell
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T23-9C Responses to emotional images: relation to the respiratory cycle
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Analysis of light entrainment pathways from the compound eye to the circadian clock of the Madeira cockroach *Rhyparobia maderae*

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Abstract

Transplantation experiment located the circadian pacemaker controlling locomotor activity rhythms to the optic lobes accessory medulla (AME). The AME is associated with seven neuropeptidergic soma groups among them the pigment dispersing factor-immunoreactive (PDF-ir) neurons. Since no direct connections between the histamine-ir photoreceptor cells of the compound eye and the AME exist, the circadian pacemaker receives indirect light-input from the ipsilateral as well as the contralateral optic lobes. The γ-aminobutyric acid (GABA)- ir distal tract, which connects the medulla and the lamina with the glomeruli of the AME, and GABA- ir median neurons of the AME, which colocalize different neuropeptides, have been suggested as candidates for light entrainment pathways to the clock. Furthermore, since different photoperiods influence the number of the PDF-ir cells, at least some of these neurons are involved in light entrainment, but their connections to photoreceptors are not known.

With triple-label-immunocytochemistry employing antibodies against histamine, GABA and PDF we analyzed the connections between the photoreceptors of the compound eye and AME neurons in search for light entrainment pathways. Furthermore, intracellular recordings were performed, combined with neurobiotin injections and immunocytochemistry. We aimed for neurons of the soma groups associated with the AME to identify light-responsive cells and to identify their individual branching patterns in relation to PDF-ir neurons. Preliminary immunostainings suggested that PDF-, GABA- as well as histamine-ir fibers branch in close vicinity in the proximal lamina. Furthermore, many histamine-ir fibers terminated in a distal layer of the medulla and projected in close proximity in the first optic chiasma to PDF-ir projections of the anterior fiber fan. The PDF-, GABA-, and histamine-ir neurons arborized in different layers of the medulla without any colocalization. In contrast to histamine- immunoreactivity, PDF- as well GABA-immunoreactivity was localized in the accessory laminae. So far, we assume that overlapping arborizations of PDF-ir neurons with histamine- ir photoreceptors are rather output regions of the clock to the compound eye, while GABA-ir peptidergic median neurons are ipsilateral light input pathways to the clock. Finally, we hypothesize that the GABA-ir distal tract may be involved in general gain control of photic information processing in all optic lobe neuropils. [Supported by DFG grants STE531/18-1,2 and STE531/21-1 to MS]

BMAL1-deficiency affects neural progenitor proliferation and fate

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The subgranular zone of the hippocampus contains a neurogenic niche which generates newborn neurons from proliferating neural progenitor cells (NPCs) and thus provides new neurons for the plastic hippocampal neuronal circuit. Previous studies have shown that the molecular clockwork is involved in controlling cell cycle entrance of quiescent neural progenitor cells. However, little is known about the role of the molecular clockwork in controlling fate of neural progenitors. In this study, mice with a deletion of the core molecular clockwork component BMAL1 (BMAL1-/-) show a significant decrease in proliferation of NPCs and a differentiation towards the astrocytic lineage at the expense of the neuronal lineage. Moreover, in vitro studies revealed that astrogliosis could be attributed to BMAL1-deficiency. Our results indicate that BMAL1 is involved in proliferation of NPCs, determination of neural progenitor fate and astrogliosis.

Convergence of morphological and physiological properties in a hub neuron in a coordinating network

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The swimmeret system of the crayfish is an excellent model to study coordination of distributed microcircuits on the cellular level. Each swimmeret is driven by its own local microcircuit, which includes a central pattern generator (CPG) that drives the motor neurons for swimmeret movement. Although these local microcircuits are anatomically separated they are very well coordinated by an identified network. The rhythmic activity always starts in the posterior microcircuit and the anterior ones follow with 1/4 of a cycle.

The coordinating network consists of three identified neurons in each microcircuit. Two types of coordinating neurons encode information about the status of the local motor output, and conduct it to all neighboring microcircuits. While passing through a ganglion, the coordinating neurons make en passant synapses to the local Commissural Interneuron 1 (ComInt1). The coordinating information elicits periodic bursts of large, brief excitatory postsynaptic potentials (EPSPs) in these neurons. In each module ComInt1 receives the strongest synaptic input, the largest EPSPs, from its nearest posterior neighbor and consecutively smaller ones from the other two microcircuits. In this way, ComInt1 decodes and integrates the coordinating information, while feeding it through an electric synapse to only one of the CPG interneurons. This makes ComInt1 a hub neuron between the neuronal microcircuits and therefore ensures the coordination of the chain of four distributed CPGs in a metachronal fashion.

The gradient of synaptic strength recorded intracellularly in ComInt1 is the reason for the fixed posterior to anterior metachronal wave observed. We hypothesized that the different sized EPSPs are due to unequally distributed synaptic contacts between the three coordinating neurons and ComInt1. For these experiments we dye filled ComInt1 and additionally marked the synaptic boutons (in two segments: A4: N=8; A3: N=5). ComInt1s morphology is very distinctive. Its soma is located in one hemiganglion and it sends its primary neurite over the midline to the contralateral side where it has its dendritic arborization. At the midline it sends one ascending and one descending dendrite parallel to the axons of the coordinating neurons, which travel through the segment. We identified three distinct regions of synaptic contacts in all probes. There are two on the anterior (AR1 and AR2) and one on the posterior branch (PR). Our results showed a statistical difference in the area covered by these synaptic regions, as one of it is always larger than the other two. In segment A4, it was always AR1, while in segment A3 is was PS. These result support our hypothesis that the gradient of synaptic strength is due to morphological features.

Another question we are currently investigating is if the status of the system excitation can alter the integration of the EPSPs. The swimmerets can move with different speeds *in vivo*. *In vitro*, we can mimic this differences by applying different concentrations of cholinergic agonists. This did not affect the timing of the bursts and the coordinating pattern. We know that the coordinating neurons are not affected by these experimental conditions. Therefore, we hypothesize that the EPSP integration in ComInt1 differs under different excitation levels. For these experiments we recorded ComInt1 intracellularly, bath applied different concentrations of cholinergic agonists, and analyzed its physiological parameters.

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Cross-modal modulation of spiking patterns in the primary somatosensory cortex of Brown Norway rats

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Optimal behavior relies on the ability to integrate environmental features that address multiple senses. A long-standing assumption has been that the integration of sensory information from different modalities takes place solely in higher association brain areas, but recent findings suggest that multisensory processing occurs already in primary sensory cortices. Phase reset of spontaneous oscillatory activity as well as the modulation of induced oscillations have been identified as mechanisms underlying cross-modal processing. They use the direct monosynaptic projections between sensory cortices as an anatomical substrate. However, the contribution of the rate and timing of neuronal firing to multisensory integration is largely unknown. Here, we assess the impact of cross-modal stimulation (i.e. simultaneous light flash and whisker deflection) on the spiking patterns in the supragranular, granular, and infragranular layer of the primary somatosensory cortex (S1) of anesthetized Brown Norway rats by performing extracellular recordings *in vivo*. Spikes from individual neurons were detected offline after high pass-pass filtering the data (>400 Hz) and clustered according to the similarity of their shapes. Cross-modal stimulation strongly modulated the temporal organization of spiking activity in all S1 layers. Furthermore, neurons in S1 showed an enhanced phase-locking to simultaneously recorded network oscillations during cross-modal conditions. Our results suggest that visuo-tactile stimulation modulates the rate and timing of neuronal activity in S1 and thereby might represent a cellular mechanism of multisensory integration.

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Cyclic nucleotide oscillations in the circadian pacemaker of the Madeira cockroach *Rhyparobia maderae*

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The neuropeptide pigment-dispersing-factor (PDF) is the most important coupling factor which signals in *Drosophila melanogaster* via activation of adenylyl cyclase, similar to its functional orthologue vasoactive intestinal polypeptide in mammals. Besides controlling circadian locomotor activity, PDF mediates circadian rhythms of the visual system and is involved in entrainment to different photoperiods. We examined whether PDF daytime-dependently elevates cAMP levels also in the Madeira cockroach *Rhyparobia maderae* and whether cAMP mimicks PDF-dependent phase shifts of locomotor activity rhythms. To determine time windows of PDF release, we searched for rhythms in concentrations of cAMP and its functional opponent cGMP in the accessory medulla (AME), the insect circadian pacemaker controlling locomotor activity rhythms and in other optic lobe neuropiles, as major input- and output areas of the circadian clock. Animals taken from 12/12 light-dark cycles as well as cockroaches reared one day under constant darkness (DD1) or two days under constant darkness (DD2) were taken into account. Enzyme linked immunosorbent assays (ELISAs) detected PDF-dependent rises of cAMP in optic lobes and daytime-dependent oscillations of cAMP- and cGMP baselines. Furthermore, cyclic nucleotide oscillations were affected when animals were transferred form light dark cycles to constant conditions. Interestingly cAMP- but not cGMP-dependent phase shifts of locomotor activity onsets in running-wheel assays resembled PDF-dependent phase shifts. Thus, we followed that PDF is released at dusk and dawn causing phase delays and phase advances, respectively, via cAMP-, but not cGMP-rises. [Supported by DFG grant to STE531/21-1 MS]

Different spatial and temporal activation patterns in the perirhinal and entorhinal cortex in response to afferent stimulation of the neocortex and amygdala

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The (para)hippocampal network is involved in memory formation, object recognition, sensory representation and spatial orientation. Two parahippocampal areas, the perirhinal cortex (PER) and entorhinal cortex (EC), receive input from several cortical and subcortical areas, including the insular cortex and the amygdala. Voltage sensitive dye imaging was used to determine the spatiotemporal activation patterns in the PER and EC in response to electrical stimulation of the insular cortex, to mimic neocortical input, and the lateral amygdala. Experiments were performed in horizontal mouse brain slices in the presence of 1 µM bicuculline to slightly reduce inhibition. Neocortical stimulation induced synaptically evoked activity in the PER and subsequently, this activity gradually propagated more caudally into the lateral EC (LEC) and finally activated the medial EC (MEC) after 40 - 50 ms. Stimulation of the amygdala first activated the deep layers of the PER and LEC; afterwards the population activity propagated towards the superficial layers of PER and LEC and into the MEC. The temporal aspect of the amygdalar activation pattern was intensity-dependent. At low stimulus intensities (50 µA), the deep layers of the PER and EC were activated 150-200 ms after stimulation of the amygdala, while high stimulus intensities (500 µA) induced a shorter latency response after 25-50 ms. Moreover, low intensity stimulation (50 - 100 µA) of the amygdala failed to activate the PER and EC in an all or none fashion in 25 - 50% of the cases. In response to high-intensity stimuli (500 µA), failures were no longer observed.
The data shows new insights into the spatiotemporal organization of the parahippocampal region in response to neocortical and subcortical inputs, suggesting that both the neocortex and the amygdala activate the same circuitry, although in a different fashion. In the near future, whole-cell patch clamp recordings on neurons in the target areas will be performed, to unravel the network interactions that are responsible for the specific spatiotemporal activation patterns observed in the voltage sensitive dye imaging experiments.

Dissecting the balance between excitation and inhibition in the CA1 and DG regions of the hippocampus

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In a local neuronal network interconnected principal neurons and interneurons maintain a critical balance between excitation (E) and inhibition (I). Deregulation of this E/I-ratio has a profound effect on network function and may ultimately lead to seizure like activity typically seen in epilepsy. We analysed the E/I-ratio in two local microcircuits of the hippocampus in response to afferent stimulation of the Schaffer collaterals (CA1) and the perforant pathway (DG).

Neurons were held under voltage clamp in the whole cell patch configuration. Linear decomposition of the synaptic conductance, evoked by afferent stimulation, allowed characterisation of the relative contribution of excitatory and inhibitory inputs in both regions (Monier et al, 2008).

Analysis of the dynamics showed more rapid rise and decay times for excitatory conductance in both circuits studied. Evoked excitatory input always preceded the inhibitory input by 3.8 ± 1.1 ms in CA1 and by 3.9 ± 0.8 ms (SEM) in DG. In DG granule cells the peak amplitude of the inhibitory conductance is smaller (Ginh) than that of the excitatory conductance (Gexc), while these values were not different in the CA1 region.

The E/I-ratio, defined as the fractional contribution of the inhibitory conductance to the total synaptic conductance over the first 150 ms after the stimulus, was different in both regions: 63% in CA1 versus 54% in DG. These findings suggest that the integration of an afferent stimulus can vary significantly between two microcircuits of the hippocampus.

Further inquiry into the relationship between the intensity of the stimulus and the peak conductance revealed differences in network recruitment. At all stimulus intensities Gexc was higher in the DG microcircuit whereas this preference was not observed in CA1. This suggests that the network in the DG, as studied by this approach, is more prone to relay the afferent stimulus via excitatory connections.

Experiments are underway to determine the influence of the stimulus intensity to network recruitment after repetitive stimulation (5 – 20 Hz) in both microcircuits. Overall, these experiments permit us to dissect the contribution of excitation and inhibition in the overall integration in two of the microcircuits of the hippocampus.

Firing patterns of identified neurons in the medial septum/diagonal band nucleus and their relationship to behaviour and hippocampal network oscillations

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The medial septum/diagonal band (MS/DB) nuclei provide cholinergic, glutamatergic and GABAergic input to the cortex including the hippocampus. The GABAergic component is thought to target exclusively GABAergic neurons in the hippocampus. It is likely that these projections are key coordinators of inter-areal cortical communication. We hypothesise that distinct MS/DB cell types provide behaviour-dependent subcortical modulation of the cortical network via regional and cortical-cell-type selective synaptic targeting, contributing to oscillatory dynamics. To test this hypothesis we record extracellularly the behaviour-contingent firing of single neurons in the MS/DB of awake head-fixed mice then use juxtacellular labelling to identify the recorded cells post hoc and establish their local and distant synaptic projection target areas and the innervated cell types. From a total of 80 extracellularly recorded neurons in the MS/DB, we labelled 14 and recovered 9 with axons projecting to the temporal lobe including the hippocampus and the entorhinal cortex. We have identified parvalbumin-immunoreactive MS/DB neurons that preferentially fired around the peak of hippocampal CA1 theta oscillations during movement and projected to retrohippocampal areas where they formed terminals. Some other neurons fired preferentially at different theta phases and projected to restricted cortical areas. Some cells that fired in rhythmic bursts during quiet wakefulness showed no relationship to another rhythmic oscillatory event known as the hippocampal sharp wave ripple. It remains to be established if these MS/DB neurons target all or only certain types of cortical interneurons.

Functional analysis of the *Rhyparobia maderae* molecular clock

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Circadian rhythms in organisms are based on molecular feedback loops that generate rhythms of approximately 24 hours. While the molecular circadian machinery in the fruitfly *Drosophila melanogaster* is well characterized, other insects are less well investigated. Although the components of the molecular circadian clock are generally conserved in all insects, in detail there are striking differences. The ancestral insect core feedback-loop mechanism of the circadian clock is thought to consist of *period* (*per*), *timeless 1* (*tim1*), *cryptochrome 1* (*cry1*), *cryptochrome 2* (*cry2*), *clock* (*clk*) and *cycle* (*cyc*). During evolution, some species may have lost (like *D. melanogaster cry2*) or duplicated (like *Acyrthosiphon pisum cry2*) single genes. Although circadian clock genes were examined in a variety of insects employing the cloning of single genes or using a transcriptome based approach, comprehensive functional studies of the molecular circadian machinery of a rather basic, unspecialized insect are still lacking.

Based on cDNA sequences from RACE PCR and a newly acquired transcriptome of *R. maderae* brain tissue, we started using RNAi to knock-down circadian genes in this cockroach species. First results of RNAi mediated *cry2* knock-down showed that many animals become arrhythmic a few days after *cry2* dsRNA injection in constant darkness (DD), although a residual rhythm persisted in some animals. In a 12:12 hours light:dark cycle (LD), animals remained rhythmic after *cry2* dsRNA injection, indicating a masking effect of light. After transfer to DD, the *cry2* dsRNA injected animals' activity strongly decreased, in contrast to *gfp* dsRNA control injected and untreated animals. After approximately one week in DD, the activity of *cry2* dsRNA injected animals increased, but was mostly arrhythmic. The effect of *cry2* dsRNA injection lasted for at least 3 weeks. These results suggest that CRY2 is part of the core oscillatory circadian system in *R. maderae*, like in other insects. However, residual rhythms indicate that either RNAi mediated gene knock-down was not complete in some tissues governing locomotor activity, or that residual rhythmicity can be maintained without CRY2.

In another approach we are currently characterizing the receptor of the peptide pigment-dispersing factor (PDFR) in *R. maderae*. PDF is a major mediator of circadian information within the clock network: it has been shown to transmit light input as well as locomotor output information and couples circadian oscillators. Its signal transduction cascade has been shown in other insects to mainly recruit a cAMP pathway. So far, we identified the *R. maderae* PDFR and the full coding sequence has been cloned to be used in a BRET based cAMP reporter system in HEK cells. In future experiments, also single cell imaging employing different pharmacological agents will be used to further characterize the signal transduction cascade of the PDFR. [Supported in part by DFG STE 531/18-1,2 to MS]

Glutamatergic system controls synchronization of spontaneous neuronal activity in the murine neonatal entorhinal cortex

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Synchronized spontaneous neuronal activity is a characteristic feature of the developing brain. Rhythmic network discharges in the neonatal medial entorhinal cortex (mEC) in vitro depend on activation of ionotropic glutamate receptors, but spontaneously active neurons are required for their initiation. Field potential recordings revealed synchronized neuronal activity in the mEC in vivo developmentally earlier than in vitro. We suggested that not only ionotropic receptors, but also other components of the glutamatergic system modulate neuronal activity in the mEC. $Ca2^{+}$ imaging was used to record neuronal activity in neonatal murine brain slices. Two types of spontaneous events were distinguished: global synchronous discharges (synchronous activity) and asynchronously (not synchronized with global discharges) active cells (asynchronous activity). AMPA receptor blockade strongly reduced the frequency of synchronous discharges, while NMDA receptor inhibition was less effective. AMPA and NMDA receptor blockade or activation of group 2/3 metabotropic glutamate receptors (mGluR2/3) completely suppressed synchronous discharges and increased the number of active cells. Blockade of glutamate transporters with DL-TBOA led to NMDA receptor-mediated hyper-synchronization of neuronal activity. Inhibition of NMDA receptors in the presence of DL-TBOA failed to restore synchronous discharges. The latter were partially reestablished only after blockade of mGluR2/3. We conclude that the glutamatergic system can influence neuronal activity via different receptors/mechanisms. As both NMDA and mGluR2/3 receptors have a high affinity for glutamate, changes in extracellular glutamate levels resulting for instance from glutamate transporter malfunction can balance neuronal activity in the mEC, affecting in turn synapse and network formation.

Influence of LFP-independent spikes on in-vivo LFPs

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The intra-cortical local field potential (LFP) reflects a variety of electrophysiological processes including synaptic inputs to neurons and their spiking activity. Recent studies have shown that spikes can influence analysis of LFP signals down to the beta band (i.e. frequencies above 10 Hz). It is important to address this spike contamination to avoid false interpretations of neurophysiological processes contributing to the LFP and potentially erroneous conclusions about the correlations of LFP measures and behaviour.

A challenge in assessing the real extent of this problem in the active brain or slice is the inherent neurophysiological coupling of spiking activity and the LFP: spikes change the LFP via synaptic input, which in turn influences spiking activity. In addition, the change in voltage occurring with every single spike propagates passively through cortical tissue and can influence the measured LFP, even if spikes cannot be detected at the recording electrode. As spiking activity is always present in the background, this influence is inevitable and undetectable.

To investigate the effect on LFP of spiking activity decoupled from any concomitant synaptic effects, we injected weak spike-like current pulses into cortical tissue of an awake behaving monkey and investigated the influence of these LFP-independent ‘spikes’ on LFPs recorded simultaneously from multiple electrodes in the vicinity of the injection site.

We designed a stimulation device to deliver spike-like current pulses with intensities as low as 5 nA. The maximum intensity used in our experiments was 100 nA, which is not known to evoke any cortical network activity. The time profiles of injected currents were based on spike waveforms extracted from 300 Hz high-pass filtered intra-cortical recordings. While recording the LFP (at 25 kHz) on the electrodes not used for stimulation, we alternated periods of no current injection with injections of either ‘spike trains’ (15 randomly distributed spikes over 100 ms) or single ‘spikes’ having different amplitudes. The frequency composition of these periods was compared statistically.

Spike triggered averaging of in-vivo LFPs revealed that natural spikes detected at one electrode of the microelectrode array directly influence the LFP recorded on electrodes at a distance of 1-2 mm. Using our stimulation set-up, we were able to reproduce this effect qualitatively, confirming that the setup is suitable for investigating spike-LFP cross-talk in the active brain of a behaving monkey.

If spikes were detectable at recording electrodes, they contaminated in-vivo LFPs down to 0 Hz. Therefore, detectable spikes must be removed before analysis of LFP. Importantly, simple low-pass filtering is not sufficient. If spikes were not detectable, they still directly contaminated in-vivo LFPs recorded at these electrodes down to approximately 100 Hz. If spiking activity is locked to a behavioural event (e.g. a trained movement), contamination due to undetectable spikes can extend below 100 Hz. These findings obviously do not exclude the existence of genuine non-spiking synaptic oscillations. However, as spiking activity is always present in the background, undetectable spikes will contribute even to low frequency components of LFPs, but the relative contribution of genuine synaptic activity and spike contamination to the LFP signal needs to be examined further.

Intra- and Inter-segmental Coordination of Rhythmic Motor Activity in the Walking System of the Stick Insect

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In stick insects it is known that locomotion is based on the interaction between Central Pattern Generators (CPGs), peripheral sensory input and central commands transferred through the connective nerves to the thoracic ganglia[1]. Proper phase coupling between CPG modules is essential for generating coordinated and behaviorally relevant motor output and numerous studies have highlighted the importance of sensory input in CPG coordination[2–4]. Nevertheless, information regarding possible central coordinating mechanisms remains highly elusive.

Hence, we used the deafferented central nervous system of the stick insect *Carausius morosus* as a model to investigate centrally mediated interactions between CPGs that control the Coxa-Trochanter joint (CTr) of the animal. The CTr-joint CPG output was assessed by recording the motoneurons that innervate the *depressor trochanteris* leg muscle of the animal. Rhythmic motor activity in leg MN pools was induced by bath application of the muscarinic receptor agonist Pilocarpine[5], so that by subsequent phase analysis of the evoked rhythmicity we could detect possible systematic coupling interactions between nerve motor outputs. The coordination of rhythmically active MNs was analyzed within each isolated and deafferented thoracic ganglion (intra-segmental) and between the three thoracic ganglia (inter-segmental) in the isolated and deafferented thoracic nerve cord.

Thereupon, our results revealed weak coupling between the activities of contralateral depressor MNs in all three thoracic ganglia. In the isolated meso-thoracic ganglion there was mainly a preference for in-phase activity, while in the isolated meta-thoracic ganglion out-of-phase activity was more frequently observed. Interestingly, rhythmic motor outputs of contralateral depressor MNs remained synchronized over longer periods when the meso- and meta-thoracic ganglia were interconnected. In this last case, the preferred phase difference between left and right rhythmic motor activities in the meta-thoracic ganglion notably varied, indicating an effect of inter-segmental information exchange on intra-segmental coordination.

Furthermore, sharp electrode recordings of meso-thoracic depressor MNs are currently performed in combination with extracellular recordings of contralateral depressor MN activity to elucidate the neural basis of coordinating mechanisms at the cellular level. With regard to the observed coupling between the activities of contralateral depressor MNs three different, still not mutually exclusive, mechanisms may exist: a) Coupling of contralateral MN pools via INs (that are not integral elements of the CPG network), b) Influence of each ipsilateral CPG on the MN pool located on the contralateral side and c) Interaction between contralateral CPGs (via commissural INs), which drive the activity of ipsilateral MN pools. Preliminary data so far bias in favor of the third hypothesis.

All in all, our results substantiate the existence of weak centrally mediated intra- and inter-segmental coupling between hemi-segmental CPGs that control the ipsilateral CTr-joint MN pools in the stick insect thoracic nerve cord.

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Intracellular calcium responses to the neurotransmitter GABA in circadian pacemaker neurons of the Madeira cockroach *Rhyparobia maderae*

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The accessory medulla (AME) is a self-sustained, synchronized network of circadian oscillators that coordinates daily physiological and behavioral rhythms in the cockroach *Rhyparobia maderae*. However, not much is known about the role of neurotransmitters in the input pathway to the circadian pacemakers. So far, only the GABA-ergic distal tract which connects the noduli of the AME with the medulla and possibly also the lamina is the best candidate for a light entrainment pathway to the cockroach clock (Reischig and Stengl, 1996). Also, injection experiments combined with behavioral analysis revealed light-like phase response curves for GABA, consistent with a role of GABA in photic entrainment of the clock (Petri et al., 2002). Moreover, extracellular recordings from the AME in vitro and in vivo revealed that AME neurons are grouped into ensembles of synchronized pacemakers via GABAergic and neuropeptidergic interactions (Schneider and Stengl 2005, 2006, 2007). To analyze possible input signals of GABA into the circadian clock, we performed Fura-2-dependent calcium imaging with primary cell cultures of the adult AME to measure GABA-dependent changes in intracellular calcium levels. Application of GABA for 60s evoked three different types of response patterns. Response type I cells expressed dose-dependent decreases in the intracellular calcium concentration following GABA application. In response type II cells GABA application doses-dependently blocked the spontaneous activity of these apparently bursting neurons. In contrast, response type III cells showed GABA-induced calcium increases. Preliminary pharmacological experiments hint that circadian pacemaker neurons express $GABA_A$ and $GABA_B$ receptors allowing for excitatory and inhibitory effects of GABA in the circadian pacemaker center.

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Intrinsic coupling modes in the ferret cortex at different levels of isoflurane

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Cortical interareal functional connectivity spontaneously fluctuates over time. In higher mammals this is expressed in alternating 'states', which can be viewed as intrinsically generated coupling modes of neuronal populations in the absence of external stimulation. Such instantaneous functional network-changes are thought to variably route the neuronal information introduced via the sensory systems. Previous studies have shown that local and cross-areal synchronisation of neuronal (population-) activity vary such that action potentials (spikes) and local field potentials (LFP) are conditionally more or less phase-locked, resp..

We recorded the spontaneous cortical activity using a custom-made 64-channel subdural electrode array (µECoG) adapted to the anaesthetised ferret's left parieto-temporo-occipital cortex (N=8). The recording sites had a diameter of 250µm and were equally spaced at 1.5mm distance. LFP-like data were filtered between 0.1 Hz and 357 Hz, digitised and down-sampled to 1.4 kHz. We systematically varied the concentration of ventilated isoflurane between 0.4% and 1.6%. MAC values were recorded and at each level the concentration was kept constant for 20 minutes to allow settling to a new network state. The last four minutes of each epoch were chosen for analysis. Additionally, visual and auditory stimulations were carried out at various isoflurane levels. All recordings were performed in a sound- and light-attenuated chamber. Data analysis was performed with Matlab, partly using the 'fieldtrip'-toolbox.

We evaluated the dynamics of cross-electrode intra- and cross-frequency coupling and found distinct effects of the isoflurane concentration on the average coupling-distance and -strength for different frequency bands. Additionally, the temporal stability of certain network states over time changed as a function of the depth of anaesthesia. Though all animals went through comparable connectivity-'states' throughout the course of the isoflurane level-increase /-decrease, the respective concentrations differed between animals.

Our results suggest that for better cross-animal comparisons of neuronal data collected in an acute experiment, not the level of anaesthetics should be equal across animals but rather the network-state This could lead to reduced inter-subject variability in experimental data.

Intrinsic Electrophysiological Properties of Locus Coeruleus Neurons

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The locus coeruleus (LC) is a nucleus bilaterally located in the dorso-rostral pons of the brainstem inheriting around 50% of all noradrenergic neurons in the brain. The majority of all forebrain areas receive direct input from the LC thereby constituting the main source for the neurotransmitter noradrenaline (NA) in the brain (J. Psychopharmacol. 2013, 27(8):659-93). Its widespread efferent and afferent projections implicate a contribution to various distinct physiological functions. Consequently, a variety of neurological disorders, such as the impairment of arousal and sleep-wake cycles, narcolepsy, and attention-deficit-syndrome are associated with the degeneration or deregulation of the LC's NA system (Brain Res Rev. 2003, 42(1):33-84). Early stages of Parkinson's disease (PD) and Alzheimer's disease (AD) are associated with neurodegeneration of the LC (J. Psychopharmacol. 2013, 27(8):659-93). Furthermore, recent work already linked the LC to the regulation of energy homeostasis since inhibition of LC neurons leads to obesity, presumably by a deregulation of energy expenditure in brown adipose tissue (BAT). BAT develops a white-adipose tissue-like phenotype and specific gene expression is altered. Most importantly, the inhibition of the LC leads to lower levels of uncoupling protein 1 (UCP-1) gene expression, which is responsible for heat dissipation from the mitochondria (Cell Metab. 2013, 18(3):445-55). In order to unravel distinct physiological roles of the LC, various animal models and techniques were used to analyze different aspects of the physiology of LC neurons over the last three decades.

Like dopaminergic neurons in the substantia nigra pars compacta (SNpc), neurons in the LC show different firing patterns (Annu. Rev. Neurosci. 2005, 28:403-50). However, in brain slices they only exhibit pacemaker firing. In DA SNpc neurons, this pacemaking is likely to contribute to degeneration in the development of PD. Comparably, very recent studies link pacemaking in LC neurons to elevated mitochondrial oxidative stress (Nat. Neurosci. 2014, 17(6):832-40). Therefore, pacemaking of LC neurons might also contribute to a characteristically early onset of neurodegeneration of the LC in the development of PD and AD.

Our long-term aims are a) to better understand the intrinsic electrophysiological properties and the underlying biophysical mechanisms, and b) how these properties are modulated under changing physiological and pathophysiological conditions. In this respect we focus on the role of LC neurons in the regulation of energy homeostasis and glucose metabolism. Here, we present a detailed analysis of the intrinsic electrophysiological properties of LC neurons that is based on perforated patch clamp recordings in acute mouse brain slices.

Investigating the molecular clock of the carpenter ant *C. floridanus*: genes, neurons and behavior

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As social insects, ants belonging to the species *Camponotus floridanus* are confronted with a number of challenges that need the right timing. These challenges range from collection of nectar, to brood care, to mating. To time such behavior, living beings employ an endogenous clock. As the rhythmic behaviors and mechanisms of the endogenous clock of *C. floridanus* have not been studied very well yet, we are striving to characterize the anatomical and molecular properties that constitute the base of the clock. We also investigate patterns of behavior related to the clock, with a focus on locomotor behavior. Here we report our first results.

Living at different latitudes: the role of *Drosophila* l-LNv in setting the phase of the evening activity peak.

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The genus Drosophila contains over 2000 species that populate very different environments, from the equator to frigid zones. To survive in these diverse areas they have evolved specific behaviors to avoid being exposed to midday heats at lower latitudes or morning chill at higher ones. Moreover, differences in locomotor activity seem to correlate with specific features of their circadian clock neuronal network (Bahn et al., 2009; Kauranen et al., 2012; Hermann et al., 2013).
Species of lower latitudes show a bimodal activity pattern, with a morning and an evening activity peak, and express the circadian photoreceptor CRY and the neuropeptide PDF (involved in the output of the clock) in both subsets of ventral Lateral Neurons (small and large LNv). On the other hand species of higher latitudes are mainly active in the second part of the day and show a reduced (if any) PDF expression in the small LNv (s-LNv) and no CRY expression in the large LNv (l-LNv).
We wondered whether differential CRY and PDF expression in the l-LNv of higher latitudes species compared to lower latitudes ones is directly responsible for their ability to better adjust to very long days and cooler temperatures, conditions they would be exposed to in a natural environment.
We used D. melanogaster and manipulated CRY and PDF expression in the brain to simulate clock neurons properties of higher latitudes species. Interestingly doing this we could provoke a behavior typical of Northern Drosophila species in a species adapted to low latitudes environments. We could also show that the l-LNv have a prominent role in setting the phase of the evening activity peak at the right time of the day.

Local neuronal phase coupling in monkey's area V4 is modulated by selective attention as if only the attended stimulus is present

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Processing of the entire visual information acquired by the eyes would easily exceed the processing capacity of the visual system. Thus, behaviorally relevant information is processed preferentially in depth, while currently irrelevant information is often suppressed. The need for such selective processing of visual information arises in part from the anatomical organization of the visual cortex. One of its fundamental properties is the convergence of afferent inputs that results in increasing receptive-field (RF) sizes of neurons along the visual processing pathways. Therefore, neurons of downstream areas receive often input originating from multiple, independent visual stimuli, which compete for processing. Selective attention is thought to resolve this conflict in favor of the behaviorally relevant, attended stimulus. Several studies showed that neuronal response strength as measured by the firing rate which encodes for specific stimulus features behaved "as if" only the attended stimulus was present, whereas the influence of the non-attended stimulus was largely suppressed. An intermediate response strength between those implied by the two different stimuli was observed when attention was directed outside the RFs, i.e. the conflict between multiple stimuli within the RF was not resolved (Moran & Desimone 1985, Science 229: 782-784; Treue & Maunsell 1996, Nature 382: 539-541). The neuronal mechanism which governs such selective stimulus processing is still not known. However, dynamic properties of the activity patterns of neuronal local populations have been suggested to modulate effective connectivity and hence functional network configuration and signal routing as required for changing the mode of operation of a neural network for processing different stimuli. This raises the question whether such dynamic activity patterns that differ for the processing of different stimuli behave "as if" only the attended stimulus is processed.

To this end, we took microelectrode recordings in area V4 of two monkeys (M. mulatta) which performed a demanding attention task. The task required the animals to focus covert attention on one of four stimuli, two of which were located within the same V4 RF. To estimate the phase coupling between local spiking activity and local population activity (LFP) of closely spaced electrodes we calculated the Pairwise Phase Consistency (Vinck et al. 2010, NeuroImage, 51: 112-122).

We found that not only the stimulus encoding firing rates but also the phase coupling between local spiking activity and LFP is modulated by selective attention "as if" only the attended stimulus is present within the RF. In contrast to the results for the firing rate we observed the lowest phase coupling when attention was directed out of the RF, despite of the presence of the preferred stimulus within the RF. Since phase coupling has been proposed to determine the strength of local interactions as well as the impact of the local population's output signals at distant sites, the present results suggest that it may serve as part of a mechanism for attention-dependent selective stimulus processing and signal routing.

Modulation of firing patterns in midbrain dopaminergic neurons

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The midbrain dopaminergic (DA) neurons in the substantia nigra (SN) and ventral tegmental area (VTA) are an essential part of the reward/hedonic system. Therefore, we are interested in the metabolic modulation of the intrinsic electrophysiological properties of these neurons (Cell Metab. 2011; 13:720-728 and Nat Neurosci. 2013; 16(8):1042-8). For this purpose we require a solid baseline from which the modulatory actions can be studied.

In vivo studies, which combined electrophysiological recordings with behavioral test, revealed that DA neurons possess three firing patterns that are correlated with the prediction and detection of rewards: (1) a single spiking pattern, (2) a burst firing pattern and (3) a hyperpolarized state in which the cells remain silent. In contrast, *in vitro* DA neurons have frequently been described to produce a characteristic, highly regular pacemaker firing pattern (Brain Res Rev. 2008; 58(2), 314-21). Notably, this pacemaker firing persists even in complete synaptic isolation. However, in the SN of rodent brain slice preparations a subpopulation of DA neurons was identified that did not show pacemaker firing, but an irregular firing pattern (J Physiol. 2010; 588(Pt 10), 1719-35). It was recently demonstrated that the proportion of irregularly, non-pacemaking DA neurons increases during ageing (J Neurosci. 2014; 34(28): 9310-8).

To analyze the cellular mechanisms that lead to this irregular firing pattern, we performed perforated patch clamp recordings on acute brain slices preparations of adult mice. Here, we found that the small conductance calcium-dependent potassium (SK) current was significantly decreased in non-pacemaking DA neurons. Additionally, the amplitude of the hyperpolarization activated (H) current is decreased compared to pacemaker DA neurons. However, single pharmacological blockage or reduction of either the SK current or the H current in DA neurons with regular pacemaking did not lead to an irregular firing pattern.

Currently, we are defining the exact role of the aforementioned currents in the generation of pacemaker patterns in DA neurons. Therefore, we simultaneously reduce the amplitude of the SK current and the H current in DA neurons with regular pacemaking. The experimental data will be used to build computational models to verify whether the observed changes in intrinsic currents are sufficient to explain the different firing patterns recorded in DA neurons.

Molecular and Functional Characterization of Lateral Horn neurons in *Drosophila melanogaster*

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In Drosophila melanogaster, the first olfactory processing centre is the antennal lobe which is consisted of multiple glomeruli. Each glomerulus is an integration of synapses between multiple olfactory sensory neurons, multi-glomerular local interneurons and uni- as well as multi-glomerular projection neurons. Each individual olfactory stimulus is encoded by the interplay and combined activity of different chemical synapses as well as the electrical synapses in multiple glomeruli. But how the information from multiple glomeruli integrates and elicits the appropriate behavior in the animal is poorly understood. The olfactory glomeruli send their projections to two higher centres in the insect brain, i.e mushroom body calyx and lateral horn, which are considered as third order neuronal cluster in the fly brain. Neurons of the higher olfactory centres integrate signals detected by different chemosensory neurons and elicit the odorant-induced behavioral responses. The mushroom body has been thoroughly studied for their role in associative learning and memory. On the other hand, the lateral horn which receives the majority of glomerular projections in a stereotyped manner is poorly explored but is thought to play an important role in mediating innate odor-guided responses (Heimbeck et al.2001). However, to date relatively little is known about the morphology and function of lateral horn neurons (LHNs). In the current study, we aim at characterizinge the molecular architecture of different LHNs and try to make a comparative map of their arborization pattern in the lateral horn in terms of their different neurotransmitter and neuromodulator identity. This will be followed by functional imaging of these neurons to a set of attractive and repulsive odors. These together will help us to investigate the mechanisms underlying coding and processing of odorant valence by these higher-order neurons.

Long-range synchronization by intermingled delta and theta oscillations.

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Long-range synchrony between distant brain regions is proposed to integrate information which is processed by local microcircuits. Theta (6-9 Hz) oscillations are a prime example of a slow activity pattern that entrains neuronal firing and synchronizes fast gamma oscillations (30-100 Hz) in distributed neuronal networks. However, theta activity, particularly in the hippocampus, emerges predominantly during movement and is less expressed during freezing or immobility. The question how cross-regional coupling might be achieved during periods of weak theta activity such as during immobile phases has remained unanswered. Here we show, using multi-site local field potential recordings from the dorsal CA1, the medial prefrontal cortex, and the ventral tegmental area in awake mice, that theta rhythms generated during mobile phases alternate with highly synchronous delta oscillations (2-5 Hz) emerging during immobile states of the animal. Both rhythmic activity patterns support long-range synchronization of neuronal population activity, interact through phase coupling, and modulate local gamma rhythms. Thus, high power theta and delta oscillations complement each other in the control of neuronal activity in distant brain regions during mobile and immobile phases, respectively, and thereby contribute to information processing in distributed neuronal networks in a behavior-dependent manner.

Movement detection systems are generally colour-blind, the startle response circuitry in goldfish is not.

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Movement detection systems are generally insensitive to colour information due to the fact that sensitivity to movement is maximal in the case of colour-blindness. In goldfish, this has been shown for the optomotor response. But directionally selective movement detection is also of high importance for triggering the teleost short latency startle response to a visual stimulus. The startle response is mediated by the Mauthner cell associated reticulospinal network. Here we use intracellular in vivo recording techniques to investigate the responsiveness of the goldfish Mauthner system and to prove its potential for decision-making. We find that, nevertheless, visual information of different wavelength is forwarded to the Mauthner cell.

The C-start is an escape response to suddenly approaching predators in most teleosts. Therefore, this reaction highly depends on movement detection. The two Mauthner cells form the centre of the Mauthner cell associated C-start network. They integrate incoming sensory information and then they decide to launch a startle response or not. If one of the two Mauthner cells is activated, a startle response will be executed. Both behavioural and electrophysiological findings show that information of a visual image regarding motion and direction is forwarded to the Mauthner cell indicating the significance of movement detection for the startle response. Here, we asked if light of a specific wavelength is exclusively used for triggering the escape response as shown for the optomotor response. We used blue, green and red light for visual stimulation and simultaneously measured the resulting PSPs at the level of the Mauthner cell. We find that light of all experimental wavelengths causes PSPs at the level of the Mauthner cell. Furthermore, both response delay and amplitude of PSPs did not differ significantly among different wavelengths, indicating that all wavelengths were equally effective in stimulating the Mauthner cell. The responsiveness of the Mauthner cell to light of different wavelengths shows that the Mauthner system receives more visual information for decision-making than, for instance, the optomotor response circuitry. We suggest this reflects the necessity of plastic decisions by the startle response circuitry, which seems to be not given for the optomotor response system.

New anatomical insight into the circadian clock network of *Drosophila melanogaster* using Flybow

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The endogenous clock of *Drosophila melanogaster* consists of about 150 neurons, which share the machinery to produce self-sustained circadian rhythms in the absence of so-called *Zeitgebers*. The molecular mechanisms and the key genes and proteins that are crucial for circadian clock function (*e.g. period, timeless, clock, cycle*) have been extensively studied and are well known. The neuronal anatomy and projection patterns composed by the different clock neuron subgroups are also sufficiently described, but due to the limitations of antibody-stainings and GAL4 driver lines, we still lack detailed anatomical information on a single-cell level.
Therefore, we use the Flybow system (Hadjieconomou *et al.*), which combines the GAL4/UAS binary system with an inducible modified Flp-*FRT* system, to provide temporal and spatial control over the stochastic expression of one of four fluorescence proteins to visualize the cell morphology of single neurons within the clock network. With this approach we want to obtain new neuroanatomical data on the single-cell level of the fly's circadian clock.

Optimal feature integration in critical, balanced networks

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Recent experimental and theoretical work established the hypothesis that cortical neurons operate close to a critical state which describes a phase transition from chaotic to ordered dynamics (e.g. [1-3]). This state is suggested to optimize several aspects of information processing (e.g. [4-6]). However, this link between criticality and cortical computation is mainly based on abstract theoretical measures while concrete examples for criticality being beneficial for active, realistic neuronal computation in the brain are virtually non-existent.

In our study we focus on visual feature integration as a prototypical and prominent example of cortical computation. We consider a network of integrate-and-fire (IAF) neurons with balanced excitation and inhibition [7] which integrates and detects contours of aligned line segments in a visual stimulus. The network consists of orientation hypercolumns with biologically plausible connectivity and serves as a model for part of an early visual area (e.g. V1 or V2).

In dependence on synaptic coupling strength, the network undergoes a transition from subcritical dynamics, over a critical state, to a highly synchronized regime. With intermediate coupling strength, the network settles into a state of irregular spiking activity with intermittent avalanches of spikes propagating over multiple cortical columns. Preferentially, those large events include columns processing the line segments forming the contour.

To quantify the network's computational capabilities, we consider a task where the contour has to be detected on the left or right part of the visual field [8]. Trial-averaged rates were increased by 13% in the hemifield presented with the contour, which is consistent with electrophysiological data from early visual areas. ROC analysis based on firing rate distributions reveals contour detection performances are maximized (with about 60% detection rate, and 50% chance level) near the critical state. In contrast to rates, synchronized events allow for near perfect detection: When spiking activity is averaged over each hemifield and fed into two IAF fire neurons which act as coincidence detectors, we find maximum contour detection rates of 99.9% around the critical point.

In short, we show that for different measures, contour detection performance is always maximized near or at the critical state. In particular, spontaneous synchronization contains far more information about the presence of a target in a stimulus than coding schemes based on trial-averaged firing rates. At the same time, our paradigm provides a unifying account for stylized features of cortical dynamics (i.e. high variability) and contour integration (i.e. high performance and robustness to noise) known from psychophysical and electrophysiological studies.

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References:

Optogenetic dissection of cellular interactions in the prefrontal cortex of neonatal mice

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The emergence of mnemonic abilities seems to require the directed oscillatory coupling within neonatal prefrontal-hippocampal networks. Hippocampal theta bursts drive the generation of discontinuous oscillatory activity in the prelimbic subdivision (PL) of the prefrontal cortex via direct axonal projections. While external glutamatergic inputs to prelimbic pyramidal neurons and interneurons were shown to selectively correlate with theta and low gamma network oscillations, respectively, a causal investigation of the cellular mechanisms underlying these activity patterns is still missing. To fill this knowledge gap, we optogenetically drove prelimbic pyramidal neurons and interneurons, while simultaneously recording the local field potential (LFP) in the PL of neonatal (postnatal day 8-10) mice *in vivo*. Layer-specific expression of light-sensitive opsins in pyramidal cells of the neonatal PL was achieved by transfection of DNA constructs into a subpopulation of progenitor cells in the ventricular zone at embryonic days (E) 12.5 and 14.5 using in utero electroporation. To target prelimbic interneurons, neurons of the ganglionic eminence were in utero gene transfected at E12.5. To reliably elicit single action potentials in prelimbic neurons expressing channelrhodopsin-2 E123T T159C (ChR2 ET/TC), the parameters of light stimulation were optimized during patch-clamp recordings *in vitro*. The effects of light-induced spiking in distinct neuronal subpopulations on the network activity in different frequency bands was assessed by recording the LFP and multiple unit activity in the neonatal PL *in vivo*. Thus, targeting individual populations of neurons in the neonatal PL by in utero gene transfer enables the dissection of their contribution to the generation of distinct patterns of network activity.

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Origin and function of depolarizing afterpotentials in stellate cells in the medial entorhinal cortex

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Supra-threshold responses in neurons of rodent layer II of the medial entorhinal cortex (mEC) represent space. While an animal explores its surroundings, these cells' membrane potentials exhibit dynamics on different time scales that include slow sub-threshold voltage ramps, theta-band oscillations and supra-threshold burst episodes with inter-spike-intervals as short as a few milliseconds. These high-frequency burst episodes within a theta oscillation have received little attention but may add substantial information to encode spatial relations.

The majority of principal neurons in layer II of the mEC are stellate cells, which display sag responses to injected hyperpolarizing step currents and resonances to oscillatory current injections. These cells also show a prominent depolarizing afterpotential (DAP) following an action potential. This additional depolarization lasts for 3-5 milliseconds and might facilitate burst episodes in stellate cells. To better understand this phenomenon, the intrinsic factors creating the DAP and its functional role were investigated.

We performed whole-cell current-clamp recordings from layer II cells in acute brain slices of the mEC of mature rats and gerbils near physiological temperatures (~35°C). In layer II, pyramidal and stellate cells differed in their response properties. Only stellate cells showed a characteristic sag response and a membrane resonance in the theta range (5 – 10 Hz). In pyramidal neurons, the DAP responses were smaller or completely absent. In stellate cells, DAPs were completely blocked by TTX and Cd^{2+}, and both the DAP width and magnitude could be influenced by hyperpolarization of the membrane potential prior to the action potential.

To elucidate the functional consequences of the DAP, its influence on facilitation of action potential generation was assayed by a paired pulse paradigm. The intensity of the first current pulse was chosen to elicit an action potential, whereas the second pulse was of various intensities so as to shift its responses from sub- to supra-threshold. Larger DAPs correlate with facilitated spiking which is consistent with enhanced responses. These data and preliminary modeling indicate that an action potential might elicit dendritic currents that flow back into the soma thereby generating the DAP. In support of this hypothesis, imaging revealed that somatic current injections evoked even distal (200 µm) dendritic calcium influx

By further data analysis, electrophysiology, calcium imaging and computational modeling, we aim at a complete understanding of the mechanisms underlying this prominent activity pattern and construct a comprehensive and consistent biophysical stellate cell model.

Photo-periodic diapause in *Drosophila ezoana*: what does it tell about circadian involvement in photo-periodic time measurement?

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Diapause is an insect physiological state characterized by low metabolic activity and arrest of growth/reproduction to survive harsh winter conditions. Diapause is induced by shortening day-length (photo-period) during summer to winter transition. A time measuring system is thus thought to underlie diapause regulation; commonly termed as photoperiodic clock. Circadian rhythm of activity-rest behaviour respond to changes in photo-period and therefore, circadian rhythms are believed to underlie the photoperiodic clock. Many species of Drosophila inhabiting the temperate zone exhibit robust adult diapause which is characterised by reproductive arrest. We studied the diapause, activity-rest rhythm and the expression of clock protein PDP in the clock neurons in *Drosphila ezoana* under the light/dark (LD) cycles with varying (a) photo-period and (b) period of LD cycle, to test the circadian involvement in diapause incidence. The diapause incidence was found to be correlated with circadian phenotype suggesting the circadian involvement in diapause regulation. However, in studies under LD cycles with 24-hour and non-24 hour period, the diapause was found to be primarily determined by the night-length (duration of dark phase of LD cycle), pointing towards the absence of circadian involvement. We hope this behavioural analysis will help us further investigate the neuronal and genetic basis of diapause.

Rapid temperature adaptation of the life-saving escape C-start is mediated by NMDA receptors at the level of the Mauthner cells in larval zebrafish

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In ectothermic animals most physiological processes and behavioral performance are highly affected by temperature fluctuations. In escape responses, rapid compensation of latency and speed after cooling would be required, particularly if predators are endotherm. However, temperature compensation in muscle and the central nervous system is slow. Here we show that larval zebrafish, unusually, can rapidly and completely compensate cooling effects in their life- saving escape behavior. Furthermore we narrowed down sites that contribute to this rapid adaptation. We simultaneously monitored the short latency escape (C-start) and the neurons (Mauthner (M)-cells) initiating this response at 28.5°C (raising temperature) and after cooling to 19.5°C, respectively. The M-cells are a pair of huge neurons located in the hindbrain of most fish and amphibians. We used simultaneous live-cell calcium imaging of both M-cells and monitored the C-start escape behavior over 10 h (100 min at 28.5°C and 500 min at 19.5°C) in semi-fixed larvae. The latency of an acoustically elicited C-start shows a rapid adaptation after an initial cooling-induced slowdown. Throughout the experiments the M-cell $[Ca^{2+}]$-signal correlates nicely with latency: it is lower in the beginning of the cold phase (ΔF/F: 22 %) and is fully compensated at the end of the experiments (ΔF/F: 40 %). The surprising correspondence of the M-cell $[Ca^{2+}]$-signal (which occurs long after the behavioral response) and latency suggests a common mechanism of adaptation. To identify this mechanism, we repeated the experiments using antidromic stimulation. Hence, instead of activating the sensory input, we evoked an action potential directly in both M-cells, still causing calcium-influxes in both M-cell somata. Again we found an adaptation after an initial decrease of the $[Ca^{2+}]$-signal, suggesting that the adaptation cannot solely be due to mechanisms at the input level. The NMDA receptor antagonist MK-801, however, blocked adaptation even in antidromic-stimulated larvae, suggesting that plasticity (mediated by dendritic NMDA receptors in the M-cells) induces the rapid and complete temperature compensation. Hence, we not only demonstrated a concurrent and remarkable rapid adaptation of a behavior and its underlying neural network to an environmental perturbation, but also suggest that this adaptation depends on NMDA receptors.

Representation of visual information in the goldfish Mauthner cell

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The representation of information is traditionally studied close to sensory organ. Only a few studies address how information is represented close to the motor output. Here we used intracellular in vivo recording techniques to study how the goldfish Mauthner cell represents visual information. We show that the Mauthner cell represents visual information in a much richer way than thought previously.

In teleost fish, the Mauthner cell associated reticulospinal network mediates life-saving escape C-starts. The Mauthner cells play a key role in driving these escapes and integrate incoming sensory information to decide whether to launch a C-start or not. Visual information is very rapidly relayed to the Mauthner cell with no time for tectal processing. We first show that it is possible to record PSPs to natural stimuli that also can be tested in behavioural experiments. Surprisingly, the Mauthner cell not only responds to looming stimuli (that signal a rapidly approaching predator), but also represents large field motion and even the movement of small objects down to 1 deg. PSPs differed both in amplitude and shape, depending on movement direction. Thus, the input structure would, in principle, allow the cell to adjust its responses to movement direction of both large and small field motion. This is surprising because the goldfish Mauthner cell is thought to only mediate escape responses, a purpose for which the representation of small field translatory movement would not seem to be required. Our results show that a much richer representation of visual information is available at the level of the goldfish Mauthner cell. Its behavioural role in the goldfish is presently unknown, but we suggest that the rich representation enables far broader plasticity in the Mauthner-associated decisions than thought previously.

Responses to emotional images: relation to the respiratory cycle

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Inspiration and expiration significantly affects the performance of different parts of human brain through reflectory influences from the upper airways. One of the most important neurophysiological mechanisms responsible for this effect is generation of electric activity, synchronous to inhale-exhale phases, by the limbic system. We assumed the existence of a connection between the breathing phases and the functioning of different systems of the brain, such as sensory system. The aim of this study was to analyse the dependence of several characteristics of event-related potentials in the human brain on the phases of respiratory cycle.

26 volunteers (17-18 years old, N=30) participated in the study. Their task was to follow the appearance of emotional images (International Affective Picture System – IAPS, the mean (SD) arousal = 6.41 (0.6)) on the screen. During this process, event-related potentials (ERP) were recorded. We analysed average signal amplitude in the time intervals 40-80, 80-120, 120-220, 220-300, 300-400 and 400-700 ms after the beginning of the exposure to the stimulus. The phases of respiration were recorded using equipment containing a heat-sensitive element, located near the nostrils of the participants. The event-related potentials accumulated in accordance with the phase of respiration in which the stimulus was given. We compared the characteristics of ERP accumulated during the inhalation and exhalation phases.

We demonstrated that the event-related potentials recorded during the inhalation and exhalation did not differ by forms of oscillations, but slightly varied in amplitude. In particular, the ERP amplitude of the latent period between 80 and 120 ms varied in the left temporal and both occipital leads in different phases of the respiratory cycle. ERP of the latent period between 400 and 700 ms had statistically significant differences in different phases of the respiratory cycle in comparison to the amplitude recorded only in the right frontal leads (Fp2, F4, F8). In all leads the amplitude of ERP was greater in the expiratory phase in comparison to the one in the phase of inspiration.

This data indicate the possible connection between the respiration process and the visual sensory system performance.

Small world and rich club dynamics of the single unit motor network and their correlation to oscillations

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Techniques to record many neurons in parallel have led to analyses of neuronal populations and their interactions, which are known to be the basis of neuronal processing. So far, it is impossible to record from the whole brain network with cellular resolution. For a reasonably large dataset, we therefore focused on the parietal-frontal network for grasp movements of macaque monkey (area AIP, F5, and M1), which is well known for sensorimotor integration and movement generation.
Three monkeys performed a delayed grasping task where they had to grasp a handle with one of two grip types. All animals were implanted with 128 to 192 electrodes (2 arrays with 32 electrodes per area) in AIP, F5, and M1. We recorded and analyzed single neuron activity during 12 recording sessions (monkey S: 6; monkey Z: 3, and monkey M: 3) and isolated on average 88 single units per dataset. To reveal functional connectivity between all single neurons we calculated crosscorrelograms of all possible pairs. We applied a combination of a cluster based permutation test with false discovery rate correction to get reliable significant functional connectivity for each whole dataset giving us functional networks. Based on the functional networks we were able to analyze their structure. For this purpose we applied graph theoretical analysis. In agreement with our hypothesis functional networks of the motor system were strongly organized in contrast to random networks. All analyzed networks were scale free and hierarchically structured and showed small world and rich club characteristics. In addition to that we found strong presents of beta (20Hz) and low frequent (4Hz) oscillations. Surprisingly, single units, which were oscillating in one of the two frequencies, were significantly in accordance with highly connected units and the rich club.
Oscillations are intensively discussed as an important mechanism to coordinate communication in the brain. Nevertheless it is basically unknown how the structure of functional communication or connectivity is coordinated by oscillations. Our results give a rough picture of the role of oscillators for functional communication. The idea arising from our findings is a coordinating role for oscillators to establish a hierarchical, scale free, small world, and rich club communication structure, which support both segregated/specialized and distributed/integrated information processing in a fast and effective manner.

Spontaneous field potential transients in the rat dentate gyrus

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The idea that the dentate gyrus (DG) functions as a gate has been around for more then 50 years, in part suggested by its wall-like position between the entorhinal cortex and the hippocampus (HC) as well as its lamellar organization. In acute ventral rat brain slices, the DG shows a small amplitude spontaneous aphasic rhythm of ~0.7 - 1 Hz (Colgin et al. 2004) termed dentate waves (DWs) whose function and underlying network mechanisms remain mostly unsolved. Using extracellular recording techniques combined with pharmacology and laminar profiles, we show that a DG intrinsic network drives this rhythm that is crucially depending on excitatory synaptic transmission and phasic inhibition. We identified a pacemaking mechanism responsible for its timing that involves HCN channels, T-type Ca2+ channels and the persistent Na current. Synaptic transmission at giant mossy fiber boutons also proves to be crucial to maintain this rhythm. Interestingly, there is a correlation between the appearance of DWs and sharp wave-ripples in area CA3 of the HC, which have been shown to be crucial for memory consolidation.

Synchronization of singing and breathing in crickets: Neuronal coupling between two rhythm-generating networks

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Male crickets sing by opening and closing their front wings and thereby produce a short sound pulse during each closing movement. In the calling song of *Gryllus bimaculatus*, chirps consisting of 3–5 sound pulses (also called syllables) are perseveringly repeated at a rate of 2–3 Hz, with a syllable repetition rate of 20-30 Hz within the chirps. The neuronal network that generates the singing pattern is located in the anterior abdominal neuromers of the central nervous system (Schöneich & Hedwig 2011, 2012), which strongly supports the idea that the stridulatory motor pattern may have evolved from abdominal ventilatory motor pattern. Singing activity considerably increases the rate of ventilatory abdominal pumping and a neuronal linkage between the stridulatory and respiratory motor pattern generators is indicated by a strict coupling of abdominal pumping cycles to the chirp rhythm (Huber 1960; Kutsch 1969; Paripovic et al. 1996).

Here we investigated the neuronal coupling between breathing and chirping rhythm by intracellular recordings of ventilatory and stridulatory interneurons in the abdominal ganglia of fictively singing crickets. The two motor patterns were monitored by extracellular recordings from transverse abdominal muscles and the mesothoracic wing nerve N3A, respectively. Although, ventilatory pumping of the abdomen usually occurred in phase with the chirp cycles of the singing activity, from time to time the two rhythms became uncoupled and resynchronised within a few cycles. At the beginning of a singing episode the two motor rhythms also needed several cycles before their typical phase relationship was established. Intracellular recordings of ventilatory interneurons revealed that they receive additional EPSPs during singing, which strictly reflected the syllable pattern of the song. We never recorded any synaptic input reflecting the ventilatory rhythm in the dendrites of singing interneurons.

Based on our data we conclude that the ventilatory and stridulatory rhythm originate from discrete pattern generating networks that are synchronized by subsequent synaptic coupling, rather than a syllable pattern generator for singing which is rhythmically modulated by the slower ventilatory rhythm. Coupling of the two rhythms is established by sub-threshold excitatory synaptic inputs from the singing to the ventilatory pattern generating network which entrain the somewhat slower ventilatory rhythm. (This study was supported by the BBSRC and the Isaac Newton Trust)

The Archerfish Predictive Start: A Setup to Couple Behavior and Electrophysiology

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The archerfish predictive start is a rapid multi-alternative decision made during hunting and based on visual information. Using virtual stimuli would not only push our ability to dissect the processing of visual information but is also required to analyze the decision-making networks. However, employing screens meets with a serious difficulty: In their fine-tuned responses the fish expect a reward being presented at the water surface at just the right time and in precisely the right position. If there is no systematic relation between what is shown on the screen and time and place of reward, the fish quickly will lose any motivation. Using screens in behavioral and training experiments therefore requires a setup that couples the motion-stimulus and the place and time of the reward.

In their decisions archerfish select turns to the later landing point of ballistically falling prey based on visual information sampled in about 30 ms. Conventional computer monitors have time intervals from 13 to 17 ms between consecutive pictures (60 to 75 Hz) and are therefore too slow for the visual stimulation of animals with such an impressive temporal resolution. To overcome this problem, we used recent LED illuminated computer monitors primarily developed for active shutter 3D systems (to enable stereoscopic vision in computer games and movies) with frame rates between 120 and 144 Hz (7 to 8.33 ms between consecutive pictures).

Archerfish exhibited reliably predictive starts when faced with movies showing fast (1.7 to 2.5 m/s) moving objects on 27'' 120 Hz computer monitors mounted horizontally above the tank.

To couple time and place of reward with the stimuli shown, we distributed four custom built automatic feeders directly above the water surface. Four different short movies – each showing an object with a trajectory that would cause ballistically falling prey to impact at the position of the feeder – were shown in random order. A trigger signal in one of the six audio channels of the movie ensured that the proper feeder opens at the appropriate time while presenting a particular movie. A further signal on another channel triggered a high speed camera (500 fps). This arrangement successfully kept the fish motivated — even after 7000 movie presentations, the archerfish still reliably showed high-performance predictive starts.

The stimuli tested in the behavioral experiments were also employed to stimulate fish visually while recording reticulospinal neurons intracellularly.

Compared to the visual stimulation with real objects, the presentation of movies on fast computer displays combined with automatic feeders has three considerable advantages: (1) Stimuli can be artificially modified. (2) The method enables averaging procedures during the electrophysiological experiments and presenting exactly those stimuli shown in behavioral experiments. (3) It is easy to systematically decouple the location and timing of the reward and the visual stimulus thus enabling mimics of physically impossible trajectories.

The energy demand during different hippocampal network activity states *in vitro*.

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The brain has a high energy expenditure and critically depends on oxidative phosphorylation in mitochondria. However, little is known about energy expenditure at specific activity states in local neuronal networks. We addressed this issue in subfield CA3 of acute slices from the mouse hippocampus (BL6 mice, p26-30) under well-defined metabolic boundary conditions.

We combined recordings of local field potential (LFP) and interstitial partial oxygen pressure (pO2) during different specific activity states, namely (i) gamma oscillations (GAM, 30-100 Hz) as induced by cholinergic receptor activation, (ii) recurrent sharp wave-ripple complexes (SPW-Rs), (iii) spontaneous asynchronous network activity (SPON), (iv) isoflurane-induced anesthesia (ISO), and (v) the absence of action potentials as a reference state of low network activity. Oxygen consumption rates were estimated by measuring pO2 depth profiles with high spatial resolution using a Clark-type oxygen microsensor and applying a mathematical model that accounts for diffusion and activity-dependent consumption of oxygen.

We find that (1) the oxygen consumption rate was highest during gamma oscillations by a factor of 2.14 on average. (2) Oxygen consumption rates during sharp wave-ripple complexes, spontaneous asynchronous network activity and anesthesia did not significantly differ. (3) For gamma oscillations and sharp wave-ripple complexes, there was a positive correlation between oxygen consumption rates and the oscillation amplitude in the local field potential. (4) Overall, oxygen consumption rates showed a positive correlation to action potential generation during the different activity states.

We conclude that in local hippocampal networks the oxygen consumption rate may vary by a factor of two during specific activity states and anesthesia, which depends on the degree of both postsynaptic potentials and action potentials.

The fitness of *Drosophila melanogaster* is influenced by the neuronal circadian clock

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Organisms are exposed to circadian rhythms of about 24 hours created by the rotations of the earth around its own axis. Those organisms that are adapted best to these rhythms have a fitness advantage.
To synchronize to circadian rhythms organisms need Zeitgebers like light or temperature. *Drosophila melanogaster* is a well known model organism and the properties of the endogenous clock is quite well characterized. Wild-type CantonS (CS) flies have a 24.44 h ± 0.02 h free running period under constant conditions and they should have a fitness advantage over clock mutant flies under natural conditions and T-Cycles of 24 hours.
To test whether the circadian clock of *Drosophila melanogaster* implies a real fitness advantage we applied a competition assay where we used wild-type CS as well as the well described clock mutants per^S, per^L and per^{01}. per^S flies have a free running period of t = 19 h and per^L flies of t = 29 h whereas the per^{01} flies are arrhythmic under constant conditions due to the lack of a functional clock.
The first competition assays were performed in a T-Cycle of 24 hours (LD12:12) to simulate natural circadian rhythmicity and to create a T-Cycle which fits best to the wild-type CS flies. For each pairing of flies (CS x per^S, CS x per^L, CS x per^{01}) 10 independent populations were founded and each population was tested with the Trikinetics locomotor activity monitors for genotype distribution. Those competition assays were not only performed in the laboratory but also outdoors under natural-like conditions, where the flies are exposed to natural illumination, humidity and temperature changes.
In a further set of the experiment the same competition pairings were used but the populations were kept under T-Cycles of 19 hours, 29 hours or constant light (LL) creating T-Cycles that fit best to the free running period of the mutants per^S, per^L and per^{01}, respectively. Due to the fact that per^{01} flies are arrhythmic under constant conditions we created an environment where wild-type CS flies become arrhythmic as well, namely LL. Under those conditions the *per* mutants should have a fitness advantage over the wild-type CS.
In the course of the competition experiments flies of different genotypes mate and produce heterozygote female offspring as the *per* gene is located on the X-chromosome. Therefore we also recorded the locomotor activity of heterozygote female flies under LD and constant conditions.

The impact of timed electrical depolarization of specific clock neurons on *Drosophila melanogaster's* circadian clock

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The circadian clock of Drosophila melanogaster consists of about 75 clock neurons per brain hemisphere, which can be divided and subclustered according to their anatomical position inside the brain. These are the dorsal neurons (DN1a/p,DN2, DN3) and the lateral neurons (s-LNv, l-LNv, LNd, LPN). Even though the function and importance of most clock cell clusters are known, the interplay between these clock neurons is not clear yet. The fact that the interaction of neurons is not rigid, but rather flexible, shows the complexity of the interaction.
To unravel the interaction between the clock neuron clusters, we depolarized certain clock neuron subgroups using thermogenetic tools (*dTrpA)*, which allows a timed electrical manipulation of the clock. The effect on locomotor activity rhythms and clock protein levels were quantified and compared between different GAL4-lines, covering different clock neuron subgroups. These phase shifts in locomotor behaviour were plotted in a phase response curve (PRC). As indicator of the state of the circadian clock, PERIOD protein levels were quantified by means of immunoreactivity in each cell cluster separately.
Our results showed that an interaction of many clock neurons is necessary to phase shift the molecular clock after a timed depolarization. This becomes apparent on PER protein levels as well as on the behavioural level.

The neuronal microcircuits of the medial septum

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The medial septum (MS) is an important modulator of hippocampal function. It is a key regulator of theta frequency oscillations (4Hz-12Hz), supports hippocampal dependent learning and adapts the firing of hippocampal neurons to the running velocity. Three main neuronal types of the medial septum are GABAergic, glutamatergic and cholinergic neurons. They are characterized electrophysiologically and by the expression of molecular markers. The connectivity between different neuronal types is not completely understood.

This study was undertaken to (i) characterize GABAergic, cholinergic and glutamatergic neurons in the mouse MS making use of PV-cre, ChAT-cre and VGluT2-cre lines with medial septal virus injection of pAAV-ChR2-eYFP and (ii) to resolve the synaptic connectivity, within the medial septal network, using optogenetic stimulation of PV, VGluT2 and ChAT neurons, accordingly.

Electrophysiological properties of fluorescently labeled PV (n=22); VGluT2 (n=30) and ChAT (n=24) neurons were determined in acute slices in the medial septum in three different mouse lines after pAAV2.1-EF1a-double floxed ChR2-EYFP-WPR (H134R) virus injection. PV neurons were distinguished from VGluT2 and ChAT neurons by fast action potential (AP) kinetics and high firing frequencies. Opposite to PV neurons, ChAT neurons fired broad APs at lower frequencies. VGluT2 neurons displayed intermediate AP properties and had a higher input resistance than PV neurons.

To predict group membership of other non-fluorescent labeled neurons inside MS, we created discriminant functions. The cross-validation procedure confirmed that 77% of fluorescent neurons were correctly classified with the used discriminant functions.

Optogenetic activation of MS PV-positive neurons elicited IPSCs in nonfluorescent neurons (success rate 54 %) with an average latency of (3.4±0.25 ms, n=62) in all classified neuronal subgroups, which were sensitive to GABAA and GABAB receptor antagonists. Thus, PV neurons made local, medial septal inhibitory synaptic contacts. In VGluT2-cre mice optogenetic stimulation evoked EPSPs and EPSP followed by IPSP in 87% of neurons recorded (identified by the absence of eYFP fluorescence) with an average latency of 3.9±0.17 ms (n=87). Predominant amount (n=43) of connected neurons were classified as VGluT2 neurons. Additionally, 12 and 1 were classified as PV and ChAT neurons. Light-evoked responses were blocked in presence of NBQX and D-AP5, NMDA and AMPA-receptor antagonists. In summary, VGluT2 neurons inside the MS provided excitatory drive to both MS PV and ChAT neurons. VGluT2 neurons also exhibited a strong recurrent connectivity. In ChAT-cre mice single light stimulation (15 ms) or 2 s train stimulation at 10 Hz did not evoke synaptic response in the standard experimental conditions (n=29). With increasing stimulation duration to 6 s slow depolarization was observed in 7 neurons consistent with volume transduction.

Taken together, we confirmed that channelrhodopsin expressing GABAergic, glutamatergic and cholinergic neurons could be directly activated by blue light (473 nm) stimulation. In addition, we resolved the synaptic interconnectivity of PV, VGluT2 and ChAT neurons in the MS.

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Theta oscillations and neuronal firing along the septo-temporal axis of the epileptic hippocampal formation

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Mesio-temporal lobe epilepsy (MTLE) is manifested in the hippocampal formation as recurrent epileptic activity (EA) and as histopathological changes.
In contrast to the anatomical changes that are persistent, EA is interspersed by periods of apparently normal brain activity. Those EA-free periods comprise oscillatory activity well studied in the healthy hippocampus. We searched for changes of network properties and of oscillatory dynamics that may render the hippocampal formation susceptible to seizures.

We investigated EA-free activity using the intrahippocampal kainate mouse model of MTLE, in which a unilateral injection of kainate into the dorsal dentate gyrus (DG) induces histopathological changes resembling those in human MTLE. The intensity of these changes such as cell loss and granule cell dispersion decays towards the temporal pole of the hippocampus.
Recently, we showed that 1) the power of epileptic activity in the DG is unequally distributed along the septo-temporal axis (Häussler et al., 2012) and 2) the coupling in theta rhythm between the histologically normal medial entorhinal cortex (MEC) and the sclerotic DG was phase-shifted in epileptic animals (Froriep et al., 2012).
To what extent this shift in MEC-DG coupling is present along the dorso-ventral axis with decreasing sclerosis is unclear. Furthermore it is unknown whether these changes are accompanied by a corresponding change of neuronal spike activity and the spectral composition of the theta-band oscillation.

To answer these questions we implanted wire electrodes and custom-made multi-site silicon probes that allow simultaneous recordings of both, local filed potential (LFP) rhythms and multi-unit activity (MUA) at several levels of the entorhinal-hippocampal (EC-HC) loop in freely behaving mice.
We found that the LFP theta rhythm can be observed at all tested septo-temporal locations in the DG of epileptic animals. Moreover, the dominant oscillation frequency was comparable to that in healthy animals and did not change across the septo-temporal axis. In line with this, we found that spiking of neurons in the non-sclerotic but strongly epileptic zone of all investigated substructures of the EC-HC loop was phase locked to the theta oscillation and at the same theta-phase as in healthy mice.
Since the intra-MEC and intra-hippocampal phase coupling of spike activity to the theta rhythm is comparable to controls, the misalignment of theta oscillations between MEC and HC entails a shift in spike timing between these regions. This could lead to a temporal mismatch of activity in neuronal subpopulations and pathological plasticity eventually rendering the hippocampal network susceptible to seizures.

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Three-dimensional Ca^{2+} imaging of neuronal network activity in the neonatal mouse visual cortex *in vivo*

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Cortical network activity during neonatal life is known to be organized in a highly discontinuous manner with bursts of activity separated by relatively long periods of silence. In the mouse visual cortex during the first postnatal week, such bursts are mainly triggered by sensory-independent retinal waves. Using electrophysiological and imaging techniques these network events were previously characterized as spindle bursts and Ca^{2+} waves, respectively. Currently, however, the spatial structure of these events is incompletely understood. Using two-photon Ca^{2+} imaging along a three-dimensional, spiral-shaped scan trajectory, we here analyzed Ca^{2+} cluster events in the upper cortical plate (CP) of mice expressing the genetically-encoded Ca^{2+} indicator GCaMP3. Using large scan volumes (500×500×200 µm³) we found that cortical network events typically exhibit a distinct spatial confinement in the horizontal plane, but mostly involve the entire depth of the upper CP. Reducing the scan volume to 100×100×100 µm³ allowed us to record network activity at single-cell resolution with a mean cell coverage of about 80%. Our data suggest that neighboring cells have a high probability of co-activation during cortical Ca^{2+} cluster events. In conclusion, three-dimensional Ca^{2+} imaging represents a valuable tool to investigate both the spatial structure and the cellular mechanisms of early network activity in the neonatal mammalian brain.

Towards recording standards of neuronal avalanches in brain slices

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The prefrontal cortex (PFC) plays an emergent role in complex cognitive functions. Indeed, our understanding of network activity which is one of the underlying preconditions for proper function of PFC is still poorly understood. During the last decade a network activity mode called “neuronal avalanches” has achieved increasing attention. Neuronal avalanches are a phenomenon seen in vitro and in vivo recordings of neural network activity where the dynamics show power-law behavior and scale invariance. Avalanches are commonly seen in complex systems and are highly suggestive for criticality. This means they operate in ranges, where the probability of high or low activity is distributed in a power-law manner with neither subliminal nor exalted network activity behavior. In this way they reach maximized information transmission, variability and network stability. In neurophysiology, these dynamics have been firstly described for the temporal-spatial distribution of local field potentials in multi-electrode recordings.
We study the dynamics of local field potentials in acute PFC slices of mice recorded by perforated multi-electrode arrays (pMEA). After 1 hour of incubation slices were transferred to the pMEA consisting of 32 electrodes, with a 30 µm electrode diameter and a 100 µm inter-electrode spacing. To keep the slices in position and in good contact with the electrodes a negative pressure was applied to the pMEA from below either manually via syringe or via controlled vacuum pump. When the slice covered all the perforations a seal was formed between the slice and the pMEA electrodes throughout the recording. Local field potentials were induced by a combined application of L-Dopa (60 µM) and NMDA (5 µM) and continuously recorded at various pressure values between 15 and 90 mbar for up to 60 min. For each electrode LFPs were identified when the peak was larger than three times of SD of the electrode noise. We quantified the avalanche size by measuring the number of activated electrodes or by summation of field potential amplitudes within a given time frame.
Our data show that the recorded activity strongly depend on the applied pressure. Therefore a standardization of the recording methods are absolutely necessary for a proper evaluation of neuronal avalanches recorded with pMEAs. Further investigations are necessary to analyze the underlying mechanisms for our observation. The pressure dependence might result from activation of mechanosensitive channels, increased extracellular glutamate concentrations released by damaged cells or higher resolution due to better tissue to electrode contacts.

Visual information transfer to the Mauthner cell without tectal processing

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The Mauthner cell, a huge identified neuron in the hindbrain of teleost fish, has been shown to be essential not only for life-saving escapes but also for rapid visually mediated decision-making. Here we test the hypothesis that the Mauthner cell receives visual information that is heavily pre-processed in the tectum. Such massive pre-processing would not only seem to be required given the complexity of some of the visual decisions but would also account for the surprisingly large delay between a visual stimulus and a Mauthner cell PSP. Taking vision specific processing into account, the delay still is surprisingly large in comparison to acoustic stimulation. So a complex processing network with many synapses would seem to be likely. Here we show that the idea of massive tectal pre-processing is wrong. By comparing the delays that occur when either the optic nerve or the tectum opticum are selectively stimulated we constrain tectal delay to about 1 ms. This surprising finding, in turn, suggests that the Mauthner cell and its associated network are the site in which much of the decision-relevant processing occurs.
Our experiments were carried out in gold- and archerfish and comprise sensory stimulation of the eye, electrical stimulation of the optic nerve and the tectum as well as intracellular in vivo recording from the Mauthner cell. We measured postsynaptic potentials in the Mauthner cell after electrical stimulation of the tectum opticum within 1 ms, suggesting a monosynaptic connection between the tectum and the Mauthner cell. Stimulation of the optic nerve causes PSPs at the Mauthner cell within less than 4 ms. Thus, there is a maximum time of 3 ms for information processing in the tectum, deducting the conduction delay. In conclusion, we demonstrate a rapid connection from the retina via the tectum opticum to the Mauthner cell. We did not find any evidence for a large upstream tectal decision-making network that pre-process visual information before it is forwarded to the Mauthner cell. This has two major consequences: First, most of the measured delay between a visual stimulus and a Mauthner cell PSP is caused by phototransduction and retinal processing. Second, the Mauthner cell must operate on incoming visual information to decide itself whether to trigger a C-start or not.

Weak Intersegmental Coupling of Rhythmic Motor Activity in the Walking System of the Stick Insect

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Walking movements result from a complex interplay of central pattern generating networks (CPGs), local sensory feedback about movements and forces generated in the legs and coordinating signals from neighboring limbs. In the stick insect, the antagonistic motoneurons of a given leg joint are driven by a CPG that can be activated by the muscarinic acetylcholine receptor agonist pilocarpine (Büschges et al., 1995). Sensory information plays a crucial role in coordinating the different CPGs of a single leg and appears to play a major role in inter-segmental coordination of leg movements as well (Borgmann et al., 2009; Büschges, 2005; Cruse, 1990). However, preliminary evidence suggests intersegmental coordination of CPGs that does not depend on sensory signals (Büschges et al. 1995). In this study we aimed to analyze the coupling of thorax-coxa (ThC) joint CPGs that control pro- and retractor motoneurons (MN) in the meso- and metathoracic ganglia, and the underlying information transfer between both ganglia.

CPG activity was monitored by recording alternating rhythmic activity in protractor and retractor MNs in deafferented thoracic ganglia of the stick insect, Carausius morosus. Rhythmic motor activity was induced by bath application of the muscarinic acetylcholine agonist pilocarpine. Activity of pro- and retractor MN pools was recorded by means of extracellular electrodes from lateral nerves nl2 and nl5. We used a new method for analyzing time series of several rhythmic systems in order to identify periods of coupling of the activity in different ThC-joint CPGs. This method provides measures for coupling strength and preferred phase relations between the different ThC-joint CPGs. Our results clearly indicate the existence of weak central coupling of contralateral mesothoracic ThC-joint CPGs as well as of ipsilateral meso- and metathoracic ThC-joint CPGs of the stick insect.

Intersegmental central coupling of CPGs would require the exchange of information through the connectives. Therefore, we started investigating the neural basis of intersegmental coupling between CPG activities of the mesothoracic and metathoracic ganglion. Extracellular recordings from the meso-meta connective showed phasic activity related to pilocarpine induced protractor MN activity. In most of the recordings single action potential units could be identified in the connective recording by their amplitude. These neurons either showed spiking activity during protractor MN bursts (N = 13) or during retractor MN bursts (N = 11).

In addition, we recorded intracellularly from neurons in the mesothoracic ganglion close to the origin of the meso-meta connective. We recorded from phasically active intersegmental interneurons descending to the metathoracic ganglion (N = 3) and from tonically active intersegmental interneurons ascending from the metathoracic ganglion (N = 6).

These neurons could potentially be involved in a neural pathway for the exchange of information between the different CPG networks. Therefore, our results provide first indications on the existence and nature of coupling between the ipsilateral meso- and metathoracic ThC-joint CPGs of the stick insect.

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Poster Topic

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Adding up the odds - nitric oxide mediates opponent assessment and the decision to flee in crickets

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Our work on crickets has shown that the amine octopamine mediates the aggression promoting effects of physical exertion, winning a fight and resource possession (Stevenson and Rillich, Frontiers in Neuroscience, 2012). By manipulating information exchange during fighting and applying nitridergic drugs we found that the gaseous neuromodulator nitric oxide (NO) is involved in opponent assessment during fighting, and promotes retreat followed by a prolonged period of submissiveness that is characteristic for subordinate animals. Confirming our earlier work, crickets deprived of visual cues (blind), or with lamed mandibles (disarmed) fight untreated crickets with almost unabated ferocity and win chances, whereas blind contestants practically always (96%) beat disarmed opponents. This finding is in full accord with the Cumulative Assessment Model (CAM, Payne, Animal Behav. 1998), which predicts than animals somehow add up the agonistic actions of their opponent's during fighting and flee when the accumulated sum exceeds some critical level. Hence, while blind receives no visual and limited physical cues from disarmed opponents, disarmed accumulates the full brunt of blind's agonistic actions and flees first. The impact of opponent signals on fighting seems to be mediated by NO. For example, treating blind with the NO-donor SNAP reduces the win chances against disarmed from 96% to 50%, while blocking NOS with LNAME in disarmed increases its win chances to 50%. Conform with predictions of the CAM, winners were found to bear a record of their opponent's actions for a short time after winning. For example, although winners are normally hyper-aggressive, they tend rather to retreat from an opponent when when rematched immediately after winning, but not when treated with LNAME. Hence, there is a susceptible period just after winning, that is dependent on NO, during which winners are on the verge of losing and will flee in response to relatively few opponent actions. In line with this idea that crickets add up incoming sensory information for the decision to retreat, winners given a single aversive stimulus (wind puff to the cerci) during the susceptible period are reverted to losers, but are unaffected by the stimulus if given after the critical period. The action of the aversive stimulus depends on NO dependent, since it is ineffective in winners treated with LNAME. We conclude that agonistic signals accumulated from an opponent during fighting lead to activation of the NO-signaling pathway, which induces retreat when a critical level is surpassed. Supported in part by the German Research Council (DFG: FOR 1363, STE 714/4-1).

Alarm pheromone-induced defensive behavior in rats: role of the bed nucleus of the stria terminalis

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The bed nucleus of the stria terminalis (BNST) is a functionally and anatomically heterogeneous structure. There is a lot of evidence that this structure is important for defensive behaviors. In rats, for example, exposure to alarm pheromone induces defensive behaviors and increases c-fos expression in the BNST (Kiyokawa et al., 2005). Goal of the present study is to investigate if pharmacological inactivation of the BNST modulates defensive behavior induced by alarm pheromone.
In a pilot experiment, we established a behavioral paradigm to investigate alarm pheromone-induced defensive behaviors in male Sprague-Dawley rats. Therefore, we used an open field with a glass dish filled with 1 ml either water or alarm pheromone in one corner, and a hiding box in the opposite corner. Alarm pheromone was obtained from the perianal region about 1-2 hours before the experiment took place (cf. Kiyokawa et al., 2006). Our data show that, in comparison to water, the exposure to alarm pheromone increases risk assessment behaviors.
Currently, we are investigating the role of the BNST in alarm pheromone-induced defensive behaviors. Therefore, we used the paradigm established in the pilot experiment and block the BNST temporarily by local microinjections of muscimol. In addition to alarm pheromone, we also expose the animals to fox urine, a predator odor that is believed to be differently processed than pheromones.

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Behavioural and neuronal correlates of visual numerosity representations in the carrion crow, *Corvus corone*

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Many animals possess numerical competence and can discriminate stimuli based on the number of items in a set ('numerosity'). Birds are also known to judge set sizes, but the characteristics of numerosity representations and scaling schemes remains elusive. To probe numerosity representations in corvids in detail, we trained two carrion crows (Corvus corone corone) in a delayed match-to-numerosity task with numerosities from 1 to 5 presented as dot patterns (controlled for visual parameters) on a touch screen. The crows had to respond whenever a test stimulus showed an equal number of dots compared to the sample stimulus.

The crows managed to discriminate numerosities in an approximate way. When performance was plotted as probability with which the crows judged the test numerosity as being equal to the sample numerosity, peak functions emerged. These behavioural performance functions showed all the characteristics of the Weber-Fechner-Law, i.e. their performance shows a clear numerical distance effect (discrimination performance improves with increasing numerical distance) and magnitude effect (greater numerical distances between quantities are required to discriminate larger absolute magnitudes). In addition, the crows behavioral filter functions were most symmetrical when plotted on a logarithmic scale, indicating non-linearly compressed representations. Based on these findings we conclude that crows – like primates and other mammals – use an analog magnitude system.

Next, we recorded single-cell activity from the telencephalic nidopallium caudolaterale (NCL) in behaving crows. Preliminary data indicate numerosity tuned neurons in the NCL. Approximately one third of randomly recorded neurons in the NCL were found to discharge in a tuned fashion as a function of the number of items in the displays.

Compared to mammals and primates in particular, the signatures of the crows' analog magnitude system show striking similarities. This is surprising, given that the endbrain of mammals and birds show remarkable differences and birds did not develop a layered neocortex as highest telencephalic integration center. The capacity of crows to deal with numerosities thus constitutes an example of convergent evolution in the realm of intelligent behaviour.

Blockade of orexin-1 receptors in the ventral tegmental area could attenuate the lateral hypothalamic stimulation-induced potentiation of rewarding properties of morphine

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The orexins (hypocretins) are lateral hypothalamic (LH) neuropeptides that have been implicated in a variety of behaviors ranging from feeding to sleep and arousal. Evidence from animal models suggests a role for orexins in reward processing and drug addiction. In the present study, we investigated the direct effect of orexin antagonist in the ventral tegmental area (VTA) on acquisition and expression of morphine conditioned place preference (CPP) induced by concurrent stimulation of LH. 132 adult male Wistar rats weighing 220-280 g were unilaterally implanted by two separate cannulae into the LH and VTA. The CPP paradigm was done; conditioning score and locomotor activity were recorded by Ethovision software. The animals received SB334867 as a selective orexin-1 receptor antagonist (0.1, 1 and 10 nmol/0.3 µl DMSO) in the VTA, just 5 min prior to intra-LH administration of ineffective dose of carbachol (62.5 nmol/0.5µl saline) and ineffective dose of morphine (1 mg/kg, s.c.) concurrently during conditioning phase (acquisition experiments) or post-conditioning phase (expression experiments). Data showed that the blockade of orexin-1 receptors in the VTA could inhibit the acquisition (development) but not expression of LH stimulation-induced morphine CPP in the rats. Our findings suggest that the orexinergic projections from the LH to the VTA are involved in development of the LH stimulation-induced potentiation of morphine rewarding properties and orexin-1 receptors in the VTA have a substantial role in this phenomenon.

Cage-based automated learning of cognitive tasks for rhesus macaques

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In cognitive neuroscience, the training of animals prior to experimentation can be a demanding, time consuming task and is often not standardized across animals. The latter may lead to different training results with potential confounding effects on the research goal. Here we test if a cage-based and automatized training is feasible, where monkeys learn cognitive tasks in an unsupervised and self-paced fashion.

We developed a cage-based training and testing system (XBI, e**X**perimental **B**ehavioural **I**nstrument) for monkeys (*Macaca mulatta*). The XBI provides a touch screen which lets the animals interact manually and in real-time with the computer-controlled visual tasks displayed on the screen. A reward system provides performance-contingent liquid reinforcement signals. Via custom-written software the XBI can be used to set up a large variety of cognitive tasks. We implemented visually and memory guided reach tasks, visual discrimination, spatial attention, gambling and match-to-sample tasks. In a group of animals, we first trained a touch-hold-release task and then a memory guided centre-out-reach task. We developed an automated training algorithm that uses a staircase procedure to teach monkeys progressively with increasing task complexity. Whenever the performance of the last 50 repetitions succeeded 80% of success, the staircase stepped up one step in difficulty, a drop of performance below 30% resulted in lowering the difficulty one step. Prior to the staircase training, during a habituation phase of two weeks, the monkeys had to find out by themselves that they can gain a reward by touching a blue square that covered most of the screen. The staircase procedure then progressed through the steps 1-15: reducing the size of the square, 16-19: randomizing the position, 20-29: incrementing hold time, 30-31: introduction of release cue, 32-37: decreasing the time for release. After reaching the highest step and either exceeding 80% performance or not stepping back for five days in a row, monkeys were offered a second staircase procedure which trained towards a memory guided centre-out reach task commonly used in sensorimotor neuroscience. In such a task, the monkey has to touch a bright square, memorize the position of a second briefly flashed peripheral square to then reach to its memorized position after an instructed delay period.

Eight male monkeys (*Macaca mulatta*) were trained between one and eight months, 1.5 hours each workday. Sweetened fruit-flavoured water was used as reinforcer, while monkeys had free access to water outside the training sessions. The average participation of the individual animals scaled from 50 to 350 trials per day, with individual values ranging from zero to several hundred trials per day. Individual monkeys stagnated at different staircase levels, but there were also common training steps of stagnation as, for instance, when memorizing is first introduced. Overall, every animal except one stepped up in the staircase over time with individual learning rates.

In conclusion, automatized training of cognitive tasks in a cage setting is possible with rhesus macaques, allowing for pre-training the monkeys in a way that is less time consuming and better standardized. The phases of stagnation in our training procedure across animals provide a hint on difficult learning steps. Knowledge of these critical steps and individual learning speeds will be used to optimize future training

programs.

Cell-type specific modulation of behaviorally relevant and distracting stimuli by dopamine D1 receptors in primate prefrontal cortex

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The prefrontal cortex (PFC) is a crucial brain structure for maintaining behaviorally relevant (target) information in working memory. We have shown previously that PFC neurons can restore target memories following interference and therefore do not need to suppress distracting stimuli. However, the neuronal mechanisms that allow the PFC to bypass distractors are not known. Here, we addressed the local circuit dynamics of target and distractor representations in PFC and investigated their regulation by dopamine, a modulatory neurotransmitter that controls many prefrontal executive functions including working memory. We recorded single-unit activity from the PFC of two rhesus monkeys trained to resist distracting stimuli in a delayed-match-to-numerosity task and simultaneously applied the dopamine D1 receptor (D1R) antagonist SCH23390 to the vicinity of the cells using micro-iontophoresis.
Our preliminary analyses show that narrow-spiking neurons (putative interneurons) initially encoded target numerosities earlier than broad-spiking neurons (putative pyramidal neurons). Following presentation of the distractor numerosity, however, target information was recovered first in pyramidal neurons. Pyramidal neurons also did not switch their preferred numerosity in the course of the trial, while interneurons were tuned to different target numerosities before and after distractor presentation. These results suggest that the recovery of target information emanates primarily from pyramidal neurons. Blocking D1R with SCH23390 enhanced the representation of both target and distractor numerosities in interneurons, showing that both stimuli were encoded similarly in these cells without particular weighting. In contrast, SCH23390 significantly enhanced coding of the target numerosity in pyramidal neurons, but had no effect on distractor stimuli. This suggests that prefrontal dopamine has cell-type specific effects on the representation of behaviorally relevant and irrelevant information.
Our results highlight the contribution of different cell types in PFC to maintaining behaviorally relevant stimuli in memory and resisting interference.

Cholinergic involvement in attentional modulation in area MT of primate visual cortex

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Attentional modulation of sensory responses in extrastriate visual cortex of primates is characterized by gain changes, i.e. multiplicative changes of firing rates for a given combination of stimuli in the receptive field and the attentional state of the animal. It has been suggested that the cholinergic system mediates such changes in local neuronal responsivity. We examined this in visual area MT, where neurons exhibit strong tuning for the direction of motion in their receptive field and firing rates are reliably enhanced by allocation of spatial and feature-based attention. We recorded from single cells in awake, behaving rhesus monkeys while they performed a spatial attention task. Two random dot patterns were displayed on a computer screen, one inside the recorded cell's receptive field and the other in the opposite visual field. In each trial, the monkey was cued to attend to one of the stimuli and to report a change in motion direction in the cued stimulus only. Firing rates were compared when the moving stimulus in the receptive field was attended vs. unattended to quantify gain changes by spatial attention.

During recordings, we used pressure injections to pharmacologically manipulate cholinergic receptors in the direct vicinity of the recording electrode. Selective antagonists were used to block the muscarinic or nicotinic cholinergic receptor subtype, or acetylcholine was injected to increase extracellular concentration. One substance was used per recording session. Injection occurred in ~10 minute blocks. The pattern of attentional modulation during injection was compared to baseline blocks recorded before injection. Control injections with saline solution did not affect firing rate.

As expected, spatial attention to the receptive field of the recorded neuron significantly increased firing rates. First results for the muscarinic antagonist, scopolamine, revealed a significant drop in firing rate during drug application. Across our population of recorded cells scopolamine also substantially reduced the attentional modulation of responses. However, since this change in attentional modulation is not significant, we currently cannot confirm the hypothesis of an involvement of muscarinic receptors in mediating response modulations in area MT by spatial attention. This is in contrast to previous work [Herrero et al., Nature 2008], which showed a clear and significant contribution of the muscarinic receptor subtype to attentional enhancement in macaque V1.

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Cortical drive of low-frequency oscillations in the human nucleus accumbens during action selection

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The nucleus accumbens is thought to contribute to the selection of goal-directed actions by integrating behaviourally relevant information from multiple regions including prefrontal cortex (Goto & Grace, 2008; Grace, 2000). Afferent input to the nucleus accumbens is likely integrated in a task-dependent manner (Calhoon & O'Donnell, 2013; Gruber, Hussain, & O'Donnell, 2009; Stuber, 2013). Studies in rodents suggest that this task-dependent integration may be regulated by inter-area oscillatory coupling (Berke, Okatan, Skurski, & Eichenbaum, 2004; Gruber et al., 2009; van der Meer & Redish, 2011). During instrumental behaviour, local field potentials in the rat nucleus accumbens and prefrontal cortex are coupled at delta frequencies (Gruber et al., 2009), possibly mediating suppression of afferent input from other areas and thereby supporting cortical control (Calhoon & O'Donnell, 2013). Here, we demonstrate a similar low-frequency coupling in humans, both at rest and in a decision-making task. We recorded local field potentials (LFP) in the nucleus accumbens of six epilepsy patients who underwent therapeutic implantation of deep brain stimulation electrodes. We found significant coherence between LFP and surface EEG at delta- and low theta frequencies. The direction of this coupling, indexed by Granger causality, indicated a consistent cortical drive of the nucleus accumbens during action selection in a decision-making task. Our results suggest that low-frequency cortico-accumbens coupling represents a highly conserved regulatory mechanism for action selection.

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Cross-modal working memory neurons in the corvid nidopallium caudo-laterale

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Many of the complex, learned behaviors depend on arbitrary associations between stimuli. Several behavioural studies demonstrate the adaptive importance of crossmodal association learning in corvids, for instance, when recognizing group members. However, neuronal representations of cross-modal, cross-temporal association (notably sight and sound) remain unknown in birds. Similar to the primate prefrontal cortex, the avian nidopallium caudo-laterale (NCL) is essential for working memory representations of sensory information and for executive control in general. Here, we present evidence that crow NCL neurons represent associations of auditory and visual stimuli across time.

We trained carrion crows (*Corvus corone*) to associate complex auditory stimuli with visual objects in a computerized procedure. For this, the crow was placed on a perch in front of a touch-screen. To start a trial, the crow had to hold its head within the range of a light barrier. After that, one of two complex auditory stimuli was played back from a speaker for 1.3 sec (sample phase). Followed by a delay of 1 sec during which the screen remained black and no auditory stimulus was present, a visual match or non-match stimulus was shown on the screen (test phase). To receive a reward from a feeder, the crow was required to peck at the test stimulus whenever it matched the previously presented, associated auditory stimulus.

Next, we recorded single-unit activity from the NCL of behaving carrion crows performing in a cross-modal, cross-temporal association task. Our preliminary data show cells selectively representing the auditory stimulus during the sample phase. In addition, many cells associated the auditory stimulus with the upcoming visual stimulus during the delay phase by selectively increased discharge rates. Association coding was significantly inverted in error trials, thus predicting the crows' behavioral choice.

These results underscore the highly integrative function of the NCL. NCL neurons bind stimuli from different modalities and bridge such associations across time. The strong correlation of neuronal activation during correct and error trials with the upcoming choice underpins the cardinal role of the NCL in executive function.

Dopamine D1 and D2 receptor modulation of numerical rule coding in primate prefrontal cortex neurons

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Numerical competence, the capability to use quantities and numbers, is based on abstract principles, or rules, of how to structure, process and evaluate quantitative information. We have shown previously that single neurons in the PFC encode abstract numerical rules. The cellular mechanisms giving rise to the rule-related neuronal activity are, however, poorly understood. Since the PFC receives strong projections from the dopaminergic midbrain modulating executive functions such as working memory, we hypothesized that dopamine receptors in the PFC are involved in regulating abstract rule coding.
Two rhesus monkeys (Macaca mulatta) were trained to compare numerosities and to switch flexibly between two abstract numerical rules based on a rule cue. The “greater than” rule required the monkeys to release a lever if the first test display showed more dots than the sample display, whereas the “less than” rule required a lever release if the number of items in the test display was smaller compared to the first test display. We recorded single neurons in the lateral PFC while simultaneously applying the dopamine D1 receptor (D1R) agonist SKF81297, the D1R antagonist SCH23390, or the D2 receptor (D2R) agonist quinpirole to the vicinity of the cells using microiontophoresis.
We report that both dopamine D1Rs and D2Rs facilitated rule coding of PFC neurons, albeit by distinct physiological mechanisms: D1R stimulation suppressed neuronal firing while enhancing responses to the preferred rule, an effect mainly mediated by narrow-spiking (putative inhibitory) neurons. D2 receptor stimulation, instead, excited neuronal firing while suppressing responses to the nonpreferred rule primarily via broad-spiking (putative pyramidal) neurons, thus also enhancing neuronal rule coding. Thus, prefrontal dopamine is essential to maintain rule coding in the PFC. These findings highlight complementary modulatory contributions of dopamine receptors to the neuronal circuitry mediating rules and goal-directed behavior.

Effects of acute and chronic administration of L-DOPA on cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm.

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Recent research has shown that pharmacological enhancement of dopaminergic function by acute administration of L-DOPA increases an optimism bias in humans. In the present study, we investigated whether L-DOPA have similar effects in rats. To accomplish this goal, the animals were trained in the ambiguous-cue interpretation (ACI) paradigm, a test allowing measurements of cognitive judgement bias in animals. In this paradigm the rats must press one lever in response to one tone to receive a reward and to press another lever in answer to a different tone to avoid punishment. Cognitive judgement bias is than tested by measuring the pattern of animals‘ responses to a tone of intermediate frequency (ambiguous-cue).
After initial behavioural training, the effects of L-DOPA on cognitive judgment bias in rats were investigated in two consecutive experiments. In the first experiment the animals received single injections of 3 different (2, 4 and 8 mg/kg) doses of L-DOPA and saline in a fully randomised latin square design. In the second experiment the animals received chronic injections of L-DOPA (8 mg/kg) for a period of 2 weeks. Control animals received corresponding injections of physiological saline.
We show for the first time, that pharmacological enhancement of dopaminergic function by administration of dopamine precursor L-DOPA, causes in rats, similar to humans, optimistic judgment bias. Our study proves translational validity of the ambiguous-cue interpretation paradigm.

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Effects of acute and chronic pharmacological manipulations of the 5-HT system on cognitive judgment bias of rats.

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The monoamine serotonin (5-HT) has long been implicated in the modulation of affective and cognitive processing and has been consistently linked to depression, anxiety and negative mood.
In the present study, we investigated the effects of different manipulations of the 5-HT system on the valence of cognitive judgment bias in rats. For this the animals received either injections of escitalopram (selective serotonin reuptake inhibitor) or PCPA (selective and irreversible inhibitor of tryptophan hydroxylase). The effects of drugs were investigated after acute and chronic administration. For the evaluation of cognitive judgment bias in rats we used the ambiguous-cue interpretation (ACI) paradigm. In this paradigm the rats are trained to press one lever in response to one tone to receive a reward and to press another lever in answer to a different tone to avoid punishment. Cognitive judgement bias is than tested by measuring the pattern of animals‘ responses to a tone of intermediate frequency (ambiguous-cue). After initial behavioural training, in the first experiment the animals received single injections of 3 different doses (0.5, 1 and 2mg/kg) of escitalopram and physiological saline, applied in fully randomised latin square design.
In the second experiment the animals were treated for a period of 2 weeks with either escitalopram or PCPA. The drugs were administered once daily (2mg/kg escitalopram and 600mg/kg PCPA). The effects of acute and chronic administration of escitalopram and PCPA were evaluated in the ACI paradigm, performed before, during and after pharmacological treatment.
Results of our study demonstrate no significant effects of neither acute nor chronic treatment with escitalopram. PCPA administration caused positive shift in cognitive judgment bias suggesting that 5-HT depletion may have pro-optimistic effects in rats. The data are discussed in terms of neurochemical action of tested compounds.

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Elucidating projection patterns and postsynaptic partners of intercalated cells in the mouse amygdala

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The amygdala plays a crucial role in the processing of fear and anxiety-related disorders. It is situated in the temporal lobe and consists of several distinct nuclei that receive sensory inputs and project to downstream regions that mediate behavioural responses associated with fear. Whereas the role of glutamatergic projection neurons has been thoroughly investigated, the function of amygdala GABAergic networks is incompletely understood. GABAergic neurons are thought to modulate fear processing via inhibition and disinhibition. Part of the inhibitory network is composed of distinct clusters of small GABAergic neurons, the intercalated cells (ITC), situated along fiber tracts surrounding the basolateral complex. Current evidence suggest that medial paracapsular ITCs (mpITCs) modulate fear processing, as they are active in fear and extinction learning, and their ablation impairs extinction memory retrieval. In addition, recent findings suggest that these cells exhibit heterogenous projection patterns. Therefore, investigation of their anatomical projection patterns and postsynaptic targets may allow a better understanding of fear and extinction memory processes.

Here we started to address two questions: 1) To which intra- and extraamygdaloid regions do mpITCs project in adult mice? 2) To which possible postsynaptic partners in the targeted regions do mpITC axons make contacts?

We address the first question using fluorescent and confocal imaging, and axonal reconstruction of labeled mpITCs in brain slices. In a large set of cells, we obtained qualitative data that show mpITC axonal projection patterns in accordance with published cell types in juvenile animals. We also observed putative new intra-amygdala projection patterns (to the basolateral complex) and extra-amygdala projections to the caudate putamen and along amygdala-striatal transition zone (Astr) to the internal capsule. In a subset of cells, we obtained 3D-reconstructions of axons using the Neurolucida software and determined quantitative parameters such as axonal length and complexity, and the number of presynaptic terminal buttons in specific target regions. For the second question, we used confocal microscopy and immunohistochemical staining for markers of distinct neuron types such as somatostatin-positive cells in the centrolateral (Cel) and centrocapsular (CeC) nuclei, as well as cholinergic cells in the amygdalostriatal transition zone (Astr), to identify possible contacts of mpITCs. Our preliminary findings suggest that mpITCs make contacts with dendrites of somatostatin-positive cells and cholinergic cells in Cel/CeC and Astr, respectively. Together, our results reveal new axonal projection patterns and give first quantitative insights into the wiring and postsynaptic partners of mpITCs in adult animals.

Environmental Enrichment during Adolescence Alters Exploration Strategies of Young Adult Rats

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Exploration, or active investigation which leads an animal to learn about its environment, is a particularly relevant activity during adolescence. For adolescent rats, exploration provides opportunity to experience, learn about, and practice investigating familiar and novel features in an environment. These experiences often lead to increased novelty-seeking and risk-taking behaviors, which are aspects of practiced exploratory behavior, across adolescence. Environmental enrichment (EE) that allows for interaction with objects and conspecifics can enhance brain development, learning and memory, and affect aspects of exploratory behavior, such as novelty seeking. In this study, we investigated how EE during adolescence affects novel location exploration in newly adult rats, and the relationship between these behaviors and neural activation in the basolateral amygdala (BLA). The BLA is implicated in the processing of fear stimuli, and thus its activity is related to novelty seeking during exploration. Between postnatal days (PND) 34-64, Long-Evans rats (n=16) were exposed to EE in cages with ramps, platforms, inanimate objects, and familiar and novel conspecifics for two days followed by a day without EE for a total of 20 EE sessions. Age-matched controls (n=16) were not enriched and experienced a home-cage. Two-trial novel location preference (NLP) testing occurred between PND 66 and 75 (15, 30, 60 min and 24 h delays). On Trial 2 after a particular delay, a familiar object at a novel location was present in the test field. Time in direct contact with and proximity to the object in the novel location was measured, in addition to total object exploration time. Post-hoc analysis of the behavioral results revealed no significant difference in proportion of time spent in contact with the familiar object at a novel location across same-day delays (15, 30, 60 min) or between groups (EE, M=0.67, SEM=0.03; no-EE, M=0.60, SEM=0.02). There was however, a significant difference between groups for the next-day delay (24 h), with enriched animals (M=0.48, SEM=0.05) spending less time at the novel location than no-EE animals (M=0.60, SEM=0.06) This EE by delay effect on novelty investigation was significant, $F(1, 56)=8.00$, $p<.01$. Further, EE animals spent less total time than no-EE controls investigating both objects for same day delays (26 s vs. 35 s, SEMs=3.0), however at the 24 h delay (i.e., the next day) EE rats spent more total time investigating both objects (37 s, SEM=3.2)) than no-EE controls (31 s, SEM=5.5). The day after NLP testing and following 2 hours in the quiet and dark, animals were sacrificed and brain tissue processed to count baseline neuronal activation using c-fos protein as an indicator. In BLA of EE brains, there was a 19% reduction in active neurons relative to controls. Behavioral results indicate a difference in exploration strategies, with EE animals investigating rearrangement of the environment in the time immediately following the change (same-day delays), but spending equal amounts of time, and more time overall, exploring the environment and the objects within it after a longer span of time (next-day delay), while unenriched rats' exploration remains relatively stable across delays. Neural data correlates with behavioral results and suggest the changes in amygdala activation that follow a history of EE contribute to altered exploration strategy through a reduction in the number of active BLA neurons.

Fighting behavior of female field crickets, natural and under pharmacological stimulation

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Female field crickets of the species *Gryllus campestris* and *Gryllus bimaculatus* show agonistic behavior in conflicts over territories just like their male conspecifics. In the field, *G. campestris* females engaged in fights over burrows and expressed all fighting levels known from male fights. *G. bimaculatus* female fights were studied in staged fights in an arena. Differences to male fights lay in the fighting strategy. In contrast to males, females fought more intensely, the smaller they were. They were also able to switch between fighting strategies.
In a further approach, we treated the female crickets with neuroactive substances to learn more about the neurological and hormonal control of their fighting behavior.

Guidance of the Focus of Attention in *Drosophila melanogaster*

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An animal may face different matters of concern simultaneously in different parts of the visual field. To respond appropriately, it needs selective visual attention, a crucial property of the visual system enabling the animal to restrict its response to parts of the visual field. Here we study the dynamics of the shifts of the focus of attention (FoA) during tethered flight. Using the polarity of Drosophila's intended turning response to a front-to-back displacement of two black bars in the left and right visual half-fields we can test, to which bar it attends. We apply two different approaches to investigate the dynamics of the FoA. First we introduce a cue on one side before the test and vary the pause between cue and test to measure how fast the cueing effect wanes. Second we vary the inter-trial-interval between the tests and analyze its effect on the length of chains of consecutive responses with the same polarity. We use the learning and memory mutants *radish*[1] and *fumin*, which display aberrant phenotypes in this paradigm to genetically and pharmacologically explore the neuronal structures involved in selective visual attention.

Human linear visual motion direction discrimination thresholds: Effects of graded deployment of spatial attention

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Attending to one location in the visual field leads to enhanced processing of the stimuli presented at that location, resulting in better performance and reduced reaction time. If it is required to attend to two locations, it is unclear whether spatial attention can be split equally between these locations or even deployed unequally in a graded manner.
In this study we investigated the influence of varying magnitudes of spatial attention on human subjects' performance in discriminating visual motion directions.
An endogenous cue was used to direct subjects' spatial attention to one of two random dot patterns (RDP), centered at 5 deg eccentricity, left and right of a central fixation point. Both RDPs had a diameter of 5 deg and moved at a speed of 8 deg/s within stationary circular apertures. Importantly, we manipulated the amount of spatial attention deployed to the two locations by varying the validity of the endogenous cue (100%, 75% and 50%) between blocks. Subjects were informed about the cue validity in a given block.
After cue presentation, the two RDP stimuli were shown. One contained the task-relevant stimulus (target), a brief coherent motion signal (75 ms), whereas the other RDP contained incoherent linear motion (distractor). The distractor was followed by a static dot pattern (75 ms) while the target stimulus was followed by a random motion mask stimulus. This was to limit the temporal window when information about the stimulus direction could be acquired, preventing subjects from sampling the two stimuli one after the other, rather than dividing their attention between them.
Subjects were asked to indicate the target stimulus location (left or right) and to discriminate the target's motion direction (up or down relative to horizontal) by pressing one of four response buttons on a game pad.
In addition to varying cue validity, the signal strength in the target dot pattern was also varied, resulting in four levels of motion coherence (100%, 80%, 60% and 40%). The experiment was performed in a blockwise full factorial manner, combining all motion coherence conditions with all cue validity conditions.

Using this task design allows us to discriminate true attentional effects from effects of modulating stimulus uncertainty by changing cue validity. As expected, motion coherence had a significant impact on subjects' performance, with discrimination thresholds improving with increasing levels of motion strength. More importantly, subjects' performance was found to improve with increasing cue validity. The two variables, cue validity and motion coherence, both seem to have an independent impact on observers' performance, with no interaction found. Our data indicate that human subjects can voluntarily and simultaneously divide their spatial attention across two stimuli, with different amounts allocated to each stimulus. This graded allocation of attention exerts a graded effect on performance.

Identification and aminergic modulation of brain interneurones with synaptic inputs from antennal mechanoreceptors in the cricket

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Mechanical stimulation of the antennae in crickets can elicit various behaviors, including aggression, that are themselves subject to modulation by the biogenic octopamine. We have identified local and descending interneurons in the cricket brain that respond to mechanical stimulation of the antennae, and found these responses to be modulated in different ways by octopamine. The contra-laterally descending brain interneurones DBNc2-2 and DBNc1-2 (cf. Schöneich, Schildberger, Stevenson, J Comp Neurol 519:1677-1690,2011) each receives direct (monosynaptic) connections from campaniform sensillae located in the scapus and usually generate a single spike in response to lightly touching the antennae with an antenna-like bristle. After bath application of octopamine, or its agonist chlordimeform (CDM), DBNc1-2 responds to the same touch stimulus with several spikes, whereas the response in DBNc2-2 is either blocked or remains unaffected. A further, as yet not fully characterised descending brain interneurone seems only to respond to antennal stimulation in the presence of CDM. These effects were reversed by washing, and reversibly blocked by the octopamine antagonist epinastine. Octopamine seems to act directly on the central synaptic connection with the interneurones, since nerve recordings from antennal afferents revealed no effect. Stains of single campaniform sensillae on the scapus revealed that their terminal processes are closely associated with the presumptive spike initiating zone of DBNc2-2 and DBNc1-2. Immunocytochemistry revealed numerous octopaminergic terminals adjacent to this site, some but fewer dopaminergic terminals but no serotonergic terminals. We are currently investigating the effects of dopamine. We speculate that octopaminergic modulation of the studied neurones will influence whether male crickets approach or retreat from conspecifics during agonistic encounters. Supported by the German Research Council (DFG: FOR 1363, STE 714/4-1).

Incentives, Accountability and Decision Making: A Neuroscientific Investigation

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Managerial decision making often involves a trade-off between the overall economic viability of a project and the distribution of a project's returns over time, as reported in accounting systems. Myopia denotes the tendency of managers to redirect resources from sustainable long-term firm value maximizing projects to short-term projects. This short-sighted behaviour is considered an important cause of dysfunctional organizational and market behaviours that ultimately result in a breakdown of individual companies and entire economic systems. Managerial myopia is, however, not well understood.

In this research we aim to enhance the understanding of myopia and, in particular, whether myopia can be cured. Starting point in our analysis is the existence of various behavioural biases that, alone or in combination, may account for myopic behaviour. People, for example, have been known to engage in hyperbolic discounting, which denotes the sharp discounting of future results. People also typically prefer smaller certain outcomes over larger riskier outcomes, a phenomenon known as risk aversion. Both effects explain myopia, as an unwarranted preference over immediate, and certain but small pay-offs over future and uncertain, but potentially big pay-offs.

Such behavioural tendencies describe the phenomenon, but do not explain it. Therefore, cures for myopia need to start with recognizing more fundamental cognitive and affective processes result in myopic behaviour. These concern the role of impulsivity, the potential role of fear associated with uncertainty, the role of affect and emotion in decision-making or the existence of cognitive limitations which hinder evaluation of future events. In traditional economics these factors are subsumed under a single heading of 'irrational decision-making', but the underlying causes are not uniform, and require a deep examination of the 'black-box' of human behaviour.

The purpose of our research is to explain how accountability (monetary incentive and social pressure) affects myopic tendencies looking at the neural activity of decision makers. We conducted a functional magnetic resonance imaging study using 30 experienced accounting and finance professionals. They performed a number of standardized cognitive tasks. We find effects of both forms of accountability on performance across most cognitive tasks. Accountability positively affects cognitive performance and reduces selective attention, impulsivity and emotional interference. Interestingly, we find that different accountability types trigger different cognitive and emotional mechanisms, suggesting that curing myopia may indeed involve addressing a number of fundamental brain processes.

Input and Output Connections to Rat Prefrontal Cortex are Reciprocally Organised but not Aligned

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Understanding the structural organisation of the prefrontal cortex (PFC) is an important step towards determining its functional organisation. Inputs and outputs of the primary visual cortex, primary somatosensory cortex and primary motor cortex in mammalian cortex contain projection sites in the same specific regions of columnar cortex. This means that their cortical inputs and outputs can be described as 'aligned'. Here we sought to investigate the alignment of inputs and outputs of rat prefrontal cortex. We injected retrograde (FluoroGold, 100nl) and anterograde (Biotinylated BDA, 100nl) tracers into sites within PFC subdivisions (prelimbic, ventral orbital, lateral orbital, dorsolateral orbital) along a coronal axis within prefrontal cortex. At each injection site one injection was made of the anterograde tracer and one injection was made of the retrograde tracer, this provided a means of labelling both the inputs and output connections associated with one cortical site. The projection locations of retrogradely labelled neurons and axon terminals was then analysed in the temporal cortex: area Te, perirhinal cortex and entorhinal cortex. We found evidence for an ordering of both the input and output connections to prefrontal cortex. As injection location of retrograde tracer was moved mediolaterally in PFC, an ordered arrangement of input projections occurring in temporal cortex was observed. We observed that anterograde and retrograde labelling in temporal cortex (i.e. PFC inputs and outputs) often occurred reciprocally (i.e. the same brain region, such as perirhinal cortex, contained anterograde and retrograde labelling). However, often the same specific columnar temporal cortex regions contained only either retrograde or anterograde labelling indicating that PFC inputs and outputs are frequently non-aligned.

MMP-9 in central amygdala improves reward learning and social motivation

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Inhibition of MMP-9 (matrix metalloproteinase 9, extracellularly operating enzyme) in the central nucleus of the amygdala (CeA) has been shown to impair appetitively motivated learning. The aim of our study was to investigate the effects of increased expression of MMP-9 in the CeA for alimentary and social motivation. We have used two models of MMP9 overexpression: a transgenic mouse and local overexpression of MMP9 by a lentiviral vector. The mice were tested in place preference and place reversal paradigms in the IntelliCage system and in the social approach test. Our results indicate that locally elevated level of MMP9 in the CeA increases motivation for seeking alimentary rewards and for exploration of a new social partner. The general overexpression results in a similar but milder phenotype. These results elucidate the role of MMP-9 in the CeA in motivation for appetitve stimuli.

Neural correlates of social interactions in the honeybee colony

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We developed a method that allowed acquiring electrophysiological data of high order interneurons of free moving honeybees in a social context. Honeybees show their most complex and fascinating behavior when interacting with hive mates within the colony. In our experiments the bees cared for the queen, nursed the brood, guarded the exit, cleaned the hive and foraged. The mini colony required at least 200 animals in a temperature controlled experimental hive, a queen and foragers. The wax comb was positioned on a tilted (17°) and heated 50 cm by 50 cm metal plate situated in an accessible all-embracing faraday cage. The colony used the honeycombs as they would naturally, workers filled some with pollen and honey, the queen laid eggs and bees slept in them. Multiple mushroom body extrinsic neurons were recorded from the A1/A2 group in the ventral aspect of the alpha lobe. The electrode was attached to the head capsule with two component non-toxic silicone. Free movement of the recorded animal was achieved by a highly flexible twisted triple of wired whose weight was counterbalanced (a very lose nylon spring). The behavior of the recorded animal was monitored in infra-red by a video camera. A custom written semi-automated MatLab script traced coordinates, social state and spatial orientation of the treated honey bee. The following social interactions were distinguished: being alone, close to another animal, within the queen group, close to the exit. The recorded bee behaved normal. Recordings of up to four units lasted up to 47 hours per animal. Spontaneous spike rates were found to be lower than the rate of the same group of mushroom body extrinsic neurons in harnessed bees. Social interactions, location on the comb and body directions were not encoded by specific neural activities of selected units but rather by the combination of several units. This is the first report on high order interneuron of activity in freely behaving honeybees inside of the colony.

Neuronal Correlates of Subjective Value in the Pigeon 'Prefrontal Cortex'

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Decision making – choosing a single behavioral option to the exclusion of all others - is a fundamental process underlying all behavior. Available options span across a vast range of dimensions and are thus difficult to directly compare to each other. This problem is reconciled by the concept of subjective valuation which postulates that all available options are assigned a unidimensional value (akin to an internal currency) which allows for easy comparison and subsequent selection of the most valuable option. Neuronal correlates of subjective value have been identified in a number of vertebrate species, most prominently in primates and rodents. Here, we show a cell-type specific correlate of subjective value in the pigeon nidopallium caudolaterale (NCL), the avian analog of the prefrontal cortex.

We trained pigeons on a sign-tracking design in which discrete stimuli predicted a food reward varying in reward magnitude and delay until reward delivery. Using the frequency of responses produced during stimulus presentation as an indicator of subjective value, we could show that subjects reliably differentiate between stimuli and assign higher values to stimuli indicating large reward magnitudes and small times to reward.

During task performance, we recorded activity from a total of 198 neurons in the pigeon NCL and analyzed their activity during the presentation of reward-predicting stimuli. To identify possible neuronal correlates of value, we correlated the modulation of firing activity with behavioral response rates. Based on waveform properties, we identified two neuronal subpopulations composed of a cluster of broad-spiking cells (82% of all neurons) with low firing rates and a cluster of thin-spiking cells (18% of all neurons) with higher firing rates. We observed no correlation between firing activity and behavioral responses for neurons of the broad-spiking cluster; however, a close correlation was observed for neurons of the thin-spiking cluster.

We report a neuronal correlate of subjective value in a discrete subpopulation of neurons in the pigeon nidopallium caudolaterale, as has previously been shown for neurons from the mammalian prefrontal cortex. We thus lend further support to the idea that both behavioral and neuronal mechanisms of decision making are highly conserved across the vertebrates.

Predicting individual aggressiveness by video-tracking analysis in freely behaving crickets

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Since it takes at least two to fight, the evaluation of aggression is usually based on the level and duration of escalation between pairs of animals. However, this gives limited information on the potential aggressiveness of each combatant, e.g. when one flees on sight. To develop a more exact measure, e.g. for assessing drug effects on aggression, we performed a video tracking analysis of aggressive and submissive crickets and found several differences in general behavioral traits, from which we can in principle predict each animal's probability of being aggressive (P-aggr.). Crickets become highly aggressive after winning a contest, but submissive after losing. Subsequent video-tracking analysis (Noldus EthoVision XT 5.8) of winner and loser behavior in an arena revealed statistically significant differences. For example, winners are quicker to enter the arena from a release area, spend more time walking, and are quicker to approach and remain longer at one end of the arena where other crickets or moving objects (small back balls) are visible behind a transparent wall. When an antenna or cercus is touched, winners also tend to turn more towards the touched body side than losers. Surprisingly, the analysis of the same animals on the day prior to experiencing a win or loss, revealed the same trends for the prospective winners and losers, indicating that there might be inherent differences in aggressiveness. We are currently working towards building a predictive model from data for 100 winners and losers using binary logistic regression. Our preliminary model has a predictive power of 75%. With refinements we aim to develop a statistical model with which an individual cricket's aggressive motivation can be predicted from video-tracked behavior with a probability of 95%. Supported by the German Research Council (DFG: FOR 1363, STE 714/4-1).

Role of dopamine D1/D2 receptors in mediating the aftereffect of wheel running

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Rats lever-press for access to running wheels implicating that wheel running per se is reinforcing. Importantly, reinforcing effects of wheel running outlast the actual occurrence of physical activity, an effect which is referred to as aftereffect. Accordingly, rats that were repeatedly confined in a specific environment immediately after termination of wheel running display a preference for this environment, a phenomenon termed as conditioned place preference (CPP). CPP involves Pavlovian conditioning, i.e., repeated pairings of the aftereffect induced by wheel running with a specific environment, creates a learned association between aftereffect and environment and, in turn, a preference for that environment. As brain dopamine systems mediate effects of Pavlovian stimuli on appetitive behavior, a role of dopamine in supporting aftereffect-induced CPP seems plausible. Therefore, we assessed whether the mixed D1/D2 receptor antagonist flupenthixol (0.25 mg/kg, i.p.) can reduce the expression of aftereffect-induced CPP.

During place preference conditioning, each rat (n=32) received 6 pairings of one chambers of the CPP apparatus with a given context and the aftereffect of wheel running as well as 6 unpaired exposures to the other chamber with a different context. During a paired trial, an animal was confined for 2h in a running wheel and immediately thereafter placed into the respective chamber. During an unpaired trial, an animal was placed for 2h in a small cage before being transferred into the respective chamber. One day after completion of place preference conditioning, each animal was given a place preference test for 10 min with free access to both chambers. Three days later, a second place preference test was performed with prior drug administration, i.e. animals received injections of either flupenthixol (0.25 mg/kg, n=16) or saline (1 ml/kg, n=16).

Our results demonstrate that rats displayed a conditioned preference for environments paired with the aftereffect of wheel running and further show that CPP magnitude was not related to the wheel running rate. Moreover, we found that the mixed dopamine D1/D2 receptor antagonist flupenthixol did not block the expression of CPP produced by the aftereffect of wheel running.

Our observation that the aftereffect of 2 h wheel running generated a robust CPP adds further support to the notion that the aftereffect is reinforcing [1]. Remarkably, we found no evidence for a relationship between CPP magnitude and wheel running rate within 2 h. However, it is not clear whether there exist linear dose-response relationships between intensity or duration of wheel running and its reinforcing efficacy as well as the resulting aftereffect size. Studies in humans on associations between activity doses and affective responses suggest complex non-linear relationships between aerobic exercise and positive or negative affect. In addition, our results show for the first time that the expression of an aftereffect-induced CPP induced by natural reinforcers such as the aftereffect of wheel running does not depend on dopamine D1/D2 receptor activation. This findings point to the possibility that opioid receptor rather than dopamine receptor activation could support the expression of an aftereffect-induced CPP. These findings contribute to an understanding of the runner's high in humans and it's the neurochemical basis.

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Spatial attention suppresses MT responses to motion onset in macaque monkeys

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Direction-selective neurons in the extrastriate cortical area MT of macaque monkeys play an important role in the processing of visual motion information. Responses of these neurons to visual stimulation exhibit two distinct components: a high-amplitude transient on-response followed by a lower rate sustained phase. Although a large number of physiological studies in the macaque documented that the allocation of spatial attention to the receptive field (RF) enhances MT sustained responses to motion, there has been little examination of how MT responses to the onset of visual motion are influenced by spatial attention.

We addressed this issue by recording the responses of 127 directionally selective, tuned MT neurons of two macaque monkeys trained to perform motion-change detection tasks: while the monkey depressed the lever of a monkey chair and kept its eye gaze on a central fixation point, a stationary random-dot pattern (RDP) was briefly shown either inside or outside the RF, cueing the location of an upcoming target. After a short blank period, two moving RDPs appeared simultaneously inside and outside the RF, both moving in the same direction. The direction of motion in each trial was randomly chosen from 12 equally spaced directions. At random time points the direction (or speed) of target and distractor (uncued stimulus) changed. The monkey was required to detect the direction (or speed) change in the target by releasing the lever and to ignore changes in the distractor to get a fluid reward.

Comparison between MT population responses to different directions when the target was inside the RF (attended) and the distractor inside the RF (unattded) confirmed previously reported findings: spatial attention enhances MT sustained response to motion. However, it revealed that spatial attention suppresses MT response to the onset of visual motion with a minimal effect on the stimuli moving at or close to the preferred direction and a maximal effect on the motion directions parallel or close to the anti-preferred direction. Moreover, we evaluated the effect of spatial attention on direction tunings across MT neurons. Our results showed that attention enhanced the amplitude of sustained tuning without any significant effect on the amplitude of tuning curve during transient phase. Sustained response to anti-preferred direction was not changed by attention whereas transient minimum response was significantly suppressed. We also demonstrated that spatial attention enhanced the peak-to-peak amplitude of the direction tuning (directional gain) in both sustained and transient phases. Our analysis showed that spatial attention altered neither preferred direction nor tuning width of MT neurons. Our results indicate that although spatial attention affects MT responses in transient and sustained phases differently, the directional gain change due to attention is the same in both phases.

The effects of devocalization on rough-and-tumble play behaviour in unfamilar adult and juvenile rats.

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When playing, rats emit 50-kHz ultrasonic vocalizations which may function as play signals. A previous study using devocalized rats provides support for the hypothesis that 50-kHz calls are functioning to promote and maintain playful interactions. However, in that study all pairs were cage mates and familiar with each other's playful tendencies which could have attenuated the play signals. The present study uses unfamiliar pairs to eliminate any chance for attenuation as well as it uses both juvenile and adult male rats to further examine the role of vocalizations in play behaviours once sexual maturity is reached. In juveniles, it was shown that 50-kHz calls are not essential for playful interactions to remain playful. However, in adults, it appears that the tactical use of 50-kHz calls is necessary to keep playful interactions from becoming aggressive in some contexts. Thus, 50-kHz calls appear to be functioning simply as an expression of affect in juveniles, whereas once sexually mature, the tactical use of ultrasonic vocalizations in male rats becomes more essential in navigating some social situations.

The neural representation of empty sets in macaque posterior parietal cortex

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A general magnitude system hosted in the posterior parietal cortex is considered responsible for the representation of quantity in primates. We have shown that numerosity tuned neurons found in those regions can account for the discrimination of numerosities. However, in the small extreme of the natural numbers, the neuronal correlates of zero remain unexplored.

Behavioral studies show that non-human primates do possess a pre-symbolic precursor of zero. In tasks requiring discrimination and ordering of simultaneously presented visual quantities, monkeys spontaneously treated zero sets as they treat other numerosities, exhibiting the so called 'distance effect' (Merritt et al, 2009). The presence of a distance effect for empty sets suggests they are manipulated according to the null quantity they represent and apprehended as part of the numerical continuum.

We trained two rhesus monkeys in a delayed match-to-sample task involving as stimuli sets of black dots presented against a gray background. Crucially, we controlled for total dot area, dot density, background shape and area, dot-background contrast and total luminance of the stimuli. When monkeys had mastered the task with numerosities 1-4, empty sets were introduced and behavioral data were collected. Subsequently, we conducted single-cell recordings in the ventral intraparietal area (VIP).

The overall performance of monkeys exhibited a distance effect. When empty sets were presented as sample in the first sessions, monkeys made significantly more mistakes when the test numerosity was 1 than when it was 2.

Preliminary analysis of single-cell recordings in VIP reveals that many purely numerosity-selective neurons responded stronger to empty sets than to other numerosities. The tuning curves of neurons responsive to empty sets were reminiscent of those of neurons tuned to small numerosities.

Our results show that single neuron responses in the posterior parietal region can discriminate empty sets from other visual numerosities. Our data shed light on the question of how null quantity is encoded in the primate brain, and how empty sets are represented in neuronal activity in relation to other cardinalities.

The role of depth cues in human place recognition

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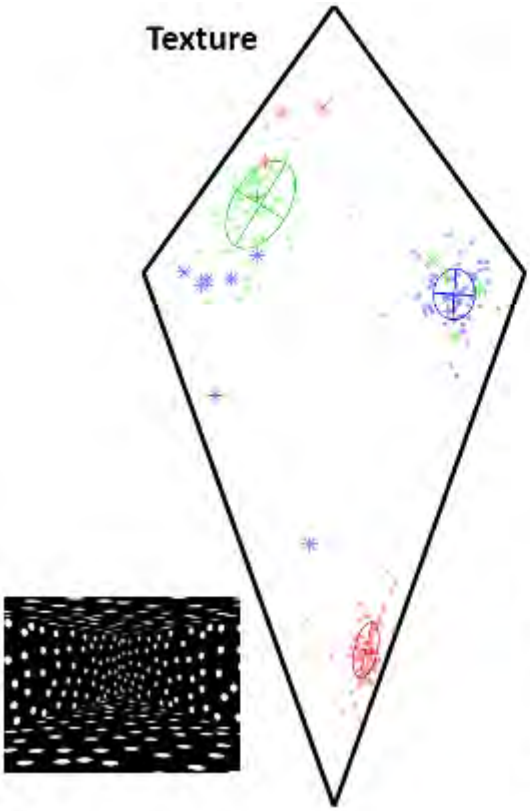
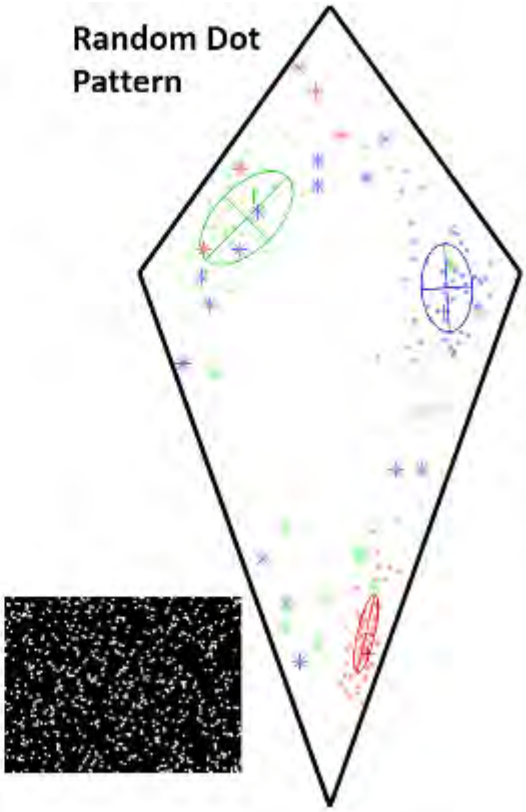
The visual recognition of known places is a central task in spatial cognition. In principle, it can be based on a large variety of cues, depending on what type of visual processing is employed and which types of features constitute the place-code in spatial memory. In insects, images, or snapshots, taken at the target place and processed with not much more than early vision operators (contrast, edges, motion, color) are considered to be the ubiquitous code for place. Snapshots have also been shown to play a role in humans (Gillner et al, Cognition 2008). However, human place recognition is thought to use also higher level visual information, including depth cues, landmark objects, and context from larger regions. Experiments on the “geometric module” (Hermer & Spelke, Cognition 1996) that have initially been taken as clear evidence of the use of depth information in place recognition, have been criticized for various reasons, not least for the fact that the experimental results could also be explained with pure snapshot models of place memory (Stürzl et al. JEP:ABP 2008). Indeed, the separation of depth from the plethora of other possible cues in behavioral experiments seems to be a crucial problem in this discussion.

In this study, we used stereoscopic presentation and dynamic random dot stimuli with limited lifetime to study the role of depth perception in place recognition. 10 subjects with normal stereo-vision were moving in a virtual kite-shaped room (21by 10 meters) presented in a mirror stereoscope. As compared to rectangular rooms, the kite-shape geometry has the advantage that all corners can be uniquely identified from geometry alone. In the “random dot” condition, room geometry was presented by limited lifetime random stereo dots and therefore was completely invisible in static monocular viewing. In addition to the stereoscopic cue, motion parallax became available when the subjects started moving, providing extra depth information. In the “texture” condition, walls were covered with a texture of white dots (about 15 cm in diameter) which also provided depth information from stereo and motion parallax. Additionally, the wall orientation could be judged from texture gradients and the four corners of the room were clearly visible due to discontinuities in the texture gradients.

In a “return-to-cued-place” paradigm, subjects were presented with views from one of four goal positions. After studying this condition, they were returned to their current starting point, with random heading, and asked to navigate to the previously studied goal, using a joy-stick. Subjects hit a button when arrival at the goal position was perceived. They were then relocated to the true goal position and a new trial started from there. Dependent variables were initial trajectory direction and position error. The random-dot and texture conditions were tested in a within subject design. Results show that finding the target place from pure depth information is possible in both conditions. Random positional errors are slightly smaller in the texture condition (dot symbols and error ellipses in the figure). The advantage of the texture condition also holds for “rotation errors”, i.e. cases in which corners or walls of the room were confused (asterisk symbols in the figure).

The results clearly demonstrate that isolated depth information can play a role in place recognition but that additional information about room corners improves performance.


Texture


Random Dot
Pattern

Transient processes and synchronization of independent ensembles neurons with human choice after the stimulus

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Stages of processes of neural activity of the cerebral cortex at which large-scale synchronization is carried out are interesting: at once for a significant volume of the cortex and between ensembles of neurons which are dissimilar in composition and properties. Synchronization automatically does not assume presence of communications between neural ensembles. The processes of neuronal activity of ensembles are nonlinear and depend on coordination with other ensembles. In particular, it is possible to investigate time correlation of rhythms with each other in connection with the external stimulus reflected in behavior. The stimulus can be displayed in two ensembles with a constant delay. For the non-linearity are the negative and positive feedback processes.

During experiment different variants of synchronization of cortical EEG are recorded. Examinees depending on a choice share on algorithm: on making a casual choice, on generated and not generated algorithm of a choice. Also the differentiation on success of the choice defined by number of accumulated points.

At processing similar synchronization on time parameter of several neural ensembles both to, and after stimulus and the subsequent choice are investigated.

Also of interest are neurons ensembles typically occur at the same time delay before and after the stimulus, but not always synchronized with each other.

Processes can be viewed on the new "stimulus" determining the occurrence of the same neuronal ensemble for some seconds before and after the stimulus.

Poster Topic

T25: Learning and Memory

T25-1A A possible role of dynamic levels of phosphorylated Apis mellifera CREB (AmCREB) in the inner compact cells of mushroom bodies in regulating the individual ability of honeybees to learn during classical conditioning
Dorothea Eisenhardt, Karin Heufelder, Katrin Gehring, Janina Feige, Yan Dyck, Paul Bauer

T25-2A A sublethal dose of the neonicotinoid thiacloprid affects navigation, memory and social communication in honeybees
Lea Tison, Marie-Luise Hahn, Nina Sophie Irmisch, Aron Duer, Uwe Greggers, Gabriela Bischoff, Randolf Menzel

T25-3A A task to probe time and distance estimation in rodents
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T25-4A Assessment of γ-Hydroxybutiric acid (GHB) action in insects
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T25-5A Changes in BDNF protein expression after fear extinction learning in the amygdala, the medial prefrontal cortex and the hippocampus
Aaron H Voss, Volkmar Lessmann, Thomas Endres

T25-6A 'Cognitive enhancement' in insects: Associative function increased by *Rhodiola rosea* food supplementation?
Birgit Michels, Katrin Franke, Ludger Wessjohann, Dushyant Mishra, Oleh Lushchak, Hanna Zwaka, Ruth Bartels, Bertram Gerber

T25-7A Comparison of classical and operant conditioning using behavior and electrophysiology during auditory learning tasks
Maité Goldschmidt, Angela Kolodziej, Andreas Schulz, Frank W. Ohl

T25-8A Decoding the brain epigenome:Chromatin readers in cognition
Eva Benito-Garagorri, Hendrik Urbanke, Jonas Barth, Andre Fischer

T25-9A Different rat hippocampal subfields show increased Arc mRNA expression after novel exploration of discrete positional or large directional spatial cues
Verena Aliane, Denise Manahan-Vaughan

T25-10A Differential analysis of gene expression with RNA-seq: regulation by training strength
Katja Merschbächer, Uli Müller

T25-11A Dorsal Hippocampal lesions boost performance in the rat sequential reaction time task:

Evaluating the role of enhanced instrumental experience
Sebastian Busse, Janine Roscher, Rainer K. W. Schwarting

T25-12A Dynamics of Memory Storage based on Cell Assemblies
Juliane Herpich, Florentin Wörgötter, Christian Tetzlaff

T25-13A Dynamics of Spatial Self-Organization of Cell Assemblies
Johannes Maria Auth, Timo Nachstedt, Christian Tetzlaff, Florentin Wörgötter

T25-14A Effects of increased acetylcholine levels on odor-induced memory reactivation during slow wave sleep in humans
Jens G Klinzing, Jan Born, Björn Rasch, Susanne Diekelmann

T25-15A Effects of oscillatory electric fields on EEG coupling: slow oscillation and spindle activity
Lisa Marshall, Dominic Aumann, Christian Wilde, Matthias Mölle, Lucas Parra

T25-1B Effects of overexpression of the (pro)renin receptor on the hippocampal formation in mice
Alexander Bracke, Kai Bente, Oliver von Bohlen und Halbach

T25-2B Retracted

T25-3B Extra-coding RNAs regulate neuronal DNA methylation
Nancy Gallus, Esther Song, Rhiana Simon, Kathrin Savell, J David Sweatt, Jeremy J Day

T25-4B Gene Regulation and Epigenetics of a Lifetime Body-Size Memory in *Drosophila*
Laura Spindler, Tammo Krause, Burkhard Poeck, Roland Strauss

T25-5B Generalization of associative olfactory memories: parametric analyses in Drosophila melanogaster
Emmanuel Antwi Adjei, Annie Voigt, Christian König, Rumeysa Taspinar, Ayse Yarali

T25-6B Genome-wide analyses on electric shock avoidance, punishment learning and relief learning in *Drosophila melanogaster*
Christian König, Mirjam Appel, Claus-Jürgen Scholz, Marcus Dittrich, Tobias Müller, Hiromu Tanimoto, Ayse Yarali

T25-7B High-resolution comparison between learned and innate olfactory behaviour in larval *Drosophila*
Michael Schleyer, Samuel Reid, Evren Pamir, Timo Saumweber, Emmanouil Paisios, Alexander Davies, Bertram Gerber, Matthieu Louis

T25-8B How to learn fast and forget slowly with dendritic spines
Michael Fauth, Florentin Wörgötter, Christian Tetzlaff

T25-9B Imaging of synaptic activity and plasticity in the *Drosophila* brain
Ulrike Pech, André Fiala

T25-10B Impact of hippocampal sclerosis lateralization on behavior in the mouse intrahippocampal kainate model of mesial temporal lobe epilepsy

Jean-Baptiste Faure, Antje Kilias, Ute Häussler, Philipp Janz, Carola Haas

T25-11B Impact of life history on fear memory and extinction in mice
Jasmin Remmes, Carina Bodden, Norbert Sachser, Hans-Christian Pape, Thomas Seidenbecher

T25-12B Impact of the cortical extracellular matrix on memory and learning flexibility
Hartmut Niekisch, Matthias Deliano, Frank W. Ohl, Renato Frischknecht, Max F.K. Happel

T25-13B Learning of declarative memory after sleep is influenced by D-cycloserine given during sleep
Marjan Alizadeh Asfestani, Surjo R. Soekadar, Jan Schwidetzky, Jan Born, Gordon B. Feld

T25-14B Locomotion patterns induced by learned odors in the honey bee (*Apis mellifera* L.)
Hiroyuki Ai, Yuta Kimura, Toshiya Yamashita, Hidetoshi Ikeno, Stephan Shuichi Haupt

T25-15B Long-term avoidance memory leads to a transient increase of synaptic complexes in the mushroom bodies of ants
Agustina Falibene, Flavio Roces, Wolfgang Rössler

T25-1C Modeling the Interaction of Long-Term Memory and Working Memory
Timo Nachstedt, Florentin Wörgötter, Christian Tetzlaff

T25-2C Neural circuit analyses of relief learning in fruit flies
Afshin Khalili, Christian König, Yoshinari Aso, Gerald Rubin, Ayse Yarali

T25-3C Neural circuits of memory re-evaluation
Johannes Felsenberg, Scott Waddell

T25-4C Neural Correlations in Brains of Freely Walking Social and Solitary Bees during Navigation in an Artificial Environment
Nanxiang Jin, Simon Klein, Randolf Menzel

T25-5C Neuronal correlates of instrumental learning in honeybees
Hanna Zwaka, Meida Jusyte, Sophie Lehfeldt, Randolf Menzel

T25-6C Neuronal correlates of unlearned calls in male and female zebrafinches
Lisa Trost, Andries Ter Maat, Hannes Sagunsky, Manfred Gahr

T25-7C Neuronal responses to mild heat and electric shock in the brain of *Drosophila*
Kristina V. Dylla, Alja Lüdke, Dana Shani Galili, Hiromu Tanimoto, C. Giovanni Galizia, Paul Szyszka

T25-8C Neuronal substrate of associative fear conditioning in the zebrafish (*Danio rerio*) – measured by c-Fos expression
Tim Ruhl, Jill Romer, Gerhard von der Emde

T25-9C Neuropeptide S receptor-deficient mice in a mouse model of post-traumatic stress disorder
Markus Fendt, Josephine Germer, Evelyn Kahl

T25-10C NOGO-A INFLUENCES STRUCTURAL DYNAMICS OF MOSSY FIBER SYNAPSES
Cristina Iobbi, Martin Korte, Marta Zagrebelsky

T25-11C Optically evoked Sharpwave-Ripple Waveforms in Hippocampal Circuits Indicate Formation of Neural Assemblies
Pascal Geschwill, Martin Both, Andreas Draguhn

T25-12C Pathway Analysis of a Visual Working Memory in *Drosophila*
Sara Kuntz, Burkhard Poeck, Roland Strauss

T25-13C PharmaKirGenetics: Neuron inhibition by small molecule-induced Kir2.1 stabilization
Christoph Thoeringer, Eva Auffenberg, Angela Jurik, Stelios Michalakis, Gerhard Rammes, Carsten Wotjak, Roland Schmid

T25-14C Plasticity in mushroom body physiology correlates with behavioral learning performance in individual honeybees.
Martin Paul Nawrot, Martin Strube-Bloss, Nobuhiro Yamagata, Joachim Haenicke, Randolf Menzel

T25-1D Plasticity of a defined mushroom body-output synapse underlies learned olfactory behavior in *Drosophila*
David Owald, Johannes Felsenberg, Gaurav Das, Emmanuel Perisse, Clifford Talbot, Wolf Huetteroth, Scott Waddell

T25-2D Processing of Competing Visual Stimuli in the Central Complex of *Drosophila melanogaster*
Stefanie Flethe, Roland Strauss

T25-3D Relief learning is controlled by dopamine release in the rat nucleus accumbens
Jorge Ricardo Bergado Acosta

T25-4D Selective retrograde amnesia of associative memory after Neuroplastin loss and severe deficits in neuroplastin-deficient mice
Soumee Bhattacharya, Rodrigo Herrera-Molina, Frank Angenstein, Karl-Heinz Smalla, Eckart D. Gundelfinger, Dirk Montag

T25-5D Sensory memory forms in the caudomedial nidopallium during song learning
Christian Flecke, Yoko Yazaki-Sugiyama

T25-6D Sleep and Predictive Coding: Can Sleep improve Information Processing in the Visual System?
Nicolas Dominic Lutz, Karsten Rauss, Jan Born

T25-7D Specific contribution of CA1 to the reconsolidation of fear memory: an optogenetic/molecular imaging study
Vanessa Lux, Olivia Masseck, Stefan Herlize, Magdalena Sauvage

T25-8D Studying the mushroom body memory matrix one cell at a time
Timo Saumweber, Astrid Rohwedder, Katharina Eichler, Albert Cardona, James Truman, Marta Zlatic, Andreas Thum, Bertram Gerber

T25-9D Synapsin functions in timing-dependent behavioural plasticity
Thomas Niewalda, Birgit Michels, Roswitha Jungnickel, Jörg Kleber, Sören Diegelmann, Bertram Gerber

T25-10D Synapsin is required to establish a strong memory in odor-sugar associative learning
Jörg Kleber, Thilo Kähne, Bertram Gerber

T25-11D The connectome of the mushroom body of the *Drosophila* larva
Andreas Stephan Thum, Katharina Eichler, Marta Zlatic, Albert Cardona

T25-12D The Importance of Visual and Olfactory Stimuli During Flower Visits in the Honey Bee
Verena Reinhardt, Christa Neumeyer

T25-13D The role of CaMKII in the formation of long-term memory in the honeybee
Christina Scholl, Wolfgang Rössler

T25-14D The role of sleep in fear extinction memory in mice
Irene Melo , Ingrid Ehrlich

T25-15D Very low birth weight piglets show improved cognitive performance in the spatial cognitive holeboard task
Alexandra Antonides, Anne C. Schoonderwoerd, Franz Josef van der Staay, Rebecca E. Nordquist

A possible role of dynamic levels of phosphorylated Apis mellifera CREB (AmCREB) in the inner compact cells of mushroom bodies in regulating the individual ability of honeybees to learn during classical conditioning

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Honeybees form a transcription-dependent long-term memory 72 h following classical conditioning which is termed late long-term memory (lLTM). A transcription factor that might take part in the learning-dependent induction of lLTM formation is the cAMP-response element-binding protein (CREB) that has been shown to play a crucial role in LTM formation in several invertebrate and vertebrate model organisms. In the honeybee, a CREB-homologous gene (AmCREB) and eight splice variants have been identified. Moreover, AmCREB is one of only twenty transcription factors in the honeybee brain that predominantly regulates distinct neurogenomic states possibly underlying behavioral changes during the age-dependent division of labor, foraging and aggression. However, its role in honeybee learning and memory formation remains elusive.

Phosphorylation of CREB by protein kinase A (PKA) enables the binding of co-factors such as the CREB binding protein (CBP) to CREB, thereby inducing CREB-dependent transcription. An increase of phosphorylated CREB (pCREB) is regarded as an indicator of transcriptional activation by CREB. Accordingly, in this study, quantitative western blot analysis and immunohistochemistry were used to examine a possible role of phosphorylated AmCREB in lLTM formation.

Surprisingly, our study did not reveal any learning-dependent alteration of the pAmCREB amount. However, examining bees of a control group that received unpaired presentations of the conditioned stimulus (CS) and the unconditioned stimulus (US) revealed that the animal's responsiveness to the CS (i.e. an odor) following the presentation of the US (i.e. a reward), corresponds with the amount of pAmCREB. Altered pAmCREB level were observed in the central brain as well as in the inner compact cells (IC), a particular Kenyon cell population in the mushroom bodies receiving multimodal sensory input. In behavioral experiments, we examined whether the bee's responsiveness impacts learning and lLTM formation and how this might relate to the level of pAmCREB. Our results suggest that the level of pAmCREB in the IC of the honeybee mushroom bodies varies between individual bees. Moreover we hypothesize that the individual level of pAmCREB affects an animal's ability to learn during classical conditioning.

A sublethal dose of the neonicotinoid thiacloprid affects navigation, memory and social communication in honeybees

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Honeybee foragers may be exposed to neonicotinoids during their foraging flights via pollen, nectar and guttation drops. It has been observed that neonicotinoids compromises navigation in honeybees. Yet, the colony relies on the foragers' ability to locate food sources and bring pollen and nectar back to the hive. The successfully returning foragers will deposit pesticide containing substances in the hive which may accumulate over time. The waggle dance may also be affected by sublethal doses of these pesticides since neonicotinoids interfere with the nicotinic synaptic transmission in the central nervous system of insects possibly altering social communication processes as well.

In order to test this hypothesis we trained foragers from two colonies of honeybees Apis mellifera carnica in observation hives to two separate feeders. One group (from the experimental colony) foraged during 2 weeks in a first experiment and 4 weeks in a second one on a sucrose solution containing a sub-lethal dose (0.02 mM) of thiacloprid, and another group (from the control colony) foraged over the same time at a feeder containing only sucrose solution. Navigation abilities of these bees were tested in a catch-and-release experiment. Bees´ waggle dances were video-recorded in both control and treated hive and the electric fields of the dancers were measured. The traffic at each feeder was estimated using light sensitive detectors at the feeders' entrances.

In addition, Proboscis Extension Reflex experiments were performed to assess whether thiacloprid has an effect on honeybees' memory recalling and memory consolidation. The retrieval of the memory was tested 1 hour (memory retention experiment) or 17 hours (memory consolidation experiment) after the dispensation of the pesticide at a dose of 69 ng/bee. Compared to the control group, the treated bees have shown a significantly lower ability to remember the odor formerly learned.

We found that chronic exposure of a colony to a field-relevant dose of thiacloprid in field-realistic conditions affects honeybees' foraging behavior, social communication and navigation. Honeybees' memory consolidation and retention was also affected by thiacloprid when given as a single dose in laboratory conditions.

A task to probe time and distance estimation in rodents

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The psychophysical properties of time and space perception share remarkable similarities. Whether those similarities stem from common neural processing and/or neural machinery, is unresolved. Tackling this problem is hard mainly because time and space perception are usually investigated with different behavioral/psychophysical paradigms making direct comparison problematic.
We introduce a behavioral paradigm that allows for investigating time and space perception under identical conditions and at behaviorally relevant temporal (supra-second) and spatial (few meters) scales. The paradigm requires movement in a virtual reality (VR) environment in combination with either timing a temporal interval or estimating a spatial distance. A running movement is natural to rodents, providing an appropriate means for stimulus estimation. But of course in such an approach, one needs to disentangle both time and space. Using VR this can be achieved: movement speed and optical flow can be de-correlated, so that the animals can not learn a mapping between stimulus duration and running distance. This we make use of, such that we can either “ask” the animal for the distance or duration it ran. In addition, a virtual test environment can be designed without prominent spatial cues thereby preventing alternative solution strategies. The estimation part is complemented by a response part in which the animal has to report if the time or distance it ran was perceived as short or long, compared to previously trained references. We used a Y-shaped maze in which the animal had to choose either the left or the right arm of the Y-maze as a response. Taken together we implement a temporal/spatial bisection task, which is a useful method to examine sensory thresholds of animals and humans in relation to various sensory stimuli.
We successfully used the virtual bisection task in experiments with Mongolian gerbils (*Meriones unguiculatus*). We did psychometric tests for temporal intervals between 2 and 4 seconds, and distances of 1 to 2 meters. Behavioral performance was characterized using psychometric analysis and probabilistic choice modeling, yielding data on psychometric parameters such as Weber fractions and just noticeable differences for both interval timing and distance estimation. The results are consistent with reports from classical non-virtual studies and hence demonstrate the applicability of our approach to probe interval timing and distance estimation in rodents. The paradigm can, in its current form, be combined with recordings to investigate the neural basis of interval timing.

Assessment of γ-Hydroxybutiric acid (GHB) action in insects

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The neurotransmitter γ-Hydroxybutiric acid (GHB) is a metabolite as well as a precursor of the γ-Aminobutyric acid (GABA). GHB is used since the early noughties as a treatment for narcolepsy. In mammals, GHB or “Liquid Ecstasy”, which is also a popular party drug since the late 1990s, can lead to amnesia and drowsiness in high concentrations, sometimes even irreversible coma. In mammalian organisms, a specific GHB-receptor exists, though it is also assumed, that the $GABA_B$-receptor is involved in the mediation of GHB-induced effects. This is of great interest since there does not appear to be a GHB-receptor in insects. In spite of this we found similar effects: high doses of GHB induce catatonia in treated honeybees (*Apis mellifera*). We tested a number of different $GABA_A$ and $GABA_B$ agonists and antagonists to investigate if the GHB-induced effects could be mediated by the GABA-receptors.

Changes in BDNF protein expression after fear extinction learning in the amygdala, the medial prefrontal cortex and the hippocampus

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The neurotrophin BDNF (brain-derived neurotrophic factor) is beside its trophic function also well known for its role in synaptic plasticity. Furthermore, recent studies suggest that it plays a key role during fear as well as fear extinction learning, which are both used as animal models for several anxiety disorders. In this respect recent data suggest that a direct application of BDNF into the medial prefrontal cortex (mPFC) can lead to an induction of fear extinction learning, even without a behavioral training (Peters et al., 2010, Science). Concordantly hampering BDNF signaling before fear extinction training in the mPFC resulted in an impaired extinction memory. In addition, overexpression of the non-functional truncated TrkB receptor in the basolateral amygdala led also to an impaired fear extinction memory (Chhatwal et al., 2006, Nat.Neurosci.). However, besides its general function in fear extinction learning the time course of BDNF signaling underlying its role in extinction learning is so far unknown. In order to further understand the involvement and the dynamics of BDNF signaling during the consolidation of fear extinction memories, we analyzed the time course of BDNF protein expression in brain regions crucially involved in fear extinction learning.
Therefore, we fear conditioned three month old C57BL/6J mice and exposed them to a fear extinction training one day later. Animals that were fear conditioned but not fear extinguished served as a control group. Afterwards mice were sacrificed at time points ranging from 0 to 120 minutes after the end of the fear extinction training. We obtained samples from the basolateral amygdala (BLA), the dorsal hippocampus (dHipp), and the mPFC with a small biopsy puncher. Thereafter the level of BDNF protein was measured by a sensitive BDNF-ELISA. These experiments revealed distinctly changes in BDNF protein expression at different time points in the three brain regions analyzed. Interestingly, the time course of protein expression differed between the analyzed brain sites: first, there was an increase in BDNF protein in the BLA 30 minutes after fear extinction training, whereas in the mPFC there was an increase of BDNF protein starting at 60 min and peaking 90 min after fear extinction training. However, in the dHipp no distinct changes in BDNF protein expression were observed. Currently we are performing pharmacological experiments in which we locally interfere with BDNF-TrkB signaling in order to further pinpoint the required dynamics of BDNF-TrkB signaling mandatory for the successful consolidation of fear extinction memory.
In conclusion, our experiments support the important role of BDNF in fear extinction learning. Furthermore, they provide novel insights into the dynamics of BDNF signaling within different brain structures of the neuronal network required for fear extinction learning.

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'Cognitive enhancement' in insects: Associative function increased by *Rhodiola rosea* food supplementation?

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Humans traditionally use extracts from *Rhodiola rosea* roots for their anti-stress and 'cognitive-enhancing' remedy. Here, we scrutinize this effect in larval *Drosophila melanogaster*. We want to investigate if food supplementation with *Rhodiola rosea* improves odour-reward associative function, including tests for sensory and motor functions that are relevant for the employed task, as well as general locomotor parameters. Furthermore, we plan to supplement fly food with either commercially available, ground tablets or extract containing *Rhodiola* root material to test for a 'cognitive enhancing' effect. *Drosophila* as a genetically tractable study case should then allow accelerated analyses of the molecular mechanism(s) that underlie the 'cognitive enhancement' conveyed by *Rhodiola rosea*. To the extent that the molecular determinants of 'cognition' are shared between animals and man, such research may have bearings for humans as well.

Comparison of classical and operant conditioning using behavior and electrophysiology during auditory learning tasks

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To gain a more fundamental understanding of the role and the mechanism of the cortico-striatal interaction underlying learning, two different forms of learning will be compared: A classical conditioning paradigm (fear conditioning) and an instrumental conditioning paradigm (avoidance learning). The planned approach provides a scenario to investigate the phenomenon of Pavlovian-instrumental transfer (PIT), a process where in the first phase an association is made between a conditioned stimulus (CS) and an unconditioned stimulus (US) by classical conditioning and in the second phase there is a transfer from classical to instrumental. Therefore, we designed a combined learning experiment with male C57BL/6J mice in which one group of animals was trained initially in a modified fear conditioning paradigm and subsequently in a customized two-way active avoidance paradigm, and a second group in a reversed order. To discover possible similarities and differences between these two basic learning scenarios we will use behavioral analyses like scoring of freezing during fear conditioning, recording of hit-rate and false-alarm-rate during avoidance conditioning. In addition to the behavioral experiments, simultaneous electrophysiological recordings of local field potentials from auditory cortex and ventral striatum will be obtained from the awake behaving animals. This approach allows dissociating the specific functions of these two behaviorally relevant brain structures and their interplay in classical and instrumental conditioning.

Decoding the brain epigenome:Chromatin readers in cognition

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Epigenetic modifications allow a dynamic interaction between the genome and the environment. These include posttranslational modifications of histones, most prominently histone acetylation, which is directly associated with chromatin structure and gene expression. In addition to proteins that control the acetylation/deacetylation balance, a set of proteins named “chromatin readers” interprets the modification pattern and recruits further factors to fine-tune transcriptional control. Although reader domains were identified in many proteins already years ago, there is virtually no information on the role of such proteins in cognition. Here we screened for protein readers that bind to histone-modifications relevant to the pathogenesis of Alzheimer’s disease. We investigate the role for one of these enzymes in the developing and adult mouse brain and demonstrate a critical role in learning and memory. Our findings unravel a new layer of regulatory complexity in the epigenetic regulation of cognition and suggest that targeting chromatin readers may be a more effective strategy to ameliorate epigenetic-associated cognitive decline.

Different rat hippocampal subfields show increased Arc mRNA expression after novel exploration of discrete positional or large directional spatial cues

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The hippocampus plays a fundamental role in learning and memory. Presumably, changes in hippocampal synaptic efficiency and neuronal networks encode spatial memory. However, the specific roles of the CA1, CA3 and dentate gyrus subregions are still unclear. The parallel map theory postulates that whereas the CA1 processes small discrete environmental features, the dentate gyrus processes large navigationally relevant landmarks. Finally, the CA3 integrates both information (Jacobs, 2012, Acad Sci USA. 109 Suppl 1:10693-10700). In line with this, in the rat brain novel exploration of discrete positional cues facilitates long-term expression (LTD) in the CA1 region and novel exploration of large directional spatial cues facilitates LTD in the dentate gyrus (Kemp & Manahan-Vaughan, 2007, Trends Neurosci. 30;111-118). In addition, the two-streams hypothesis suggests that the subregions of the hippocampus are differentiated to enable processing of information from the 'where' and the 'what' streams. Information related to an item's features and spatial characterisitcs are encoded in two separate streams in the parahippocampal region: the 'what' stream and 'where' stream and this information is then integrated in the hippocampus (Mishkin et al., 1983, Trends in Neurosciences. Trends in Neurosci. 6:414–417). Anatomical and functional data suggest that such a distinction between the 'what' and 'where' pathway may be observed in the CA1 and CA3 regions (Sauvage et al., 2013, Behav Brain Res. 254:22-33).The 'what' stream could comprise the distal CA1 (close to the subiculum) and the proximal CA3 region (close to the dentate gyrus). Whereas the proximal CA1 and the distal CA3 region might form the 'where' stream within the hippocampus. In the dentate gyrus a distinction between the 'what' and 'where' pathway might be possible as well (Chawla et al., 2005, Hippocampus. 15:579-586).

In the present study, we implemented behavioral learning paradigms that facilitate the expression of robust hippocampal LTD to explore the functional basis of both hypotheses. We used fluorescence in situ hybridization (FISH), to map the activity-dependent mRNA expression of the immediate early gene Arc in the hippocampus, which is related to learning and synaptic plasticity. This technique allows the analysis of different hippocampal subregions in the same animal.

Arc mRNA expression war increased in the CA1 region after exploration of discrete spatial features and in the dentate gyrus after exploration of large spatially-distinct landmarks. These results are in line with the parallel map theory of spatial representations and support that these hippocampal subfields may contribute different components of a spatial representation. Interestingly, the increase of Arc mRNA expression after novel exploration of discrete spatial feaItures was restricted to the distal CA1 region of the 'what' stream. Furthermore, in the dentate gyrus we analyzed the Arc mRNA expression in the upper vs lower blade of the dentate gyrus. Here we observed that the increased Arc mRNA expression was restricted to the lower blade. Thus, our results confirm both the parallel map and two-streams theories of hippocampal function.

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Differential analysis of gene expression with RNA-seq: regulation by training strength

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Regulation of gene expression plays an important role during development and numerous physiological processes including learning and memory formation. Gene expression is regulated by activation of the transcription machinery and the status of the chromatin structure. Our findings have shown that the temporal dynamics of learning-induced changes in chromatin modifications such as histone acetylation depend on training strength. The accordant differentially expressed genes are not yet identified.
To address this matter we adapted and applied a technique that enables in vivo labelling of mRNA transcribed during a defined time window after conditioning. Using olfactory associative learning in honeybees we induced a long lasting memory by strong training and a transient memory by weak training. By deep sequencing of labelled mRNA from bees subjected to these training scenarios we identified genes that are differently regulated depending on training strength.

Dorsal Hippocampal lesions boost performance in the rat sequential reaction time task: Evaluating the role of enhanced instrumental experience

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It is commonly accepted that the hippocampus plays a major role in declarative memory across various species, for example, spatial memory in rodents. However, the interplay between hippocampal function and nondeclarative learning and memory, like procedural stimulus-response (S-R) or sequential learning, is less clear. Recently (Eckart et al., Hippocampus 22'12, 1202-1214), we showed that excitotoxic lesions of the rat dorsal hippocampus led not only to the expected deficits in a spatial and presumably declarative task, that is, the object-place recognition test, but also to substantial improvements in terms of both, speed and accuracy in a rodent adaption of the human serial reaction time task (SRT), that is, a task where performance is usually attributed to striatal mechanisms (Eckart et al., Neurotox. Res.17'10, 487-298). This effect was subsequently replicated by us in a rat model of mesial temporal lobe epilepsy with classic hippocampal sclerosis (Will et al., Behav. Brain Res. 247'13, 65-72). The designs of both experiments, however, which included fixed test durations per training day, led to the fact that lesioned animals gained more instrumental experience, since they were substantially faster and more accurate than controls. This factor may partly have accounted for their enhanced performance. In order to rule out such a potential confound, we performed the present experiment on rats with ibotenic lesions of the dorsal hippocampus, where we kept the amount of correct instrumental responses and reinforcement on the same level as in controls. Nevertheless, rats with dorsal hippocampal lesions clearly outperformed controls in the SRT, indicating that the higher levels of instrumental experience, which rats with similar lesions had in our previous experiments, did not substantially account for the lesion-induced boost of performance in the rodent SRT. In line with our previous lesion findings, these data support the hypothesis that loss or impairment of hippocampal function can enhance specific task performance, especially when it is dependent on procedural (probably striatum-dependent) mechanisms with minimal spatial requirements.

Dynamics of Memory Storage based on Cell Assemblies

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The ability of learning and memory are essential properties of adaptive neural circuits. Thereby, the hypothesis is that the adaptive process of synaptic plasticity induces changes at the synapses connecting the neurons (named Synaptic-Plasticity-and-Memory hypothesis; [1, 3]). This yields a formation of strongly interconnected subgroups of neurons, so-called cell assemblies [2]. Such cell assemblies represent the learned memory item.

As known from everyday life, after learning, humans and animals show the remarkable ability to connect, generalize, and discriminate learned memories. How these processes are realized on the neuronal level based on cell assemblies is still widely unknown.

In this work, we use a model dependent on the interaction between synaptic plasticity and scaling [5, 6]. This interaction yields the formation of cell assemblies showing dynamics comparable to human memories [4]. For simplicity we abstract this model by the methods of mean-field theory to a set of differential equations describing the dynamics of adaptive populations of neurons. Given this reduced model we analyze, on the one side, the constraints and resulting dynamics of how these adaptive populations become cell assemblies. Interestingly, these constraints are quite general as, for instance, the need of recurrently connected nonlinear neurons. Furthermore, in contrast to loosely connected groups of neurons, the formation of cell assemblies enables the population to follow the dynamics of a hysteresis. On the other side, the reduced model enables us to analyze if and how several interacting cell assemblies (each described by its own set of differential equations) can show the processes of connecting, generalizing, and discriminating memories based on the investigated hysteresis. Thereby, we are able to provide constraints on model parameters and learning signals leading to the different processes.

Thus, this work is a further step of connecting the dynamics of memories on psychological scale to the hypothesized dynamics of cell assemblies on neuronal scale and, therefore, provides a further evidence for the Synaptic-Plasticity-and-Memory hypothesis [2, 3].

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Dynamics of Spatial Self-Organization of Cell Assemblies

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The formation of memory is still an unsolved question in neuroscience. Cell assemblies (CAs), subgroups of highly interconnected neurons, and their basic properties are very likely to play a key role in this mechanism [1]. So far, many models have been proposed to investigate the CAs origin and their various functional effects [2]. However, in those approaches randomly placed or predefined CAs are used, but their self-organized occurrence and spatial arrangement mostly remain unanswered. A promising solution to fill this gap is the combination of CAs and the principle of self-organizing maps (SOMs) [3]. We use this approach to form assemblies not only showing self-organization in spatial emergence but also in input specific self-rearrangement. The latter is likely to contribute to modelling stimulus discrimination which also is a long-standing question in neuroscience [4].

Our study uses a model of two neural layers, a smaller input layer consisting of several independent neurons and a plastic recurrent layer. Random excitatory connections from the first to the latter allow projection of different input patterns. The network neurons dispatch excitatory synapses to neighbouring neurons of a certain radius while inhibition is realised via one globally and mutually connected neuron population with non-plastic synapses. The neurons are represented by a rate-based leaky integrator model. All excitatory synapses, that are the projectors as well as the interconnections in the network, are adapted by Hebbian plasticity in combination with synaptic scaling [5].

Activation of differing subsets in the input layer yields weight growth of the projective synapses through time correlation and scaling. As a result, also the network neurons build up clusters of enhanced connectivity strength according to the input pattern and depress the efficacies between the latter. During alternate training of several patterns, we notice overlapping input patterns result in overlapping CAs. Thus we see the network not being able two discriminate between these stimuli. With increasing experience, however, separation slowly sets in and eventually tells differing but similar inputs apart. Our work focuses on the separation dynamics and results promise strong conjunctions of separation speed with the network parameters and training intensity.

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Effects of increased acetylcholine levels on odor-induced memory reactivation during slow wave sleep in humans

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Sleep is known to improve memory consolidation, a function assumed to rely on the reactivation of newly acquired memory representations mainly during slow wave sleep. External cues like odors or sounds have been used to facilitate memory reactivation. In the odor reactivation paradigm, odor cues are presented during a learning task by which the acquired memories become associated with the contextual odor. Presenting the odor again during slow wave sleep (compared to presenting an odorless vehicle) enhances the consolidation of the associated memories and improves later recall performance.

The neural mechanisms underlying the beneficial effect of odor-induced memory reactivation are not well understood. One influential idea is that odor cues presented during sleep act as context reminders, which bias spontaneous hippocampal memory reactivation in favor of the associated memory traces. This reactivation allows information encoded in hippocampal circuits to be communicated via long-range connections to other brain areas so that over time they become integrated into distributed neocortical networks for long-term storage. This hippocampal-neocortical information flow has been suggested to depend essentially on the suppression of acetylcholine, the levels of which drop to a minimum during slow wave sleep.

In the present study, we aimed to investigate the role of cholinergic suppression in the beneficial effect of odor-induced memory reactivation during slow wave sleep. We pharmacologically increased acetylcholine levels by administering the acetylcholine-esterase inhibitor physostigmine. In the presence of an odor, healthy male subjects learned a visuospatial 2D object-location task in the evening before going to bed. At sleep onset, we started intravenous infusion of physostigmine for a period of 40 minutes. During 20 minutes of slow wave sleep, we presented the odor cue (or odorless vehicle) in 30-seconds on/off intervals. After a total sleep time of about 40 minutes, participants were woken up and subjected to an interference learning task followed by memory recall of the original task.

We expected that under the administration of physostigmine, odor reactivation would no longer be effective in improving memory performance. In a pilot study, we found that physostigmine indeed neutralized the effects of odor-induced memory reactivation: subjects who received the odor during sleep did not show superior memory performance at recall compared to subjects who received the odorless vehicle. These findings suggest that physiologically decreased levels of acetylcholine during slow wave sleep enable odor cues to facilitate hippocampal replay and the associated hippocampal-neocortical information flow. The poster will present our pilot data as well as the results of a placebo-controlled study with 32 subjects which is currently conducted to verify our preliminary findings.

Effects of oscillatory electric fields on EEG coupling: slow oscillation and spindle activity

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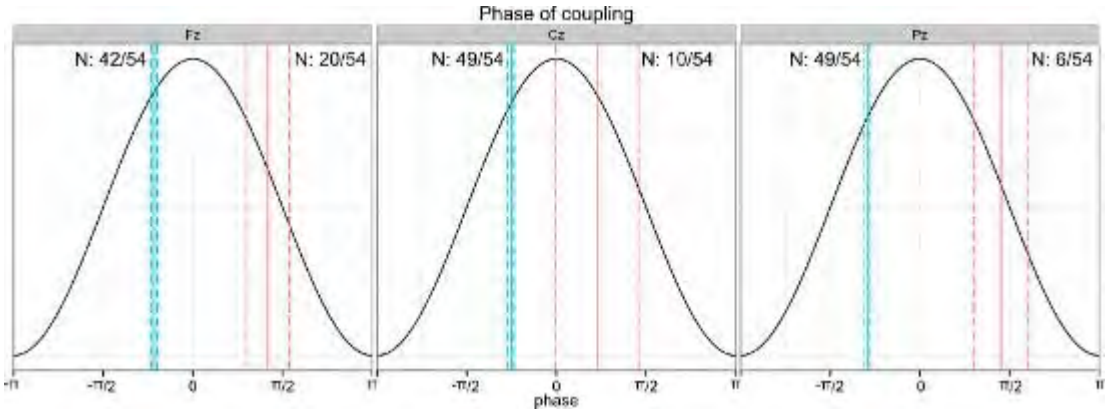
Transcranially applied weak electric oscillatory stimulation can induce responses in behavior and brain electric activity in healthy and diseased subjects. Effects have been attributed to the ability of oscillatory stimulation to entrain endogenous EEG activity, e.g. (1). The present study aimed to analyze endogenous EEG activity during and following slow oscillatory transcranial direct current stimulation (so-tDCS).Methods: In two different studies healthy human subjects conducted memory tasks (associative, perceptual) before and after nocturnal sleep. During the initial phase of nocturnal non-rapid eye movement (NREM) sleep so-tDCS (i.e., with a stimulation frequency close to that of the endogenous slow oscillation of ~0.8 Hz) was applied within five 5-min blocks, separated each by 1 min stimulation-free intervals. Oscillatory stimulation had either an anodal or cathodal offset. Stimulation electrodes were applied either bilaterally over the dorsolateral prefrontal cortex (DLPFC; F3 and F4, return currents at M1 and M2, respectively) or unilaterally over the visual cortex (O1 (O2) with M2(M1). Memory performance was measured as the difference in performance between recall and learning. EEG analyses involved power spectra, time-frequency representations, and cross frequency coupling (CFC, (3) between the slow oscillation stimulation signal (a), or endogenous EEG activity in the slow oscillation frequency band, center frequency, fc=0.75 Hz (b), and slow (9-12 Hz) or fast (12-15 Hz) sleep spindle activity.Results: As expected analyses of CFC between endogenous EEG activity filtered using fc =f 0.75 Hz and spindle activity revealed a strong coupling between the slow oscillatory and fast spindle activity (average coherence at Cz and Pz: 7.8, z-transformed), and a relatively weak coupling between the slow oscillatory and slow spindle activity (average coherence at Fz: 3.6). The phase at which fast and slow spindle activity coupled to endogenous slow oscillatory activity was as previously described for slow oscillations and sleep spindle events, cp. (3); Fig). Preliminary analyses of stimulation over the DLPFC indicate weaker CFC between the so-tDCS signal and endogenous sleep spindle activity than for CFC with the endogenous slow oscillation activity. The phases at which slow and fast spindle activity are coupled to the so-tDCS signal appear to deviate from the endogenous phase pattern. On the other hand, CFC between the endogenous slow oscillatory frequency band and sleep spindle activity during the stimulation block is comparable to that of the sham condition. During stimulation preliminary analyses indicate significant CFC within the third stimulation block for anodal so-tDCs (coherence, slow spindle activity, Fz: 2.8, $p<0.008$; average coherence, fast spindle activity, Cz and Pz: 2.4, $p<0.024$, uncorrected for multiple comparisons). Similar results are reflected in spectral power. Both studies revealed overnight improvement in memory consolidation under sham conditions ($p<0.05$).
Conclusion: The presented preliminary results suggest that effects of weak oscillatory stimulation may not be facilitate a constellation of oscillatory activity identical to the endogenous pattern.

Fig .CFC during endogenous NREM sleep between slow oscillatory and slow (continuous red line) or fast spindle (green) activity at Fz, Cz and Pz. Dashed lines indicate +/-SEM. Numbers indicate number of subjects with significant coupling relative to all subjects tested.

(1) Fröhlich & McCormick 2010 Neuron (2) Canolty et al 2006 Science (3) Mölle et al 2011 Sleep.


Phase of coupling
Fz
Cz
Pz
N: 42/54
N: 20/54
N: 49/54
N: 10/54
N: 49/54
N: 6/54
-π
-π/2
0
π/2
π
phase

Effects of overexpression of the (pro)renin receptor on the hippocampal formation in mice

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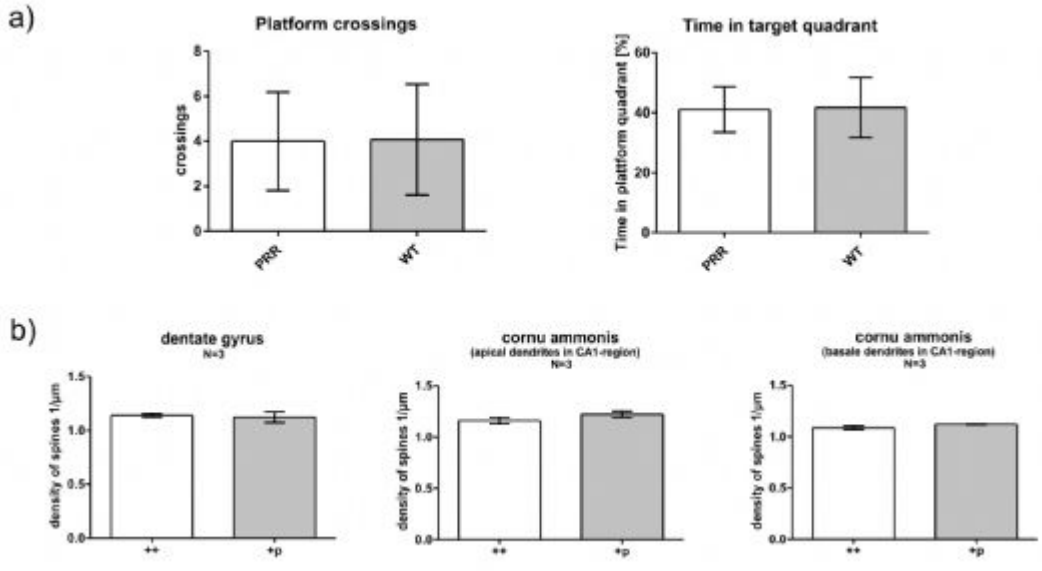
(Pro)renin receptor ((P)RR) mRNA has been detected in a variety of tissues including heart, liver, kidney, and brain. Within the brain (P)RR mRNA has been found, among others, in the thalamus, cortex and hippocampus. We recently studied the expression of (P)RR protein in detail and found that (P)RR protein is strongly expressed within the dentate gyrus. Moreover, we have shown that (P)RR co-localizes with doublecortin (DCX) but not with Sox2, hinting for a role of (P)PR in late stages of adult neurogenesis. Using an knock-down approach, we could indeed demonstrate that (P)PR strongly influenced neurogenesis within the adult hippocampus. In order to get a more detailed view in the involvement of (P)PR in adult neurogenesis, we were analyzing mice that over-express (P)PR by estimating the number of proliferating cells (using phosphohistone H3 as a specific marker) and the numbers of newly formed neuronal cells (using DCX as a specific marker). Since (P)PR protein was also found to be expressed by pyramidal cells in the CA1-area, we further analyzed the impact of over-expression of (P)PR on dendritic spines. In addition, we further analysed whether over-expression of (P)RR translates into behavioural alterations as compared to wild-type littermates. The results so far indicate that over-expression of (P)RR does not alter spatial learning behaviour in the Morris water maze test (a) and does not have an impact upon dendritic spine densities neither in the dentate gyrus nor in area CA1(b).



Extra-coding RNAs regulate neuronal DNA methylation

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Epigenetic mechanisms are essential regulators of the function and information storage capacity of neurons. DNA methylation, a potent epigenetic modification, is highly dynamic in the developing and adult brain, and active DNA methylation and demethylation is required for synaptic plasticity and long-term memory. Although neuronal activity induces alterations in DNA methylation patterns, it is presently unclear how the methylation status at individual genes or even individual cytosine nucleotides can be targeted for modification. Here, we report that neuronal extra-coding RNA (ecRNA) is regulated differently than overlapping mRNA, is sensitive to neuronal activity, and binds to DNA methyltransferases. Intriguingly, genome-wide sequencing approaches reveal that the transcription of extra-coding RNA species from protein-coding genomic loci is a ubiquitous phenomenon in neurons, and is associated with decreased promoter methylation and enhanced mRNA expression, demonstrating a potential mechanism for site-specific regulation of DNA methylation status. Finally, we show that ecRNA presence also regulates expression of overlapping messenger RNA species. These results suggest that ecRNAs are fundamental regulators of the establishment and perpetuation of DNA methylation patterns in neuronal systems, and reveal a promising avenue for therapeutic targeting in neurological and cognitive disease states.

Gene Regulation and Epigenetics of a Lifetime Body-Size Memory in *Drosophila*

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One of the last steps in the neurodevelopment of a *Drosophila* fly is to learn and consolidate the own body size. We have shown that naïve (dark-reared flies) do not know their body reach and try to surmount gaps that clearly exceed their reach by far. We found that naïve flies learn their body size from the parallax motion they create while walking in visual patterns; they keep this body-size memory for the rest of their life. We could identify sets of orthogonally connected neuronal bundles in the central brain of the fly (protocerebral bridge; pb) that require opposing cAMP/PKA signalling levels and activation of the transcriptional regulator CREB to establish the individual body reach. We consider two – not mutually exclusive – scenarios how differential CREB regulation can result in a fixed long-term memory. As one possible mechanism, and as a last step of neuronal differentiation, CREB signalling might induce permanent changes in synaptic connectivity of the orthogonally connected neurons. On the other hand, opposing activity of CREB in the two populations may lead to permanent differential changes in the expression levels of CREB target genes or genes downstream of those. In terms of the latter hypothesis, we speculate that the lifetime body-size memory might be consolidated through epigenetic mechanisms to ensure a fixed change in opposing gene regulation. Although DNA methylation has not been observed in developing or adult *Drosophila*, most genes involved in epigenetics of vertebrates have their counterpart in flies and some of them have been shown to be involved in memory formation (e.g. Kramer et al., 2011). A genetic screen was initiated to identify possible candidates executing epigenetic regulations. Transgenic lines are used to induce mRNA knock-down against ca. 100 genes, putatively involved in *Drosophila* epigenetics. RNA interference is limited to the pb neurons that require CREB activity for memory consolidation. We will present behavioural and histological data on histone acetylases and deacetylases that affected body size memory in our knock-down approach. Moreover, we show that dose compensation on gonosomal genes is necessary to consolidate this memory. This approach will elucidate which epigenetic modifications are required to consolidate this unusually long-lasting memory in flies.

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Generalization of associative olfactory memories: parametric analyses in Drosophila melanogaster

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Animals associate initially neutral stimuli with significant events to be able to act predictively. Fruit flies, for example, when trained with pairings of an odour and electric shock, later on avoid the odour as a predictor for punishment. Other odours induce such conditioned avoidance only to the extent that they are perceived by the fly as similar to the trained one. The magnitude of such behavioral generalization correlates well with the similarity of neuronal representations at various stages of the olfactory pathway (Niewalda et al. 2011, Campbell et al. 2013; for bee, see Guerieri et al. 2005).

Given an aversive life-episode, the generalization vs. specificity of the formed memory is critical for adaptive behaviour: Too much generalization may lead to avoidance of stimuli that actually do not predict punishment; whereas given too much specificity, the animal may fail avoiding the punishment-predicting stimulus, if it varies even only very little from the original. Indeed, in rodent models and humans, behavioural generalization has been suggested as relevant for the behavioural consequences of trauma.

With this in mind, we initiated parametric analyses on the generalization of fly associative olfactory memory. All our experiments followed a common four-group design: Flies were

trained with an odour A, and tested with the same odour A; or
trained with another odour, B, and tested with that very odour; or
trained with odour A, but tested with odour B; or
trained with odour B but tested with odour A.

In this design, any conditioned avoidance in the groups that experienced the odour-switch between training and test reflects generalization; the specificity of the olfactory memory is in turn reflected in the extent to which this switch of odours interferes with conditioned avoidance.

Using this design, we tested the dependence of generalization on (i) the time that elapses between training and test as well as (ii) the repetition of training. Currently, we are gearing up to testing for effects of (iii) the intensity of reinforcement on generalization. All these three parameters are proposed to be critical for rodent- and human-behaviour in the aftermath of trauma (Siegmund & Wotjak 2006).

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Genome-wide analyses on electric shock avoidance, punishment learning and relief learning in *Drosophila melanogaster*

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Minimizing exposure to noxious stimuli has an obvious survival value. It is thus unsurprising that even simple animals possess multiple behavioural strategies aiming towards this end. The most basic form of such behaviour is the innate, reflexive escape from a noxious stimulus, as exemplified by fruit flies avoiding a maze-arm that is applied with pulses of electric shock. In addition, cues that precede a noxious event are learned as predictors for “punishment”, and later on pre-emptively avoided. Interestingly, also cues that occur at the offset of pain are learned, but as signals for the “feeling of relief”, resulting in approach behaviour. Indeed, fruit flies form such opponent memories of an odour, depending on whether it occurs before or after shock during training (Tanimoto et al. 2004, Yarali et al. 2008; for rodents and man, see Andreatta et al. 2010, 2012; for an cross-species review, see Gerber et al. 2014).

Here, we used the fruit fly as a model to comparatively map out the genetic effectors of these three kinds of behaviour, i.e., innate electric shock avoidance, punishment learning and relief learning. We employed a genome-wide approach using 38 nature-derived inbred fly strains. The flies within each strain were genetically identical, whereas across strains, both gene-sequences and gene expression levels substantially varied (Ayroles et al. 2009, Mackay et al. 2012). We found these strains to vary also in terms of all three behaviours we looked at. Thus, for each kind of behaviour we looked for genes whose expression levels and/ or sequences co-varied with the behavioural scores.

Regarding shock avoidance, 514 such “candidate genes” were identified. We independently scrutinized the shock avoidance-role of 14 of these using mutants, validating 7. In addition, integrating our association results with external protein-protein interaction data yielded a shock avoidance-associated network of 38 genes. Both this network and the original candidate gene list contained a substantial number of genes that affect mechanosensory bristles, which are hair-like organs distributed across fly’s body. Also, three of our validated candidate genes were bristle-relevant. These results may potentially point to a previously unrecognized role for mechanosensory bristles in shock sensation. Thus, the follow-up on the association analyses for shock avoidance not only demonstrated the value of the emerging candidate gene list (i.e. these genes stood independent scrutiny by reverse genetics), but may also have provided a novel, attractive hypothesis concerning nociception (i.e. the role for bristles).

Thus, we are currently gearing up to independent testing and gene-network analysis of candidate genes for punishment and relief learning. In the future, some of the validated candidates may perhaps prove relevant for these opponent kinds of memory in rodents and man.

High-resolution comparison between learned and innate olfactory behaviour in larval *Drosophila*

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Drosophila larvae are an attractive study case when trying to understand processes of learning and memory. It has been shown, using group-based measures of preference, that animals prefer an odour previously paired with reward relatively more than an odour that previously was presented separately with reward (Scherer et al. 2003; Saumweber et al. 2011; reviewed in Gerber & Stocker 2007; Schleyer et al. 2013). Such learned olfactory behaviour can be understood as a search for reward as it is suppressed in the presence of the sought-for reward (Gerber & Hendel 2005; Schleyer et al 2011). Notably, innate olfactory behaviour is unaffected by the presence versus absence of reward (Schleyer et al. 2011). In any event, it remained unclear which particular changes in locomotion bring about such learned behaviour, and how it relates to the locomotor programs of innate olfactory preference.

Monitoring larval behaviour in high spatial and temporal resolution revealed that larvae innately orient in odour gradients through a sequential organization of stereotypical behavioural modules, including runs and directed turns (Gomez-Marin et al. 2011). We therefore focus on three basic questions: How fast do larvae run? When do they initiate a turn? Where-to do they turn? We ask these questions regarding either (i) memory-based up- and down-regulations of olfactory preference, or (ii) the concentration-dependent up- and down-regulation of innate olfactory preference. Furthermore, in a model-based generative approach we test whether the observed modulations in those three parameters are sufficient to mimic larval behaviour by a simulated agent. These experiments can provide an interesting study case as to exactly how memory modulates innate behaviour to organize adaptive search. This should be interesting with regard to robotic applications.

How to learn fast and forget slowly with dendritic spines

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The majority of cortical synapses resides on dendritic spines. These spines have, on the one hand, been shown to be involved in learning and long-term memory but, on the other hand, undergo a remarkably high turnover [1, 2]. This seems contradictory, as it is not clear how the information is preserved while its substrate (synapses or spines) is exchanged.
To investigate this phenomenon we use a simple stochastic model of structural plasticity: We assume that there is a certain number of potential synaptic locations from one neuron to another.
At each of those locations synapses (spines) are created with a constant probability and removed with a probability depending on the number of existing synapses. Hereby, the stationary distribution of the number of synapses between two neurons is fully determined by the removal probabilities or vice versa.
Experiments investigating these probability distributions in the cortex found that the majority of connections has either zero or multiple synapses while one or two contacts are very improbable [e.g., 3-5]. Using information theoretic measures we show that such bimodal distributions enable information storage over time-scales many orders of magnitudes higher than the involved probabilities. Thus, in this system the conflict of rapid spine turnover (probabilities) and long-term memory is resolved by storing the information collaboratively in multiple synapses.
In the following, we will consider the bimodal stationary distributions as the working point of the system. Then, we can model external signals, as, e.g., increased or decreased activities during learning, as changes of the removal probabilities (e.g., mediated by synaptic plasticity [6]).
The changed probabilities, in turn, yield a new stationary distribution to which the system converges.
For learning signals, which yield unimodal stationary distributions (only connected or only unconnected), we find that learning is orders of magnitude faster than forgetting. This provides a possible solution to the plasticity-stability dilemma in learning and memory on the time-scale of structural changes.
Finally, we demonstrate that this simple model is consistent with spine creation and removal statistics observed in various learning and memory related experiments [1, 2].

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Imaging of synaptic activity and plasticity in the *Drosophila* brain

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Drosophila represents a key model organism for dissecting neuronal circuits that underlie innate and adaptive behavior. However, this task is limited by a lack of tools to monitor physiological parameters of spatially distributed, central synapses in identified neurons. We generated transgenic fly strains that express functional fluorescence reporters targeted to either pre- or postsynaptic compartments. Two-photon *in vivo* imaging of olfactory projection neurons in the *Drosophila* brain, using olfactory stimuli, demonstrates the functionality of either sensor and of dual-color combinations. We show that the usage of the sensors do not only distinguish pre- and postsynaptic activity, but also detect differential experience-dependent changes in pre- and postsynaptic activity. The technique facilitates the physiological analysis of synaptic connections across defined groups of neurons in intact *Drosophila*, thereby advancing the dissection of synaptic signaling that underlies neuronal processing.

Impact of hippocampal sclerosis lateralization on behavior in the mouse intrahippocampal kainate model of mesial temporal lobe epilepsy

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Mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) is a frequent disabling form of refractory epilepsy in humans (Engel, 2001). MTLE-HS is highly lateralized and leads to cognitive impairments that are to some extent related to the sclerosis side (Witt et al., 2014). Focal seizures, unilateral hippocampal sclerosis and granule cell dispersion are shared features of human patients with MTLE-HS and the mouse intrahippocampal kainate model (Bouilleret et al., 1999; Haussler et al., 2012).
To assess the effect of the hippocampal sclerosis side on seizure severity and behavior in an MTLE model, we injected kainate in the left (n = 10) or right (n = 7) dorsal hippocampus to induce MTLE in adult male C57Bl/6 mice. Spatial memory (Morris water maze test), anxiety (light/dark box test) and novel environment exploration (open field test) were assessed four weeks after injection, i.e. during chronic epilepsy. Seizures were then recorded after electrode implantation in left and right dorsal hippocampi.
Left (LKA) and right (RKA) kainate-injected mice swam a longer distance to reach the hidden platform than saline mice and did not show improvement over days in the water maze. This could be explained either by a dysfunction of their spatial memory itself, by an incapacity to understand the task, or by a high level of anxiety. KA-treated mice spent more than 50% close to the walls of the water maze ("thigmotactic" behavior) vs. less than 10% in saline mice, which could reflect a high anxiety level or misunderstanding of the task goal. However KA-treated mice performance improved when the platform was visible, excluding the hypothesis of a misunderstanding of the goal, as well as motivation and vision problems. Anxiety assessment with the light/dark box test revealed a longer time spent by RKA in the "safe" dark closed compartment compared to LKA and saline mice. This data could reflect a higher level of anxiety in RKA mice, possibly due to extended lesions or spreading seizures in the ventral hippocampus and amygdala, which are the two major brain structures relevant for anxiety and belong to the mesial temporal lobe where seizures occur. Finally, all groups explored a large open field equally reflecting neither novel environment exploration impairment nor gross motor deficits.
This study is the first demonstration of a lateralization effect of hippocampal sclerosis on behavior in a model of MTLE. Sclerosis lateralization predominantly impacts on anxiety, with RKA mice expressing an anxious behavior compared to LKA mice, while water maze performance is altered in the same way, independently of the injection side. When this performance could be explained by higher anxiety level in RKA mice, LKA mice seem unable to learn the location of a hidden platform arguing for a navigation system impairment. This result likely puts the role of the lesion extent into perspective.

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Impact of life history on fear memory and extinction in mice

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Cumulative stress throughout life time is one of the predominant environmental risk factors for several psychiatric disorders, such as depression or anxiety disorders. So far different hypotheses exist that address the correlation between early and adult chronic stress and the individuals' vulnerability to psychiatric diseases. The match/mismatch hypothesis of psychiatric disease states that the early life environment shapes coping strategies in a manner that enables individuals to optimally face similar environments later in life (Santarelli et al. 2014).
In the present study we aim to determine the influence of a matched/mismatched life history on fear memory and extinction in mice. Therefore, five groups of C57Bl/6J male mice underwent differential treatment (AA = adverse early + adverse late environment, BB = beneficial early + beneficial late environment, AB = adverse early + beneficial late environment; BA = beneficial early + adverse late environment, C = Control) from prenatal stage till the age of 75±2 days before being tested for fear memory and extinction processes in a Pavlovian fear conditioning paradigm.
To determine whether life history modulates neurophysiological activities in brain regions related to fear and anxiety, we recorded from the infralimbic region of the medial prefrontal cortex (IL, mPFC) and the lateral amygdala (LA), respectively. Fear memory and extinction of conditioned fear was observed in parallel to recordings of neuronal activity during phases of retrieval (R1), extinction learning (R2-R6), recall of fear extinction (E1-E2) and spontaneous recovery of fear (SR).
First results indicate an impairment of fear extinction in animals raised in an AA and BA environment which is accompanied by theta coupling of IL and LA during R1, R6 and E1. In contrast, animals with a beneficial early and late background in their life history showed a trend towards enhanced extinction learning. Interestingly, animals initially raised adversely and afterwards encountering a beneficial environment display similar behaviour when compared to control animals.
These preliminary data suggest that environmental factors influence fear related behaviours, contributing to a differential outcome in fear memory and extinction depending on the individual life history in mice.

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Impact of the cortical extracellular matrix on memory and learning flexibility

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Remodeling of synaptic networks are indispensable key events during learning and memory formation and re-consolidation throughout life. The extracellular matrix (ECM) has been considered to serve for such stabilization of synaptic networks in the adult brain. Whether the ECM might thereby govern learning-related plasticity, life-long memory re-formation and higher cognitive functions is largely unknown.

Recent research from our lab has enlightened a new role of the mature ECM actively organizing the balance between structural stability and functional synaptic plasticity. We investigated the impact of local enzymatic removal of the ECM in auditory cortex of adult Mongolian gerbils on cortex-dependent auditory re-learning behavior induced by the contingency reversal within a frequency-modulated (FM) tone discrimination shuttle-box task. Relearning during states of weakened ECM was improved, but did not generally impact the retrieval of previous acquired memories. Hence, ECM-removal opened short-term windows of enhanced activity-dependent reorganization promoting complex forms of behavioral strategy change during learning (Happel et al., PNAS, 2014). This first study implicated a novel function of the cortical ECM as a potential regulatory switch to adjust the balance between stability and plasticity in the adult, learning brain.

We now investigate the molecular basis of enhanced synaptic plasticity derived by intrinsic ECM-modifications. Reduction of ECM *in vitro* has been implicated in regulating synaptic short-term plasticity by enhanced synaptic exchange of postsynaptic receptors (Frischknecht et al., Nat Neurosci, 2009). To foster the insights into intrinsic plasticity mechanisms during learning, we analyze proteolytically induced ECM remodeling in the adult, learning brain using post-training quantitative Western blot analysis. We will discuss relationships between learning performances and respective inherent ECM-protein dynamics, with an emphasis on comparisons of initial acquisition learning and long-term retrieval.

Learning of declarative memory after sleep is influenced by D-cycloserine given during sleep

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Sleep has beneficial effects on consolidation and subsequent learning of declarative memory. However, the neurochemical processes remain elusive. In the hippocampus, long term potentiation (LTP) is mediated by N-methyl-D-aspartate-receptors (NMDA) containing the NR2A subunit and long term depression (LTD) by receptors containing NR2B. The NMDA-receptor co-agonist D-cycloserine (DCS) preferentially acts through NR2A containing receptors favouring LTP over LTD. DCS aids sleep-dependent memory consolidation but may perturb subsequent new learning. In a double-blind, placebo-controlled, balanced crossover study participants learned two lists of word-pairs and then orally received DCS or placebo before sleeping 8 hours. The next evening, they learned two new lists of word-pairs, one list was composed of completely new word-pairs, the other of cues taken from one of the original lists which were paired with a new word (interference list). Afterwards, participants were asked to retrieve the original word pairs learned before sleep. Interestingly, our preliminarily results (n=10) indicate that learning after sleep is significantly improved after sleep with DCS independent of interference. Results including the whole sample with will be presented on the poster.

Locomotion patterns induced by learned odors in the honey bee (*Apis mellifera* L.)

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Learned odors thus influence the behaviour of bees. This has been widely investigated using classical conditioning (Menzel, 1999: Giurfa and Sandoz, 2012). The flight trajectories towards an odor (CS) with which a bee had been associatively rewarded during classical conditioning were investigated in a wind tunnel. The bees flew significantly around the odor source with upwind zigzagging and circling (Chaffiol et al., 2005). Bees also approach the CS in a Y-maze (Martin 1964, Carcaud et al, 2009). Walking patterns towards odor sources were also investigated on a locomotion compensator (Kramer, 1976). Bees that had been trained associatively to a particular odor at a particular concentration using a sugar reward could orient towards this conditioned odor at the given concentration. Kramer suggested that this is possible by switching over from negative to positive anemotaxis at the learned concentration (Kramer, 1976). Osmotropotaxis together with osmoklinotaxis are of vital importance in orientatioj of honeybee (Martin, 1965) Using a simpler test, the residence times in scented zones of a four-armed olfactometer, it could be shown that bees spent more time in zone with an odor previously associated with a sugar reward (Sandoz et al., 2000). However in these studies, the odor-induced walking behaviors, that may be indicative of odor-source localization strategies have not been analyzed quantitatively. While honeybees almost exclusively move by flight outside the hive, they walk inside the hive, for instance to communicate with hive mates. This is therefore a natural context in which walking orientation towards associatively learned odors can occur.

Honeybees were differentially conditioned using an odor associated with a reward (CS+) and an odor not associated with a reward (CS-) and their locomotor pattern was investigated. Conditioned bees preferred the CS+ section in a four-armed olfactometer. Detailed locomotor patterns were analyzed with conditioned bees tethered to walk on a trackball locomotion recorder. Stimulation with CS+ induced local walks around the virtual position in which the stimulus was received, with slow progress both forward and laterally, resulting in small cumulative values of forward and lateral displacement integrated over 1 min following the start of a recording. During the olfactory stimulus alternated turns with about 20 degrees turn angle and about 6 Hz were induced. The turn angle after CS+ stimulation is significantly larger than that during CS+ stimulation. We call this walking pattern “local search” (for an odor source). In contrast, CS- stimulation as well as stimulation with an odor unrelated to conditioning induced walks at higher speed covering a larger area that had no clear relation to the stimulus position, with fast progress both forward and laterally. The turn angles during CS- stimulation are significantly larger than during CS+ stimulation. The walking pattern by CS-, that could not be triggered by stimulation with air alone, represents also odorant-induced arousal and is called “exploratory locomotion”. Reward-associated odors thus induce a specific walking pattern that may be designed to efficiently localize a trophallaxis donor carrying a known odor to beg for nectar. This work is supported by the Ministry of Education, Science, Technology, Sports and Culture of Japan (Grant Number 22570079).

Long-term avoidance memory leads to a transient increase of synaptic complexes in the mushroom bodies of ants

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Long-term behavioral changes related to learning and experience have been shown to be associated with structural remodeling in the brain. Leaf-cutting ants learn to avoid previously preferred plants after those are experienced to be harmful for their symbiotic fungus, a process that involves long-term olfactory memory. We studied the dynamics of brain microarchitectural changes after long-term olfactory memory formation following avoidance learning in *Acromyrmex ambiguus*. Quantification of synaptic complexes (microglomeruli, MG) in olfactory regions of the mushroom bodies (MB) at different times after learning revealed a transient change in MG densities. Two days after learning, lip MG density was higher than before learning. At days 4 and 15 after learning — when ants still showed plant avoidance — MG densities had decreased to the initial state. Changes were observed in the olfactory lip but not in the visual collar in which MG densities remained unaffected. Furthermore, enriched experience such as the simultaneous collection of several, instead of one, non-harmful plant species resulted in a decrease in MG densities (pruning). The results indicate that learning and sensory experience affect the synaptic architecture of the MB calyces via different processes. While sensory experience leads to MG pruning in the MB olfactory lip, long-term memory formation appears to involve growth of new MG followed by the elimination of others. *Supported by DAAD (A/11/76441) and DFG SFB 1047 'Insect timing' (projects B6 and C1)*.

Modeling the Interaction of Long-Term Memory and Working Memory

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Learning and memorizing past information are critical features of neural networks. Several models have been proposed to explain the storage and retrieval of different kinds of information [1, 2] . These models differ in terms of the types of memories they can store, in terms of the time scales on which they operate, and in terms of the persistence of the stored information. Depending on the concrete choice, they are able to explain different features of memory systems observed in humans and animals. In particular, there are neural models for the long-term memory (LTM) system [2] and other neural models for the working memory (WM) system [1]. While there are some controversies whether LTM and WM are realized by distinct brain regions, there is no doubt about the fact that these two system are at the same time tightly coupled and interact continuously [3]. It is widely unclear, however, how this interaction is actually realized. Here, we demonstrate a possible schema to simulate and explain the interaction between LTM and WM such as to exploit the abilities and advantages of the individual components.

To model LTM, we employ the well-known idea of cell assemblies [4]. According to this idea, declarative knowledge translates into correlations of neural activities of groups of neurons. Common correlations are stored by ways of strong excitatory synapses in between these neurons. Groups of highly interconnected neurons are called cell assemblies. If the synapses in a recurrent network are governed by Hebbian plasticity, cell assemblies emerge in an unsupervised manner based on correlations observed in the network input.

For modeling WM, a completely different type of neural networks is used: Reservoir computing networks are a powerful class of artificial neural networks [5]. They consist of a large reservoir of randomly and recurrently connected neurons and a set of input neurons. Due to the huge variety of different signals present in the reservoir, these networks can serve as universal function approximators. They can perform complex tasks including temporal memory tasks. The influence of past inputs fades away within short time scales.

In the proposed interaction schema, a third component is introduced: The representation layer contains a set of neurons each of them representing a certain entity, for instance, a certain number or a special context. It receives signals from both the cell assembly network as well as the reservoir. The reservoir is trained to perform certain arithmetic operations. During operation, the cell assembly network learns to detect and remember associations between context signals and computed results. This enables the system to remember results of earlier calculations and to reuse them for later tasks. Thus, the system is able to perform computation based on short-term memories of the operands as well as to overcome the limits of the short-term memory by storing computed results into the long-term memory system.

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Neural circuit analyses of relief learning in fruit flies

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A painful event leaves behind two opposing kinds of memory. Stimuli that precede pain later on induce aversive behaviour, whereas stimuli that follow the offset of pain are remembered pleasantly. Such timing-dependent opponency seems to be an evolutionarily conserved property of associative learning [reviewed in Gerber et al. 2014]. We study the neuronal bases of this property using the fruit fly as a model.

Flies, when trained such that an odour precedes shock, later on avoid it as a signal for punishment. Quiet on the other hand, if an odour is presented after the shock during training, flies associate it with the relief at shock-offset and show conditioned approach [Tanimoto et al. 2004, Yarali et al. 2008].

Olfactory punishment learning is fairly well studied in the fly. During training, the odour signal and the reinforcement signal, carried by identified dopaminergic neurons, converge on a brain structure called the “mushroom body”. Upon this convergence, mushroom body output synapses towards pre-motor centers are modified to enable conditioned avoidance.

We found that relief learning, just as punishment learning, relies on mushroom body function, too. Following up on this finding, we now aim at uncovering a minimal mushroom body-centered circuit, within which relief-reinforcement is signaled, relief memories are laid down, stored and retrieved. Previous attempts at identifying a neuronal reinforcement pathway for relief learning had been unsuccessful [Yarali & Gerber 2010], likely due to the inadequacy of the neuronal interference tools used. We now use a novel set of transgenic tools, which enable us to express a blocker of action potentials in almost all mushroom body-associated neurons, in very small groups at a time, to then screen for effects on relief learning. We present preliminary results with respect to neurons that are efferent to the mushroom bodies, and thus are candidates for “reading out” relief memories.

In the future, detailed fly circuit maps of relief-, punishment- and reward- learning can be integrated into mathematical models, to point to circuit principles that may be evolutionarily conserved, and/ or may be implemented in robotic devices.

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Neural circuits of memory re-evaluation

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Retrieving a memory makes it malleable and entails its re-evaluation. This property of memory has been considered as a strategy to target maladaptive memories in humans, by either producing a memory of opposing valence (extinction learning) or by disruption of the initial memory (interference with reconsolidation). Even though the underlying neuronal processes are of high interest, they are poorly understood. The relatively simple central nervous system and the sophisticated genetic toolbox of the fruit fly *Drosophila melanogaster* make it an ideal system to investigate the neural circuits involved in memory re-evaluation. Fruit flies can learn to associate an odour with reward or punishment and they form a long lasting memory of this association. The activity of discrete dopaminergic neurons is needed to assign either a positive or a negative value to an odour during learning. These dopaminergic neurons project to different zones of the major integration centre of the fly brain, the mushroom body. Altering the activity of various sub-divisions of the mushroom body associated network has permitted us to identify a role in memory re-evaluation.

Neural Correlations in Brains of Freely Walking Social and Solitary Bees during Navigation in an Artificial Environment

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Single walking bees (bumble bee *Bombus terrestris* and solitary bee *Osmia cornuta*) are trained to navigate in an artificial environment (AE) containing a visual local cue and panorama patterns. Animals memorize both local cue and panorama irrespective of the spatial relationship between these two kinds of visual cues. Based on this finding, we set out to investigate the neural correlates in the brains of bees navigating in AE. We aim for high order neurons (mushroom body extrinsic neurons), because neurons in this region integrate different kinds of highly processed sensory cues. So far we found that a neuron unit in a bumble bee crossing a color-contrast border in both directions became much more active. We also found in a solitary bee (*Osmia cornuta*) that her locomotion and sensory simulations were synchronized with changes of the neural activities of mushroom body extrinsic neurons.

Neuronal correlates of instrumental learning in honeybees

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The focus of our work is the search for neural correlates of instrumental learning in honeybees in a virtual environment. We record multiple units of mushroom body extrinsic neurons over several days in the behaving animal.

The mushroom body (MB), a higher-order integration center in the insect brain, is known to be involved in the consolidation and retrieval of memory. MB extrinsic neurons are expected to recode the sensory input according to its value. Putative inhibitory neurons of the protocerebral calycal track (PCT) feed-back from the MB output to the MB input region. They change their response when bees learn visual and olfactory stimuli. Thus we expect read-out from the MB to be involved in instrumental learning.
We achieve extracellular recordings in animals controlling their visual environment by the movement of a floating ball that they walk on. The virtual environment consists of colored objects that can selectively be rewarded. In addition odors can be introduced into the environment. The animal´s walking trace through the virtual environment is recorded simultaneously with the recordings.
To determine whether we created a close to natural learning situation in the virtual environment, in precedent behavioral experiments, we analyzed bees that were first trained in free flight in a T-maze and then introduced into the virtual environment for a test situation. In the virtual environment, bees recapitulated learned behavior from the natural situation: animals chose the prior rewarded object also in the virtual situation when corresponding visual patterns to the natural training situation were presented.
In current experiments naïve animals are instrumentally trained in the virtual environment. Animals learn to choose selected objects while we record at the same time. When approaching one selected object, the bees are rewarded. During instrumental learning and in test situations, changes in the neural activities related to reward expectation were observed.

Neuronal correlates of unlearned calls in male and female zebrafinches

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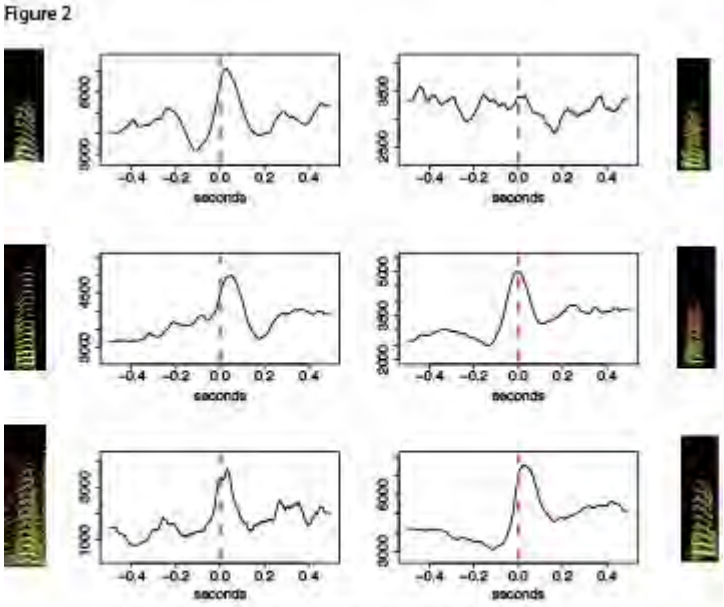
In songbirds, the emergence of the ability to produce learned vocalizations coincides with the evolution of the forebrain vocal control system, an interconnected network of brain nuclei that shapes the song during learning and organizes the motor output when singing. These structures are missing or have only rudimentary neural homologs in non-learning relatives.
We present evidence that, in addition to learned song production, social calling patterns also involve the song control system. We first describe patterns of calling between individual zebra finches living in dense groups using wireless backpack microphones and show that one type of unlearned soft calls, the stack call, is exchanged almost exclusively between bonded partners. To study the neural activity underlying this communication pattern we performed neuronal recording from free ranging males and females (fig. 1), interacting with their partners.
Local field potentials and multiunit activity recorded both from males and females show a strong association with the production of stack calls.
Thus, the song control circuit in males could have evolved from a more basic system necessary to differentiate between the partners call and that of other group members and develop a pattern of precisely timed close range vocal exchange.

Figure 2



Rectified local field potetentials (LFP) of RA in six females, aligned to their respective stack calls (red dashed line). Note that the right topmost figure shows no response to own call in the LFP. The electrode missed the focal area and was placed caudal from RA.

Neuronal responses to mild heat and electric shock in the brain of *Drosophila*

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Drosophila melanogaster aversive associative learning has been studied in various classical conditioning paradigms. In the most common paradigm an odor is used as the conditioned stimulus and electric shock as the unconditioned stimulus. However, flies are also able to form an aversive odor memory when mild heat is used as an unconditioned stimulus. So far, it was unclear if mild heat reinforcement is mediated by the same neuronal pathway as electric shock. Behavioral experiments with transgenic flies revealed that temperature-sensitive anterior cell (AC) neurons are involved in the signaling of mild heat but not electric shock while subpopulations of dopaminergic (DA) neurons are involved in both.

We used *in vivo* calcium imaging with the calcium indicator GCaMP3 *(UAS-GCaMP3)* to record from the somata of AC neurons *(dTrpA1^{SH}-GAL4)* and DA neurons *(TH-GAL4)* during mild heat and electric shock stimulation. Our results show that AC neurons respond reliably with an intracellular calcium increase to mild heat but not to electric shock. DA neuron responses to mild heat were similar across the different DA clusters but changed upon repeated stimulation. DA neurons differed in their response strength and response latency to electric shock. In some cases, the sequence of stimulus delivery appeared to influence the response strength in DA neurons. We also measured the effect of temperature increase on GCaMP3 signals in antennal lobe olfactory receptor neurons *(Orco-GAL4)* and found that the raw fluorescence as well as odor responses were reduced upon mild heat stimulation. This suggests that temperature increase reduces the fluorescence of GCaMP3 and therefore masks neuronal responses in calcium imaging. Summing up, we found that mild heat and electric shock differ in their effect on neural activation. Our calcium imaging data supports the behavioral data that revealed converging neuronal circuits for the two aversive reinforcers.

Neuronal substrate of associative fear conditioning in the zebrafish (*Danio rerio*) – measured by c-Fos expression

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Skin damage causes the release of a pheromone (Schreckstoff) that elicits inborn anti-predator escape and fear reactions in shoaling fish, such as zebrafish. Chondroitin fragments are odorants that trigger this behavior. Control over inducement of fear responses can be transferred through conditioning to other stimuli that do not initially provoke anti-predator behavior. Presentation of a neutral visual stimulus in combination with the alarm pheromone leads to an association between the two stimuli. For successful association, it takes only one combined stimulus presentation and the initial neutral stimulus is transformed into a learned elicitor of fear behavior in the fish. When investigating neuronal substrate of learning in fish in the past, mostly lesion studies were conducted, which indicated an involvement of the lateral telencephalic pallium (Dl) and especially the medial telencephalic pallium (Dm) in emotional learning. These two pallial regions are known to be interconnected and communicate during a learning process. In this project, we wanted to find out whether there are additional brain regions involved in associative fear conditioning, which might constitute a memory formatting neuronal network. In a single trial, zebrafish of one group (I) were taught to associate the presentation of a neutral color-stimulus (red light) with the occurrence of Chondroitin. In order to validate substance effectivity, we compared the behavior of group I with control animals (0) that experienced only red light. In a second group (II), fear behavior was additionally investigated with red light presentation only one day after associative learning. We found high efficiency of alarm pheromone presentation on fish fear behavior in groups I and II. On the second day, presentation of the red light stimulus elicited fear behavior in group II at comparable level as on the day before with pheromone presentation. Furthermore, the brains were investigated by immunohistochemical labeling of the expression of the c-Fos protein, which is overexpressed in active neurons. We found an increase of c-Fos in both pallial regions, Dm and Dl, in groups I and II. We also inspected subpallial brain regions (striatum) and nuclei in the diencephalon: thalamus, posterior tuberculum, and hypothalamus.

Neuropeptide S receptor-deficient mice in a mouse model of post-traumatic stress disorder

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Fear optimally prepares the brain and body for dangerous situations, which helps humans and animals cope with potentially dangerous events. Dysfunctions within the mechanisms underlying fear can lead to maladaptive fear. Some clinical manifestations of such maladaptive fear are post-traumatic stress disorder or panic disorder. Several clinical studies identified a polymorphism in the neuropeptide S (NPS) receptor gene that is associated with an increased incidence of panic disorder. Moreover, the identified risk allele of the NPS receptor interacts with unfavorable developmental conditions (here: childhood maltreatment).

These findings motivated us to investigate the phenotype of mice lacking the NPS receptor in an animal model of post-traumatic stress disorder. In this paradigm (Siegmund and Wotjak, 2007), mice are fear conditioned with a single very intense electric stimulus (the 'traumatic experience'). After an incubation time of 4 weeks, the animals are then tested for the specificity of their fear memory and whether they are hyper-sensitive to novel stimuli. These mice then express a generalized fear memory as well as hypersensitivity to novel stimuli – both symptoms of human post-traumatic stress disorder. Control mice that are tested after 1 week, do not express generalized fear memory and hypersensitivity.

Our first data generally confirmed the published incubation time effect on behavior (generalized fear memory). Further¬more, corticosterone plasma levels strongly increased after the incubation time. However, NPS receptor-deficient mice seem to be not very different to their littermates: We only found a more specific fear memory after 1 week and increased anxiety measures after 4 weeks. Further data are currently collected.

NOGO-A INFLUENCES STRUCTURAL DYNAMICS OF MOSSY FIBER SYNAPSES

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Nogo-A has been shown to negatively regulate activity-dependent synaptic plasticity in the hippocampus, especially by restricting long-term potentiation at the Schaffer collaterals-CA1 pathway. Moreover it is involved in maintaining the mature dendritic architecture of CA3 pyramidal cells. Interestingly, Nogo-A is expressed not only in oligodendrocytes and in pyramidal neurons of the CA1 - CA3 regions of the hippocampus, but also in granule cells of the dentate gyrus. Granule cell axons, the mossy fibers projecting to the CA3 pyramidal cell dendrites, are unmyelinated, thus providing an interesting model for analyzing the role of only neuronal Nogo-A in regulating functional and structural synaptic plasticity. Moreover, the mossy fibers are characterized by the presence of huge presynaptic structures having unique properties when compared to other synapses in the brain. In particular, they have been shown to regulate both synaptic excitation and inhibition of signal transduction to the CA3 circuitry of the hippocampus, as the main terminals innervate the excitatory CA3 pyramidal cells, whereas the filopodial extensions emerging from the terminal contact inhibitory interneurons. In addition, the mossy fiber synapses have been demonstrated to rearrange their morphological structure and functional connectivity in response to experience and to play an important role in hippocampal dependent memory.

Although the knowledge about the properties of the mossy fiber synapses is growing, the molecules involved in the regulation of the activity-dependent synaptic plasticity remain to be fully determined. In this work we elucidate whether Nogo-A plays a role in regulating the structural plasticity of mossy fiber synapses. We performed time-lapse confocal imaging of organotypic hippocampal slice cultures derived from transgenic mice expressing a membrane targeted form of eGFP in a subpopulation of neurons through the brain (Thy-1 mGFP transgenic mice). We compared the short- and long-term structural reorganization of mossy fiber terminals and their filopodia extensions following an acute versus a chronic treatment with a specific Nogo-A function blocking antibody.

Our results show that the acute Nogo-A neutralization increases the short-term motility of the core region of the mossy fiber terminals. Conversely, the chronic treatment with Nogo-A blocking antibody leads to a reduction of the size of the mossy fiber terminals as well as of the length of their filopodia extensions. Hence, Nogo-A differently regulates the structural dynamics of the mossy fiber synapses, depending on the time-scale.

In conclusion, this result represents the first insight into the role of neuronal Nogo-A in regulating the structural plasticity of mossy fiber synapses. Current experiments are aimed at addressing the role of Nogo-A in regulating activity-dependent functional plasticity at the mossy fiber-CA3 pathway of the hippocampus.

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Optically evoked Sharpwave-Ripple Waveforms in Hippocampal Circuits Indicate Formation of Neural Assemblies

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It is widely believed that the formation and maintenance of neuronal ensembles in the brain may reflect mnemonic processes such as memory encoding and long-term memory storage. The defining feature of these groups of neurons is their temporally coordinated activity pattern during a certain task in relation to an underlying neural oscillation. The hippocampus, a brain region which is closely associated with episodic and spatial memory contains spatially tuned neurons, so called place cells and is a widely used model system for studying neuronal ensembles. The main reason for this is the observation that these place cells display firing in a certain sequence when an experimental animal follows a trajectory through an open field. These sequences are replayed during resting periods in a time-compressed manner along with so-called sharpwave-ripple complexes (SPW-R). These oscillatory signatures replace the underlying theta frequency bands (4-12Hz) which dominate exploratory behaviours. Taking rules of classical Hebbian Plasticity into account, this suggests, that those neurons which fire in a certain time window increase the strength of their synaptic connections ultimately leading to a stabilization of the memory trace. We use optogenetic tools together with a pattern illumination system in an *in-vitro* approach to artificially induce SPW-R in an acute hippocampal slice preparation. We found that optically evoked field potential signatures closely resemble spontaneously generated SPW-R and determined the minimum area that has to be illuminated in order to generate these potentials. These data could provide first insights into the working principles of neural assemblies.

Pathway Analysis of a Visual Working Memory in *Drosophila*

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Flies, like other higher organisms, use orientation strategies to navigate in their complex environment and are not just guided by their acute sensory input. They rather integrate information and memorize it to achieve goal-driven behavior. Here, we analyze the working memory of idiothetic and visual information that a walking fly uses to navigate to a former target that got out of sight. Previous studies have shown that this visual orientation memory for attractive targets is localized in the ellipsoid-body of the central complex in the adult brain. Former experiments revealed that the cGMP-dependent kinase encoded by *foraging* (*for*) and the *ignorant* (*ign*) kinase, the flies´ orthologue of Ribosomal-S6-kinase2 (RSK2), are required for this kind of working memory. Moreover, various rescue experiments confirmed that FOR and IGN are only necessary in one of the four known types of ring neurons (R3) to enable a proper orientation memory. Furthermore, a genetic and epistatic interaction study revealed that these two kinases interact in the same signaling pathway and that FOR acts upstream of IGN kinase (Kuntz et al., 2012).

Mutants of *ellipsoid-body-open* (*ebo*) display a defective working memory as well. *ebo* encodes Exportin6 (EXP6), which is required for the export of actin from the nucleus. In *ebo* mutants, actin accumulates in the nucleus and thereby suppresses the interaction of the transcription factors dSRF and dMRTF. But surprisingly, the *ebo* phenotype could be rescued in all four subtypes of ring neurons. The rescue in any type of ring neurons indicates that the transcriptional complex of dSRF and dMRTF has to regulate the transcription of a gene/enzyme that produces a diffusible memory factor (Thran et al., 2013). Here we show that nitric oxide (NO) is involved in the working memory. NO is produced by the nitric oxide synthase (NOS). NOS expression is found in the ellipsoid body and furthermore, *NOS* mutants have a reduced working memory. Extended genetic interaction studies revealed that Nitric oxide synthase (NOS), the Guanylyl cyclase (GycA99B) and FOR are sharing the same signaling pathway to enable this visual working memory. Moreover, we demonstrate that FOR-IGN pathway is required to activate the transcriptional activating factor CREB. Additionally, the *ign* null mutant ($ign^{58/1}$) has less CREB-activity and the $ign^{58/1}$ memory deficit can be rescued by overexpressing CREB in R3 ring neurons. These results demonstrate that transcriptional regulation by CREB is not only required for long-term memory formation but also for this ultra-short term memory. We therefore hypothesize that CREB activates gene expression of competence factors, which enables the ring neurons to perform high-speed decision-making.

PharmaKirGenetics: Neuron inhibition by small molecule-induced Kir2.1 stabilization

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Existing methods for neuronal silencing, e.g. DREADDs and optogenetics, have their disadvantages and, therefore, we searched for an alternative strategy to exert selective, remote and reversible inhibitory control on neurons and circuits in living animals. We adapted a pharmacogenetic method for direct pharmacological control of protein stability. Briefly, mutants of the human FKBP12 protein undergo rapid and constitutive degradation in cells, and this instability is conferred to other proteins fused to these destabilizing domains (DD). A synthetic ligand (shield1) binds to the destabilizing domains and shields them from degradation, allowing fused proteins to perform their cellular functions.

Here we attached DD to Kir2.1, a strong inwardly rectifying potassium channel crucially involved in regulating neuronal excitability and action potential cessation, and expressed DD-Kir2.1 via a pIRES2-EGFP vector in HEK293T cells to prove in vitro effects. We also probed our approach in vivo creating a virus, AAV-DD-Kir2.1, to transduce thalamic neurons in mice. Intracellular recordings in acute brain slices revealed DD-Kir2.1 expression/stabilization by ligand-mediated increases in RMPs, changes in cell resistance and excitability. Behaviorally, we could demonstrate neuronal silencing of transduced striatal neurons using the paradigm of stereotypical amphetamine-induced unilateral rotations, that could be reversibly blocked by systemically administered shield1.

In conclusion, we could establish a novel pharmacogenetic approach that enables selective, remote and reversible inhibition of neuronal cell with little invasiveness and thus may serve an interesting tool in behavioral neurosciences.

Plasticity in mushroom body physiology correlates with behavioral learning performance in individual honeybees.

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We used two complementary experimental approaches to study neuronal correlates of learning at two different sites within the mushroom body (MB) of the honeybee. We find neural plasticity at the input to the MB and in the activity of MB output neurons. In both cases the magnitude of the physiological plasticity strongly and significantly correlates with the individual behavioral performance across animals. Our results indicate that learning-induced plasticity at different stages of the MB circuitry underlies the behaviorally relevant encoding of a stimulus ‘value’ at the MB output as a prerequisite for the expression of a conditioned behavior.

We used a differential conditioning paradigm (Bitterman et al., 1983) where an odor (conditioned stimulus , CS+) is repeatedly paired with a reward (unconditioned stimulus, US) while a control stimulus (CS-) remains unrewarded. In this paradigm individual honeybees can rapidly learn the association (Menzel 2012; Pamir et al., 2014) as evidenced by the expression of a conditioned response to the CS+ odor.

In a first setting, we used Ca-imaging of projection neuron boutons in the MB (Yamagata et al., 2009) while performing a differential conditioning protocol. We quantified physiological plasticity by the absolute difference in the single bouton Ca-responses to repeated CS+ and CS- presentations post and pre conditioning. We find that both, the responses to CS+ and CS- can change significantly as a result of learning and that individual boutons could equally increase or decrease their activation level for both stimulus types. In order to relate bouton plasticity to behavioral plasticity we quantified the behavioral learning performance in each individual animal. Surprisingly, the level of plasticity strongly and significantly correlates with the performance level across individuals.

In a second approach, we performed extracellular single-unit recordings from MB extrinsic neurons (Strube-Bloss, Nawrot & Menzel, 2011) that provide the MB output in the context of differential conditioning. When comparing single neuron responses to the CS+ and CS- before and after conditioning we find that individual neurons can develop a novel response to either stimulus or they increase or decrease their stimulus strength. Overall the average response to the CS+ increases while the average response to the CS- remains unchanged. Again, we find a strong and significant positive correlation of the single neuron plasticity and the behavioral learning score across individual animals. The early neuronal responses precede the behavioral response by at least ~200ms, underpinning a causal relation.

Our results demonstrate that the magnitude of plasticity at the level of single circuit elements – boutons at

the MB input site and neurons at the MB output site – is predictive of the individual behavior.

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Plasticity of a defined mushroom body-output synapse underlies learned olfactory behavior in *Drosophila*

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During olfactory learning in fruit flies dopaminergic neurons assign value to odor representations in the mushroom body Kenyon cells. Here we identify a class of six downstream glutamatergic neurons whose dendritic fields overlap with identified dopaminergic neuron projections. This anatomy and their odor-tuning suggests that these neurons pool odor-driven Kenyon cell synaptic outputs. Like that of mushroom body neurons, this output is required for expression of appetitive and aversive memory performance. Moreover, appetitive and aversive olfactory conditioning bi-directionally alter the relative odor-drive of these neurons. Directly blocking these neurons in naïve flies mimics appetitive conditioning, being sufficient to convert odor-driven avoidance into approach, while optogenetically activating these neurons induces avoidance behavior. We therefore propose that this junction is a key site of learning-relevant synaptic plasticity for odor-guided behavioral choice.

Processing of Competing Visual Stimuli in the Central Complex of *Drosophila melanogaster*

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Learning and memory are among the most intriguing adaptation processes that have evolved in animals and humans. One form of memory, a visual working memory, is investigated here on a neural and molecular level. Working memories are ephemeral but nevertheless important forms of short-term storage of current intentions and currently incoming sensory information, which are kept for just a few seconds. Different information must be encoded in specific neurons with particular properties and biochemistry.

A specific form of visual working memory is studied here in the model organism *Drosophila melanogaster*. In a virtual-reality panorama flies are confronted with two identical attractive visual objects, initially under ±60° azimuth angle to the fly, which are, however, just alternatingly visible. The slightly longer-shown visual object will attract wild-type flies, which can ignore the alternative object after a short while. A defective ellipsoid body within the central complex of the fly brain can cause zig-zagging behavior; the respective mutant *ellipsoid body open* (*ebo*$^{-}$) flies keep approaching always the currently visible object. Stabilization of the walking direction in wild-type flies can be explained under the assumption that visual information is integrated in a specific manner in a neural network involving the ellipsoid body.

This integration process requires the S6KII kinase encoded by *ignorant* but is otherwise biochemically and neuronally distinct from the visual working memory enabling flies to pursue the approach to a visual target that became invisible during approach (detour memory; Neuser et al. 2008; Kuntz et al. 2012). Rescues of the ellipsoid body open mutation with regard to competing visual stimuli are effective in R1 or R2 ring neurons of the ellipsoid body, but not in the R3 system, the seat of the detour memory. Ten flies of each strain had to complete ten approaches each; the length of each walking trace was measured and the number of arrivals at the more salient object was determined. Furthermore, the role of electrical synapses in the integration network is different. Eight innexin genes are found in *Drosophila* which encode different kinds of gap junctions. Either the gene for *inx6*, *inx7* or *inx8* has been knocked-down with the help of a UAS-RNAi-construct. The GAL4-system was used to direct the knock-down to the R3 ring system of the ellipsoid body. None of the innexins is required to bring about avoidance of zig-zagging behavior. We conclude that different forms of visual working memory reside in the different ring systems of the ellipsoid body.

Relief learning is controlled by dopamine release in the rat nucleus accumbens

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Contrary to popular belief, fearful situations do not exclusively induce aversive memories. In backward fear conditioning for instance, the conditioning stimulus (CS) is presented close in time after the termination of an unconditional stimulus (US) and will acquire a positive valence behavior that can be measured by a startle response attenuation. Backward fear conditioning is also called “relief learning” since the CS is presented during the relief from the aversive event. Previously, we have revealed a crucial role of the nucleus accumbens (NAc) in conditioned relief memory. Temporary inactivation of the NAc in rats strongly reduced the acquisition and expression of conditioned relief. In humans, functional brain imaging showed an activation of the NAc during the expression of conditioned relief. The GABAergic spiny cells of the NAC express dopamine D1 and D2 receptors, receive important dopaminergic input from the ventral tegmental area, and this input have been strongly related with reward learning.

The aim of the present study was to investigate the role of dopamine transmission within the NAc during relief learning. In brief, adult male Sprague-Dawley rats were locally microinjected into the nucleus accumbens core region with a D1 receptor antagonist (SCH-23390) or a D2 receptor antagonist (raclopride) before the acquisition of relief learning. Relief conditioning consisted of 15 presentation of electric stimuli (US) followed by a light stimulus (CS). One day later, the rats were put into the startle chamber to test the effects of the light CS on the startle response (retention test).

Our data clearly indicate that both D1 and D2 receptors are involved in relief learning. However, D1 receptor blockade was more effective than D2 receptor blockade. We consider that our results can help to understand the contribution of relief feelings to pathological emotional memories after traumatic experiences.

Selective retrograde amnesia of associative memory after Neuroplastin loss and severe deficits in neuroplastin-deficient mice

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The neuroplastin (Np) cell recognition molecules are single pass membrane glycoproteins of the Ig-superfamily. Two isoforms, Np55 and Np65, result from alternative splicing of the neuroplastin mRNA, and carry 2 and 3 immunoglobulin-like domains, respectively. Np55 is expressed in a wide range of tissues but Np65 is restricted to neurons (Langnaese *et al*. 1998; Smalla *et al*. 2000). In cultured hippocampal neurons, Neuroplastins induce synaptic Ca^{2+} responses by interaction with the fibroblast growth factor receptor 1 (Owczarek *et al*. 2010, 2011). The maintenance of long lasting LTP *in vitro* can be disturbed by the recombinant ectodomain of Np65 or by anti-Np65 antibodies (Smalla *et al*. 2000; Empson *et al*. 2006). Np65 colocalizes with $GABA_A$ receptors affecting receptor mobility and synaptic strength (Sarto-Jackson *et al*. 2012). Importantly, as neuroplastins participate in the formation and maintenance of synaptic structures, the proper balance of inhibitory and excitatory synapses depends on neuroplastins (Herrera-Molina *et al*., 2014). Polymorphisms in the *neuroplastin* gene likely to modify the expression of neuroplastins were found to be associated with schizophrenia (Saito *et al*. 2007) or a reduced thickness of the brain cortex and verbal and nonverbal intellectual abilities in adolescents (Desrivières *et al*. 2014).

To investigate the functional importance of neuroplastins, we generated loss-of-function mouse mutants. Homozygous Np-deficient mice ($NPTN^{-/-}$) are viable but display a reduced life expectancy and lower body weight in comparison to their wild-type ($NPTN^{+/+}$) littermates. Anatomy and morphology of the $NPTN^{-/-}$ mouse brain appear generally normal with all major structural entities present. However, the number of glutamatergic synapses in subfields of the hippocampus is reduced. In comparison to controls, $NPTN^{-/-}$ mice display significantly elevated serum levels of the major stress hormone corticosterone and a dysregulation of the Hypothalamic-Pituitary-Adrenal axis. $NPTN^{-/-}$ male mice are incompetent in producing offspring and display substantially reduced levels of testosterone. The behavioral analysis of $NPTN^{-/-}$ mice revealed multiple deficits related to motoric capabilities, anxiety, fear, associative learning, sensorimotor gating, hedonia and social behavior.

Neuron-specific *neuroplastin* gene inactivation in adult mice revealed selective deficits in associative learning. Furthermore, *neuroplastin* ablation induced after the acquisition of learning tasks caused complete retrograde amnesia specifically for the acquired associative task.

Our results show that neuroplastins serve essential functions during development in multiple organs and constitutive loss of neuroplastins results in profound pleiotropic deficits. The acute loss of neuronal Nps causes specific associative learning deficits and establishes a role of neuroplastins in assessing an associative memory trace. Inducible neuroplastin-deficiency may, thus, be employed to study molecular

mechanisms of memory.

Sensory memory forms in the caudomedial nidopallium during song learning

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There are some similarities between human speech acquisition and bird song learning. Like humans learn to speak, birds learn to sing from their experience of adult conspecific vocalizations in early developmental stages. In zebra finches *(Taeniopygia guttata)*, premier model species in the field of bird song learning, exclusively male birds learn to sing in the developmental critical period. During the sensory phase of critical period male juveniles listen and memorize a tutor song. This memory is then used for sensory-motor matching to gradually shape the bird's own song during the later sensorimotor phase. Increasing evidence suggests that the neural substrate for the tutor song memory is located in the caudomedial nidopallium (NCM), an area homologous to the mammalian auditory-associated cortex. In this study we sought a trace of tutor song memory in the neuronal activity in NCM by performing electrophysiological recordings using an 8-channel multi-electrode array. Male juvenile zebra finches were isolated from father together with the mother and siblings at 10-12 days post hatching (PHD), before the start of the sensory period and thus before a memory of the tutor song could be formed. Starting at 55 PHD they were tutored by a male conspecific (mostly the father) for five days which was sufficient for establishing a tutor song memory, but was not long enough for changing the bird's own song significantly. At the day following tutoring they were subjected to electrophysiological recordings. During the recordings neuronal units throughout NCM were probed with varieties of conspecific and heterospecific songs, including the tutor song, familiar and unfamiliar zebra finch songs, and tested for biased responses to the experienced tutor song. The short tutor exposure allowed us to clearly differentiate neuronal responses to an auditory experienced song from vocally mimicked song. In tutored birds a fraction of neuronal units, about 8 % (10 out of 10 individuals), showed biased responses to the tutor song. By contrast, in the age matched controls with no tutor song experience only about 2 % of units (6 out of 11 individuals) were biased to the tutor song. Tutor song-biased units were predominantly located in the medial and ventral part of NCM. Our results suggest that sensory memory for the tutor song forms in the auditory-associated cortex of male juveniles within a few days of auditory experience.

Sleep and Predictive Coding: Can Sleep improve Information Processing in the Visual System?

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Predictive coding theories describe the idea that the brain generates predictions of upcoming sensory events based on previous experiences, e.g. visual perceptual memories. As memory consolidation has been shown to be sleep-dependent, we hypothesize that sleep compared to wakefulness improves the development of predictive coding. Specifically, we test whether performance improvements during initial learning of a visual statistical learning paradigm are consolidated during sleep, but not during an equivalent period of wakefulness. The behavioural study described here will establish the experimental parameters for subsequent EEG and fMRI experiments on implicit memory consolidation.

Specific contribution of CA1 to the reconsolidation of fear memory: an optogenetic/molecular imaging study

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It is believed that once a memory is reactivated, it enters a labile state during which it is prone to modifications before returning to a more stable state after reconsolidation. Blocking reconsolidation, for example with drugs, can permanently 'erase' fear memories and this concept is the basis for new reconsolidation-based treatments for anxiety and Post-traumatic stress disorders.
While the amygdala is known to play a crucial role in the reconsolidation process, less is known about the specific contribution of the hippocampus, especially that of the CA1 subfield, which is crucial for the retrieval of fear memory.
To investigate the role of CA1 in the reconsolidation of fear memory, we combined optogenetics with high resolution molecular imaging. Following reactivation of the fear memory by reexposure to the conditioning context, we aimed at blocking the restabilization of the memory by inhibiting cell firing in CA1 in a temporally and spatially precise manner. We showed that mice displayed significantly less freezing behavior when exposed for the third time to the conditioning box the following day. This result suggests that CA1 is crucial for reconsolidation. CA1 levels of cellular activation were also studied to bring further support to this finding.

Studying the mushroom body memory matrix one cell at a time

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We focus on the neuronal substrates of associative ODOR-SUGAR learning in larval *Drosophila*. The sensory pathways as well as memory trace formation at the mushroom body, a third order 'cortical' brain area, are reasonably well understood. However, two important aspects lack a systematic understanding:
Which specific, individual neurons map reward information onto the mushroom bodies?
Which specific, individual neurons read out information from the mushroom bodies to organize learned behavior?
In a collaborative project we investigated Mushroom Body Extrinsic neurons (MBEs), using the genetic toolkit available at the Janelia Farm Research Campus. We inspected a collection of about 800 sparsely expressing GMR-Gal4 strains, selected based on the anatomical annotation of multicolor flip-outs and established a single-cell atlas of all the ~45 mushroom body extrinsic neurons.
Using the Janelia GMR-Gal4 collection as well as the Split-Gal4 technique, we performed an inhibition screen with strains containing at least one MBE using *shibire* and *Kir* to block synaptic output. Our results suggest that dopaminergic input of single cells towards the medial lobe of the mushroom body and two corresponding output neurons are necessary for appetitive olfactory learning and may well define a functional micro-circuit.
To unravel the associative architecture up to the subcellular level, an ongoing project generated a complete volume of a first instar larval brain using serial section transmission electron microscopy at 3.8 x 3.8 x 50 nm resolution. Identifying and reconstructing MBEs in this volume as well as reconstructing all the pre- and postsynaptic partners of task-relevant MBEs will enable us to identify the network from olfactory and gustatory sensation all through to the motor system.
We will now combine single cell activation with fine-grained behavior analysis, and formalize our findings into a computational model. This will aid the implementation into a bio-inspired robot for memory-based adaptive search.

Synapsin functions in timing-dependent behavioural plasticity

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We study the oppositely valenced memories that result from differences in associative timing, in the fruitfly Drosophila melanogaster. If an odour is delivered before an electroshock, flies form ‘negative’ memories that can support escape from the odour during a subsequent test. In contrast, if the odour is applied after the electroshock, at the moment of ‘relief’, a ‘positive’ memory is formed that can support learned approach (Tanimoto et al 2004). These kinds of learning, called punishment-learning and relief-learning, respectively, can likewise be established in rat and man (review Gerber et al. 2014). In the fly, punishment-learning has been exhaustively studied, while much less is known about relief-learning.

In an attempt to understand these types of processes on the neurogenetic level, we focus on Synapsin, an evolutionarily conserved presynaptic phosphoprotein. In Synapsin-less flies (syn97 deletion null-mutant; review Diegelmann et al. 2013), punishment-learning is significantly reduced and relief-learning is abolished. This phenotype is due to a genuine dampening in associative ability rather than to alterations in the temporal properties of coincidence detection. A lack of Synapsin also leaves sensory and motor faculties intact, further strengthening the attribution of the observed phenotype to a defect in associative processing. The phenotype in punishment- and relief-learning can be phenocopied by a downregulation of Synapsin using RNAi. We further show that both punishment- and relief- learning can be re-established by restoring Synaspin expression acutely and locally, in the mushroom body.

We conclude that Synapsin features in both punishment- and relief-learning. Similar commonalities between the molecular mechanisms of trauma-related punishment- and relief-learning in man would be of practical concern: systemic treatments could unwittingly reduce both types of memory, with a net effect that would be difficult to predict in valence.

Diegelmann et al 2013. Maggot learning and Synapsin function. J Exp Biol 216: 939-51.

Gerber et al 2014. Pain-relief learning in flies, rats, and man: Basic research and applied perspectives. Learn Mem. 21:232-52.

Tanimoto et al 2004. Event-timing turns punishment to reward. Nature 430:983.

Synapsin is required to establish a strong memory in odor-sugar associative learning

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One of the brains more fascinating features is to allow the organism to learn and to remember. Here, we focus on the role of Synapsin, using odor-sugar associative memory trace formation in larval *Drosophila* as a study case.

Synapsin likely plays a major role in associative memory processes. Synapsins belong to a family of conserved phosphoproteins associated with the cytoplasmic side of synaptic vesicles (Greengard et al., 1993; Hilfiker et al., 1999; Hosaka et al., 1999). *Drosophila* Synapsin is encoded by only one gene (syn; CG 3985) and is expressed in most or even all neurons in the larva and adult *Drosophila*. Synapsin is required for proper associative function. Adult flies as well as larvae lacking Synapsin show up to 50 % less associative learning as compared to wild-type animals. Notably, Synapsin phosphorylation seems to be important in the mode of operation of this protein (Schwaerzel et al., 2003; Riemensperger et al., 2005; Schroll et al., 2006, Michels et al., 2011). It loosenes the attachment of reserve-pool vesicles to the cytoskeleton and alows their recruitment to the readily-releasable pool of synaptic vesicles.

We specifically focus on the question in which way the Synapsin null mutant (*syn*^{97}) phenotype of *Drosophila* larvae depends on certain parameters of an odor-sugar associative learning experiment. To systematically investigate this topic we varied parameters which are known to affect memory scores in the wild-type, namely odor concentration (Mischra et al., 2011) as well as sugar concentration (Schipanski et al., 2008) and the time interval between training and test.

Our results suggest that odor-sugar learning in *syn*^{97} mutant larvae, different from wild-type, hardly benefit from increasing odor concentration and sugar concentration. Specifically in the *syn*^{97} mutant associative learning remained at low levels across the range of tested odor and sugar concentrations. In contrast, the wild-type learning scores increased for higher concentrations of odor and sugar. Thus, in the absence of Synapsin *Drosophila* larvae can learn and remember, yet in order to benefit from an increased salience of odors or of the reward for establishing stronger memories Synapsin is required: Without Synapsin the upper limit in mnemonic capacity is lower.

The connectome of the mushroom body of the *Drosophila* larva

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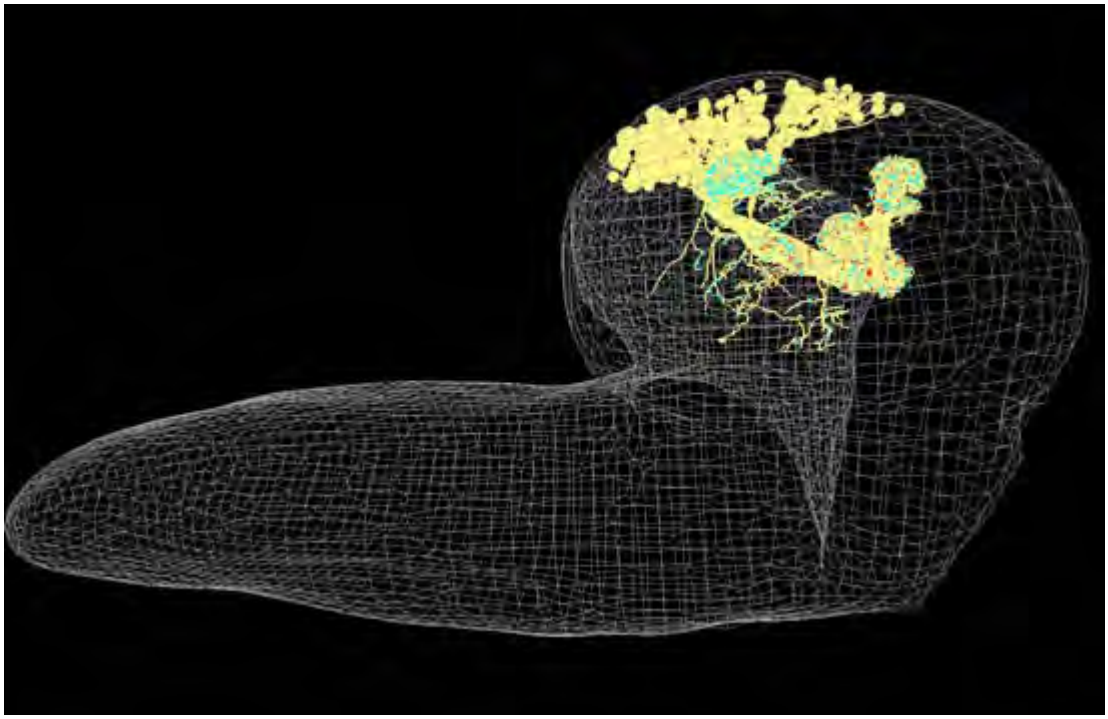
Insect mushroom bodies are central brain structures that integrate olfactory, visual, gustatory and mechanosensory information. It was also shown that the mushroom body intrinsic neurons, the so-called Kenyon cells, harbor a memory trace after associative olfactory conditioning. Thus, the current model for Drosophila melanogaster suggests that rewarding and punishing environmental stimuli are linked with olfactory information within the intrinsic mushroom body Kenyon cells to establish learning dependent modifications.

Identifying the cellular morphology and synaptic connectivity of intrinsic mushroom body Kenyon cells is therefore crucial for understanding learning dependent modifications in this higher brain center. In collaboration with the Cardona lab (HHMI Janelia Farm) we have started to reconstruct the entire set of intrinsic mushroom body Kenyon cells of the simple Drosophila larval brain. The Cardona lab has made available to the larval research community a complete volume of a first instar larval brain, generated by Rick D. Fetter for the Fly EM Project Team using serial section transmission electron microscopy. Given the 3.8x3.8x50-nm resolution of the EM volume and the custom-made analysis software, we are now reconstructing all intrinsic mushroom body Kenyon cells and their synaptic partners up to the single synapse level. Taken together, the aim of the project is to generate the complete wiring diagram of the intrinsic mushroom body Kenyon cells of the Drosophila larval brain.

Ultimately, the establishment of the connectome of the larval mushroom body of Drosophila will support generating functional models, which can be tested in behavioral assays, and thus in the longer run to understand how learning dependent modifications are established in insect brains.



The Importance of Visual and Olfactory Stimuli During Flower Visits in the Honey Bee

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Flowers attract honey bees using colour and scent signals. Bimodality (having both scent and colour) leads to increased visitation rates, but how the signals influence each other in a foraging situation is still controversial. We trained and tested free-flying bees in an open arena with scent-and-colour stimuli, and studied four basic questions:

When faced with conflicting scent and colour information, will bees choose by scent and ignore the "wrong" colour, or vice versa? It turned out that the result depends on stimulus quality: if the colours were very similar, bees chose by scent. If colours were very different, bees chose by colour. We used the same scents, lavender and rosemary, in both cases. We analysed choices, but also pre-choice behaviour ("aborted choices").

Our second question was: Are individual bees hardwired to use one sensory modality and ignore the other, or can this behaviour be modified, depending on which cue is more readily available in the current foraging context? To study this question, we picked colour-preferring bees and gave them extra training on scent-only stimuli. Afterwards, we tested if their preference had changed, and if they still remembered the scent stimulus they had originally used as their main cue. We came to the conclusion that a colour preference can be reversed through scent-only training.

Our third question was: Do bees learn bimodal stimuli as the sum of their parts (elemental learning), or as a new stimulus which is different from the sum of the components' parts (configural learning)? We trained bees on bimodal stimuli, then tested them on the colour components only, and the scent components only. We performed this experiment with a similar colour set, and a very different colour set, but used lavender and rosemary for scent stimuli in both cases. Our experiment yielded unexpected results: with the very different colours, bees showed elemental learning, but with the similar colour set, bees exhibited configural learning and did not recognize the components alone – even though their memory of the bimodal compound was excellent.

Finally, we looked at reverse-learning. We reverse-trained bees with bimodal stimuli to find out whether bimodality leads to better reverse-learning compared to monomodal stimuli. We performed this experiment with both colour sets, always using the same two scents (lavender and rosemary). It turned out that bimodality does not help bees to "get the task" and anticipate the switch. Generally, bees trained on the different colour set performed better than bees trained on the similar colour set, indicating that stimulus salience influences reverse-learning.

The role of CaMKII in the formation of long-term memory in the honeybee

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The calcium-calmodulin dependent protein kinase II (CaMKII) is a protein essential for memory acquisition and learning. It is often declared as a "molecular memory switch" as it remains activated even after the initial excitation of the neuron is no longer present. It "switches" to a calcium independent constitutively active state, thereby providing a mechanism for a molecular memory.
In honeybees CaMKII was shown to be highly abundant in the mushroom bodies (MBs). These brain neuropils are known to be centers for sensory integration, association and learning in many insects including the honeybee. To investigate a potential role of CaMKII in memory formation of honeybees, we used RNA interference to knock down, and two different CaMKII inhibitors, to disrupt the function of the protein. To specifically and effectively reach the MBs, the 3 substrates were injected directly in the brain via the ocellar tract. 8 hours (siRNA) or 1 hour (inhibitors) later the bees were subjected to classical olfactory conditioning to train the bees to associate an odor with a sucrose reward. Afterwards we tested whether the bees were able to transfer the learned association into short term memory (= STM, tested 1 hour later), early long term memory (eLTM, tested 24 hours later) and late long term memory (lLTM, tested 72 hours later). The results for the bees injected with the two inhibitors and the siRNA showed a learning acquisition equal to the control groups. All groups displayed an intact STM, but both stages of LTM - eLTM and lLTM - were significantly impaired after injection of siRNA and the inhibitors compared to the control groups. This suggests the necessity of functional CaMKII in the MBs for the induction of LTM in honeybees.
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The role of sleep in fear extinction memory in mice

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Abnormal fear is a hallmark of several psychiatric disorders, such as phobias, general anxiety and post-traumatic stress disorder. Treatment of fear-related pathologies is based in exposure therapies, where stimuli associated with the phobic and/or traumatic event are repeatedly presented under safe and controlled conditions, which can lead to a gradual extinction of the fear response. In rodents, Pavlovian fear conditioning and extinction are widely used models to study mechanisms of acquired fear memory. Sleep is suggested to have an important role in memory consolidation, including emotional memories. In both humans and rodents, sleep following fear conditioning can improve fear consolidation as well as discrimination between fearful and non-fearful stimuli. In addition, sleep was also suggested to support the consolidation of fear extinction memories. Here, we want to further investigate the role of sleep in the behavioral expression of fear extinction memory, strength and discrimination.

We assessed the role of sleep in fear extinction memory consolidation in mice, using Pavlovian discriminative auditory fear conditioning and extinction. We found that animals trained in the beginning of their resting phase and undergoing undisturbed sleep following the extinction training, presented significant and context-specific retrieval of fear extinction memory. On the other hand, mice that were sleep deprived in the first 5 hours following extinction training, failed to show fear extinction retrieval on the next day. These results indicate, that during the circadian resting time, sleep deprivation impairs the consolidation of fear extinction memories. Moreover, we are currently testing animals trained at the beginning of their active phase. Preliminary results indicate that animals that are left unperturbed after learning also retrieve extinction memory. Laboratory mice also sleep during the active phase, however in considerable less amount than during the resting phase. Therefore, we are currently testing animals that are kept awake after extinction training during the active phase.

In addition, we are starting to evaluate activity patterns and sleep stages following extinction training, in mice undergoing sleep or sleep deprivation conditions using video monitoring and EEG recordings. We will correlate these with efficacy, persistence, and stimulus discrimination of extinction memory retrieval.

Very low birth weight piglets show improved cognitive performance in the spatial cognitive holeboard task

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Low birth weight (LBW) is common in humans and has been found to cause lasting cognitive and developmental deficits later in life. It is thought that the primary cause is intra-uterine growth restriction due to a shortage of oxygen and nutrients supply to the fetus. Pigs appear to be a good model animal to investigate long-term cognitive effects of LBW, as LBW is common in commercially farmed breeds of pigs. Moreover, pigs are developmentally similar to humans and can be trained to perform complex tasks. In this study, we trained ten very low birth weight (vLBW) piglets and their ten normal birth weight (NBW) siblings in a spatial cognitive holeboard task in order to investigate long-term cognitive effects of LBW. In this task, four out of sixteen holes contain a hidden food reward, which allows measuring working memory (short-term) and reference memory (long-term) in parallel. Piglets were trained for 46-54 trials during the acquisition phase, followed by a 20-trial reversal phase in which a different set of four holes was baited. Both groups acquired the task and improved their performance over time. A mixed model repeated measures ANOVA revealed that vLBW piglets showed a better reference memory performance than NBW piglets in both the acquisition and reversal phase. Additionally, the vLBW piglets fell back less in working memory scores than the NBW animals when switched to the reversal phase. These findings are contrary to findings in humans. A higher food motivation of the vLBW animals may be one of the factors responsible for their better performance. Moreover, vLBW pigs had lower hair cortisol concentrations than NBW pigs in flank hair at 12 weeks of age. These results could indicate that restricted growth causes compensatory mechanisms to arise in early development that result in beneficial effects for vLBW piglets, increasing their low survival chances in early-life competition.

Poster Topic

T26: Computational Neuroscience

T26-1A A 3D Dynamic Model for the Study of Central Drive of Antennal Movements in Insects
Nalin Harischandra, André F. Krause, Volker Dürr

T26-2A A Simplified Model for Oscillatory Population Dynamics in Visual Cortex
Daniel Harnack, Klaus Richard Pawelzik, Udo Alexander Ernst, Sina Tootoonian

T26-3A Basal ganglia dynamics during movement initiation: a computational model for transient beta oscillations
Amin Mirzaei, Arvind Kumar, Daniel Leventhal, Nicolas Mallet, Ad Aertsen, Joshua Berke, Robert Schmidt

T26-4A Boosting Coordinated Reset Stimulation by Slowly-Varying-Sequences: a computational study
Magteld Zeitler, Peter A. Tass

T26-5A Challenging a dynamical threshold equation for action potential initiation for its generality
Lukas Sonnenberg, Jan Benda

T26-6A Channel Mechanisms for a Hierarchy of Timescales Across the Human Cortex
Benjamin Staar, Alberto Bernacchia

T26-7A Computational characterization of Axon Carrying Dendrites Cells: Electrophysiological properties and synaptic integration
Antonio Yanez, Alexei V. Egorov, Tobias Rau, Christian Thome, Andreas Draguhn, Martin Both

T26-8A Computational modeling of LTP and concurrent ‘heterosynaptic’ LTD in the dentate gyrus in vivo
Peter Jedlicka, Lubica Benuskova, Wickliffe C. Abraham

T26-1B Effect of Alzheimer disease on the dynamical and computational characteristics of recurrent neural networks
Claudia Bachmann, Tom Tetzlaff, Susanne Kunkel, Abigail Morrison

T26-2B Effects of neuronal diversity illustrated by current- and voltage-clamp experiments in a virtual laboratory
Aubin Tchaptchet, Hans Albert Braun

T26-3B Efficient coding of rewards in strategic decision-making
Florin Ionita, Alberto Bernacchia

T26-4B From randomly connected to spatially organized multi-layered cortical network models
Johanna Senk, Espen Hagen, Sacha van Albada, Markus Diesmann

T26-5B Functional role of opponent, dopamine modulated D1/D2 plasticity in reinforcement learning
Philipp Weidel, Abigail Morrison, Jenia Jitsev

T26-6B Genetic networks specifying the functional architecture of orientation domains in V1
Joscha Liedtke, Fred Wolf

T26-7B Hybrid scheme for modeling local field potentials from point-neuron networks
Espen Hagen, David Dahmen, Maria L. Stavrinou, Henrik Lindén, Tom Tetzlaff, Sacha van Albada, Markus Diesmann, Sonja Grün, Gaute T. Einevoll

T26-8B Impact of parametric uncertainties in computational models for deep brain stimulation
Christian Schmidt, Ursula van Rienen

T26-1C Impaired homeostatic regulation of feedback inhibition associated with system deficiency to detect fluctuation in stimulus intensity: a simulation study
Faramarz Faghihi, Ahmed Moustafa

T26-2C Integrating Touch and Vision in Stick Insects and Insectoid Robots
Thierry Hoinville, Nalin Harischandra, Volker Dürr

T26-3C Lability and constancy of orientation tuning in the visual cortex depends on the functional architecture
Juan Daniel Florez Weidinger, Wolfgang Keil, Siegrid Löwel, Fred Wolf

T26-4C Mesoscopic population dynamics of spiking neurons derived from single cell properties
Tilo Schwalger, Moritz Deger, Wulfram Gerstner

T26-5C Neuronal morphology and spike onset rapidness modulate the dynamic gain in cultured hippocampal neurons
Elinor Lazarov, Michael Gutnick, Fred Wolf, Andreas Neef

T26-6C Online Parameter Estimation using GPU Super-Computing
Thomas Nowotny

T26-7C Shaping phase space of neural networks via connectivity
Maximilian Schmidt, Jannis Schuecker, Markus Diesmann, Moritz Helias

T26-8C State dependent modulation of dopamine function in the striatum
Marko Filipovic, Lars Hunger, Kai Du, Jeanette Hellgren-Kotaleski, Gilad Silberberg, Robert Schmidt, Arvind Kumar

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T26-7D Tonic conductance changes in the Central Amygdala influence fear generalization
Martin Angelhuber, Paolo Botta, Andreas Luthi, Ad Aertsen, Arvind Kumar

T26-8D Ultra-fast response to external electric pulses explained by neural morphology
Andreas Neef

A 3D Dynamic Model for the Study of Central Drive of Antennal Movements in Insects

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An important aspect of autonomous behaviors in animals is the active exploration of their environment using different sensing systems, allowing them to achieve context-dependent control of actions. One sensory system involved is active touch. In this study, we are developing a general framework of coupled Central Pattern Generators (CPGs) to be used in the context of movement generation for active tactile exploration. CPGs are biological neural networks that produce rhythmic outputs without rhythmical inputs. Stick insects show rhythmic tactile exploration behavior during walking with coordinated movement of two joints per antenna. The phase difference of the distal scape-pedicel (SP) joint and the proximal head-scape (HS) joint is about twenty degrees with the SP joint leading. The neuronal network that generates this rhythmic antennal movement is unknown, but it has been localized in the brain and shown to be sensitive to the muscarinic acetylcholine agonist pilocarpine. Here, we develop a 3D dynamic, skeletal model of the stick insect head and antennae with physically realistic parameters. It is driven by a CPG consisting of two layers: a rhythm generator and a pattern-forming network. The CPG is modeled using a system of coupled non-linear oscillators, and is able to successfully produce biologically realistic movements (joint kinematics) of the skeletal model. We found that the phase lead of the SP joint could vary from about 10 to 30 degrees without disrupting the characteristic, elliptical trajectory of the antennal tip.

In real stick insects, ablating proprioceptive hair fields on the antenna has no effect on the overall pattern of inter-joint coordination but affects the working-ranges of both antennal joints. Our model is able to simulate the effect of these ablations on the joint kinematics by changing the amplitude and offset of the corresponding oscillator in the CPG network. We propose that these hair fields control the local effective stiffness of each joint by acting through a negative feedback loop. Possible pathways could either act directly on the muscles actuating the corresponding joint, or act on the CPG that drives these muscles. The current skeletal system can be easily extended by adding a muscle model to control the joint movements in order to obtain a fully functional Neuro-Musculo-Skeletal-Control system for the insect head-antennal system. We conclude that the 3D dynamic model and the simulator, is capable of mimicking neurophysiological phenomena and will be useful in experiments on adaptive tactile exploration systems in future investigations in silico.

A Simplified Model for Oscillatory Population Dynamics in Visual Cortex

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A remarkable feature of the brain is its ability to flexibly process information in a context-dependent manner. A hallmark example is selective visual attention. It allows to focus on relevant information, and to ignore distracting features of a visual scene, effectively routing information through the visual hierarchy **[1]**. Building on the hypothesis of 'Communication through Coherence' **[2]**, we previously developed a biologically plausible, hierarchical network model of attentional selection in the visual system **[3]**. In this model, phase relations between neuronal populations self-organize to facilitate information transmission from an attended stimulus and impede transmission from unattended ones.

We now show that neuronal population dynamics of this biophysically detailed model can be described in a reduced fashion: every population can be characterized solely by a mean firing rate and phase, following the approach in **[4]**. In a suitable working regime, the parameters of the full model can be mapped onto the parameters of the reduced model. We demonstrate that the main characteristics of the full model, namely self-organization of relative phases and attention dependent information routing, are well captured in this reduced version.

A benefit of the reduced model is that it is mathematical tractable: Considering two competing populations in one layer converging onto the same population in a second layer, the parameter ranges in which the system reaches stable steady state solutions are determined. Also the steady state rates and phases can be found analytically. We furthermore show that under specific circumstances, arbitrarily small differences in the mean rates of competing populations in the first layer have a very strong impact on their respective phase difference to the second layer population. This allows for efficient selective information transmission and flexible switching between different routing configurations by a weak attentional signal.

By inverse mapping onto the parameters of the full model, we then determine the theoretical bounds in which efficient information transmission by coherence can occur in spiking neural network models.

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Basal ganglia dynamics during movement initiation: a computational model for transient beta oscillations

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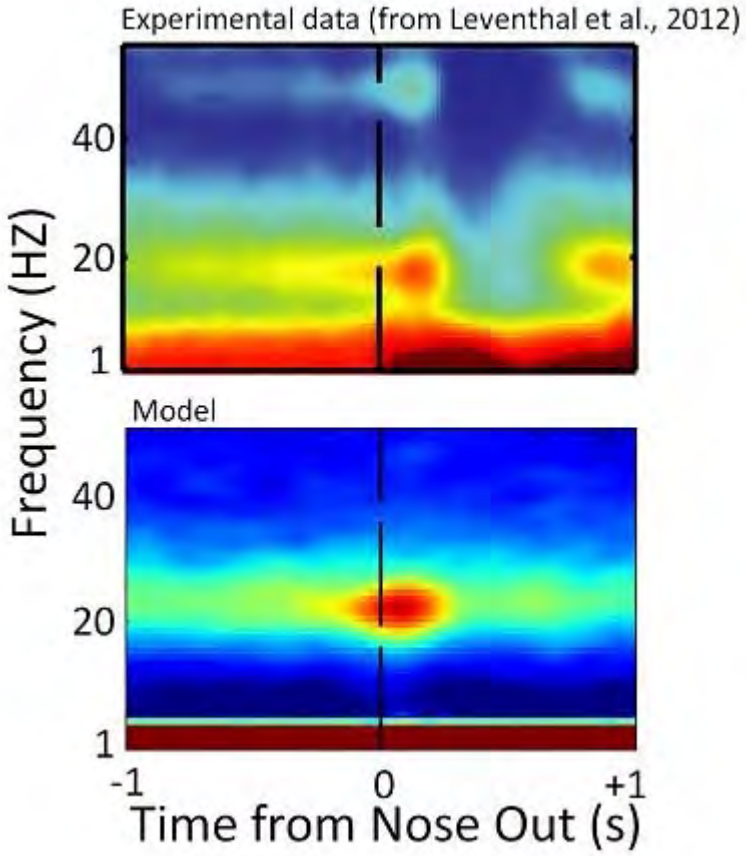
Beta oscillations are often considered as pathological activity in Parkinson's disease and have also been implicated in motor suppression of healthy animals. However, recently we demonstrated that behavioral correlates of beta oscillations are more complex as, for example, beta power also increased in rats when sensory signals prompted them to quickly initiate movements [1]. To study the underlying neural mechanisms and the complex relation between beta oscillations and behavior, we combined computational modeling of basal ganglia networks with electrophysiological recordings from behaving rats. Previous computational models showed that increased inhibitory input from striatum to globus pallidus (GP) can generate pathological beta oscillations in the subthalamo-pallidal network [2]. To test whether the same model can also reproduce the experimentally-observed transient beta oscillations, we identified rat striatal medium-spiny neurons that increased their firing rate when the rat initiated movements. This striatal activity was then used as an estimate for inhibitory input to the GP in our network model. Indeed, we found that the movement-related increase in striatal activity leads to transient beta oscillations in the model with a time course that closely matches the experimentally measured oscillations (see Figure). We also studied the effect of additional excitatory input to the subthalamic nucleus (STN) in the model. It turns out that not only the amplitude, but also the relative timing of the inputs to GP and STN affect the amount of beta power in the model. Furthermore, we found that brief stimulation of STN can generate rapid beta selective phase reset in our network model similar to what we already reported for the experimental data [1].

We conclude that the striatal indirect pathway can drive transient beta oscillations during movement initiation and that transient beta oscillations in behaving animals share some of the underlying mechanisms with pathological beta oscillations in Parkinson's disease.


Experimental data (from Leventhal et al., 2012)
40
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1
Frequency (HZ)
Model
40
20
1
-1
0
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Time from Nose Out (s)

Boosting Coordinated Reset Stimulation by Slowly-Varying-Sequences: a computational study

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Several brain disorders are characterized by an abnormally strong neuronal synchronization. Current challenges are to unravel the mechanisms behind this pathological synchronization and to develop treatments to stop or even reverse the processes that lead to highly synchronized neuronal activity.
The Coordinated Reset (CR) stimulation was developed to selectively desynchronize neuronal activity and is based on the ideas that neuronal synchrony is related to the connectivity within the neural network and that the connection strengths can change throughout life via spike timing-dependent plasticity (SDTP). Within the pathological network several subpopulations are stimulated separately in a timely coordinated way. The spatiotemporal sequence in which all stimulation sites are stimulated exactly once is called the stimulation site sequence, or briefly sequence. In the conventional CR approach the sequences are applied in random order or simply kept fixed. This neurostimulation method can cause a long-lasting anti-kindling [3], i.e. an unlearning of pathological connectivity and synchronization.
Unlearning causes, similar to learning, a rewiring of the relevant subpopulation. Besides STDP, optimal repetition is also a fundamental principle of learning, but is not included in the conventional CR approach. Here we investigate the effect of repeating one sequence many times before the next sequence is applied, on the anti-kindling of the highly synchronized network. This new method is called the slowly-varying-sequence (SVS) CR stimulation. Further, we also investigate the effect of an increased maximum inhibitory coupling strength, since the balance between excitation and inhibition is a candidate mechanism for neuronal synchronization.
We use a computational neural network with strong short-range excitatory and weak long-range inhibitory dynamic couplings. Simulation results show that over a wide range of stimulation intensities the SVS approach significantly boost the long-lasting anti-kindling. Doubling the maximum-allowed inhibitory connection strength significantly reduces the average connection strength for both CR-algorithms, but it did not significantly improve the desynchronization.

Challenging a dynamical threshold equation for action potential initiation for its generality

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Excitable neurons generate action potentials whenever the membrane potential exceeds the current firing threshold. In general a neuron's firing threshold is not at a fixed membrane potential but depends on the state of sodium inactivation, delayed rectifier activation, adaptation currents, synaptic conductances, etc. Quadratic and exponential integrate-and-fire models reproduce neuronal firing dynamics remarkably well, but either assumed a constant threshold or a threshold dynamics that has not been derived from a more detailed Hodgkin-Huxley-type model. Recently, Platkiewicz and Brette derived an approximation for the firing threshold and compared it with simulated data of a single conductance-based model.

We investigated the generality of the proposed equation using five different Hodgkin-Huxley-type models including both type I and type II excitability. A comparison of numerical measurements of the real firing threshold at any time with the solution of the threshold equation shows that the threshold equation consistently underestimates the real threshold by 2mV to 14mV. In every model the membrane potential occasionally crossed the calculated threshold without triggering any spike. In some models the calculated threshold was almost permanently lower than the current membrane potential. The discrepancies also depend on the mean and the variance of Ornstein-Uhlenbeck noise inputs. While in some models the difference between the two thresholds increased with the mean input, in other models it decreased. The effect of the input variance varied strongly between the different models. We conclude that the threshold equation derived by Platkiewicz and Brette does not generalize to other conductance-based models or even stimuli.

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Channel Mechanisms for a Hierarchy of Timescales Across the Human Cortex

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In the mammalian visual system, a hierarchy of cortical areas processes increasingly complex features of stimuli, with an increase in spatial scale. Recent observations suggest that the increase in spatial scale is accompanied by an increase in the temporal scale, with neurons in higher areas showing slower dynamics[1]. However, the underlying physiological mechanisms are not understood. Here we investigated the hypotheses that differences in ion channel expression across cortical areas determine the observed change in temporal dynamics.

We analyzed the freely accessible set of microarray and RNA-sequencing transcriptome data on human brains, provided by the Allen Brain Atlas[2]. We tested a set of of 66 ion channel-related genes for differential expression between primary sensory areas and association areas in order to isolate candidate channel mechanisms. The data set was comprised of genes coding for the a-subunits of voltage-gated sodium-, potassium- and calcium channels (Na_V, K_V and Ca_V), transient receptor potential cation channels (TRP), calcium activated potassium channels (K_{Ca}), hyperpolarization-activated cyclic nucleotide-gated (HCN) ion channels, as well as ionotropic glutamate and GABA receptors (AMPA-, NMDA- and GABA-receptors).

After testing for differences in expression, we used single-compartment Hodgkin-Huxley models to assess how the resulting subset of channels influences the timescale of neural dynamics. Preliminary results suggest that the change of temporal scale across cortical regions is not due to variations in ion channel expression, but the result of network effects.

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Computational characterization of Axon Carrying Dendrites Cells: Electrophysiological properties and synaptic integration

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Neuronal processing is classically divided into dendritic input, somatic integration and axonal output. In this view, neurons process information by receiving input from the
dendrites, integrate it in soma and finally producing an output at the axon. The axon initial segment (AIS), where action potentials (AP) are initiated is usually located adjoining to the soma, although other configurations have been reported (Grubb 2011; Häusser, Stuart. Racca Sakmann 1995). We have recently shown (Thome, 2014) that the axon of about 50 % of CA1 pyramidal neurons branch off from a basal dendrite rather than the soma. We used NEURON simulations of a biophysically realistic pyramidal CA1 neuron to see how this might affect the integration of synaptic input at such an axon carrying dendrite (AcD) compared to a simple dendrite. The axon of our study neuron was connected to the AcD at a variable length from the soma, from which more basal dendrites could emerge. Our simmulations show that that indeed the threshold of AP activation is lower for AcD input. Furthermore, this reduction is enhanced when multiple branches emerge between soma and AIS. In a physiological context of network oscillations, i.e., with perisomatic inhibition, the difference in input required for eliciting an AP was also largely reduced between simple dendrites and the AcD. Additionally, synaptic input coming from the AcD also facilitated more dendritic spikes than nonAcD inputs. In summary, this novel morphologic feature causes distinct electrophysiological properties that can be explained by a computational analysis.

Computational modeling of LTP and concurrent 'heterosynaptic' LTD in the dentate gyrus in vivo

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Hippocampal long-term potentiation (LTP) and long-term depression (LTD) are thought to be key synaptic mechanisms of learning and memory. Here we use compartmental granule cell models to better understand LTP and concurrent 'heterosynaptic' LTD in the dentate gyrus of freely moving rats. We show that observed LTP and LTD can be accounted for by a spike-timing-dependent plasticity (STDP) rule combined with a fast Bienenstock-Cooper-Munro (BCM)-like metaplasticity rule. Our simulations suggest that the interplay of STDP-BCM plasticity rules and ongoing pre- and postsynaptic background activity determines not only the degree of input-specific LTP elicited by various plasticity-inducing protocols, but also the degree of associated LTD in neighboring non-tetanized inputs.

Effect of Alzheimer disease on the dynamical and computational characteristics of recurrent neural networks

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Recurrent circuits of simple model neurons can provide the substrate for cognitive functions such as perception, memory, association, classification or prediction of dynamical systems [1,2,3]. In Alzheimer's Disease (AD), the impairment of such functions is clearly correlated to synapse loss [4]. So far, the mechanisms underlying this correlation are only poorly understood. Here, we investigate how the loss of excitatory synapses in sparsely connected random networks of spiking excitatory and inhibitory neurons [5] alters their dynamical and computational characteristics.

By means of simulations, we study the network response to noisy variations of multidimensional spike-train patterns.

We find that the loss of excitatory synapses on excitatory neurons (decrease in excitatory-excitatory indegree; vertical arrow in the figure 1) lowers the network's sensitivity to small perturbations of time-varying inputs, reduces its ability to discriminate and improves its generalization capability [6].

A full recovery of the network performance can be achieved by firing-rate homeostasis, implemented by an up-scaling of the remaining excitatory-excitatory synapses (horizontal arrow in the figure 1). By studying the stability of the linearized network dynamics, we show how homeostasis can simultaneously maintain the network's firing rate, sensitivity to small perturbations and its computational performance.

Figure 1: Loss of excitatory-excitatory synapses (vertical arrow) impairs discrimination capability (gray coded). Recovery of discrimination capability by firing-rate homeostasis (up-scaling of remaining excitatory-excitatory synapses; horizontal arrow).

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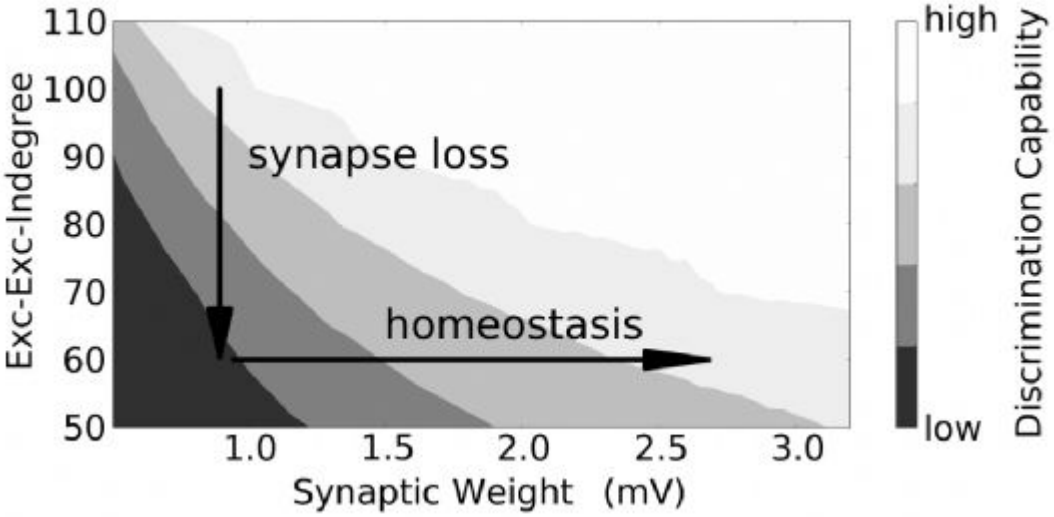
110
100
90
80
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60
50
Exc-Exc-Indegree
synapse loss
homeostasis
1.0
1.5
2.0
2.5
3.0
Synaptic Weight (mV)
high
low
Discrimination Capability

Effects of neuronal diversity illustrated by current- and voltage-clamp experiments in a virtual laboratory

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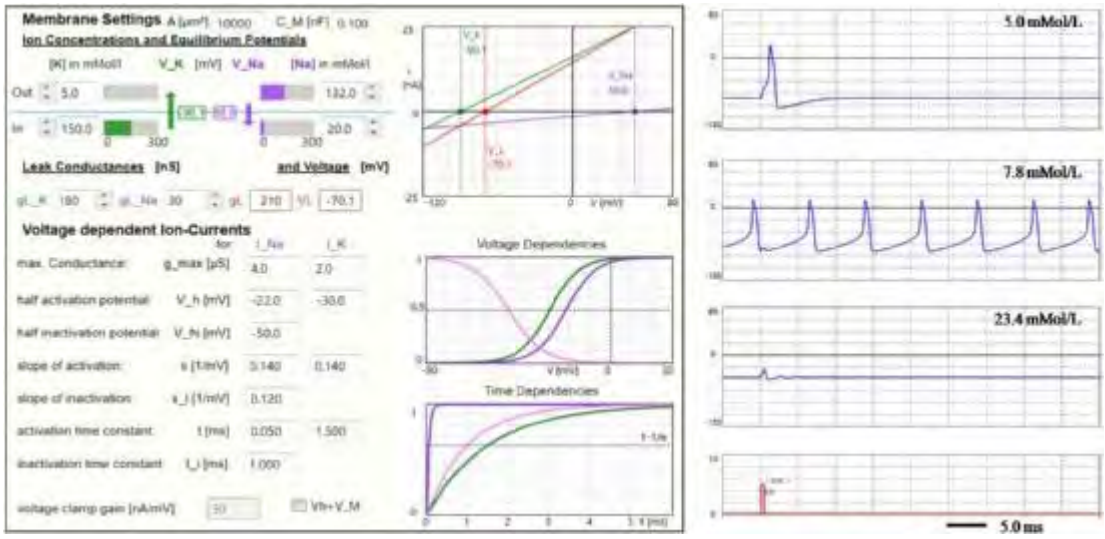
No neuron is reacting in exactly the same way as any other one – what each experimental neurophysiologist very well knows. The neurons' dynamic states and their responsiveness depend on the type and number of ion channels that are embedded in the neuronal membrane as well as on the size of the neuron determining the membrane capacitance. The latest version of the teaching tool SimNeuron takes into account such neuronal diversity. The parameters of the neurons are randomly chosen in a physiologically reasonable range. In the current version this concerns the (in-) activation parameters of voltage dependent Na^+- and K^+-conductance for spike generation as well as so-called passive membrane properties like leak conductances and the membrane area (Fig). It has been taken care that all neurons will initially have a stable membrane potential while a password protected "neuron editor" allows to change the neuron parameters to convert, for example, an initially stable neuron into a pacemaker cell. The thereby induced alterations of the neurons' responsiveness can be examined in the virtual voltage- and current-clamp labs. Fully functioning demo versions can be downloaded from www.virtual-physiology.com

Figure: Part of the "neuron editor" for parameter changes (left) and an example of increased external K+-concen-tration (right): from stable potential to spon¬taneous firing to a depolari¬zation block (from top to bottom). The last figure on the right side shows the giving impulse current (5 nA in 0.4 ms).



Efficient coding of rewards in strategic decision-making

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It has been proposed that one fundamental task accomplished by the brain is to encode sensory stimuli efficiently. This view is supported by the observation that neural activity in sensory areas maximizes information transmission. However, most of those observations have been obtained in subjects that are anesthetized, passive, or engaged in tasks that do not require cognitive functions such as memory and decision-making. It remains unclear whether the principle of efficient coding applies to higher brain regions, such as the prefrontal cortex, while subjects are involved in cognitive tasks.

We study the response of 681 (non-sensory) cortical neurons to reward events during strategic decision-making in primates. We showed previously that the dynamics of those responses is highly heterogeneous and spans two orders of magnitude, from 100ms to 10s [1]. We hypothesize that this variety of responses is a consequence of efficient coding. We build a model of a neural circuit, and we compute the synaptic matrix that maximizes information transmission. We show that the model matches the experimental observations in terms of both the marginal and the joint statistics of timescales and amplitudes of the neural responses.

Our results suggest that the efficient coding hypothesis predicts not only the spatial properties of neural response (e.g. receptive fields), but also its temporal properties, on a broad range of timescales, and it can be applied to non-sensory areas. While the efficient coding hypothesis predicts maximum information, it does not address the question of how this information is used. Future work will focus on the question of how efficient coding of reward is used for decision-making, with the overarching goal of bridging the gap between sensory coding and behavior.

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From randomly connected to spatially organized multi-layered cortical network models

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In vivo recordings of the electrical activity in neural tissue (e.g., spiking activity and local field potentials (LFP)) have exposed both spatially confined and propagating features [1]. Such activity emerges from complex interactions between local and remote neurons and ongoing physiological processes on broad spatiotemporal scales. Typically addressing the temporal dynamics of cortical networks, spiking point-neuron network models can generate asynchronous irregular firing at rates in line with *in vivo* observations, as demonstrated for instance by Potjans & Diesmann [2]. Their model represents 1 mm² of early sensory cortex with four cortical layers, each containing one excitatory and one inhibitory neuron population, randomly connected without spatial dependencies with probabilities derived from experimental data. However, geometrical constraints on connectivity become increasingly relevant when upscaling the area covered by the network since local connections are typically in the range of = 500 µm depending on neuron type, species, and cortical area [4].

Here, we laterally extend the multi-layered network model of [2] to the mesoscopic scale (~ 10 mm²). As illustrated below, the local connectivity between network populations is specified using finite spatial connection kernels with radial distance dependency. In order to fully investigate the effects of such spatial structure on the dynamics of the multi-layered network, we vary the kernel type and also introduce distance-dependent synaptic transmission delays (using the NEST [3] Topology Module). The current model covers 4x4 mm² of cortical surface, comparable to the area covered by chronically implanted multi-electrode arrays (Blackrock Microsystems, Salt Lake City, UT, USA). We aim at incorporating realistic neuron and synapse densities for detailed assessment of the effect of key parameters on stationary and non-stationary pattern formation for various network states [5,6,7]. The systematic network analysis requires high-performance computing facilities, in particular for extensive parameter scans to account for lacking data, but is feasible using hardware available today.

Preliminary results show that introducing distance-dependent conduction delays in the network leads to pattern formation that may underlay observed features from *in vivo* LFP recordings. Further work is needed to quantify the exhibited dynamics and investigate its parameter dependence.

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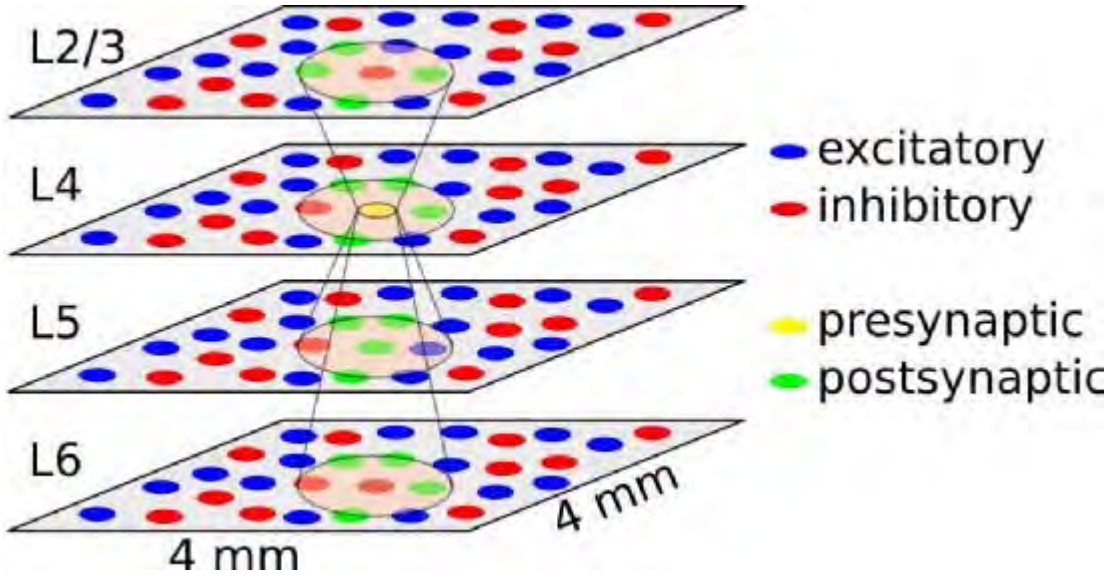
Norway (NFR) through NevroNor, eNEURO, Notur, NN4661K.



Functional role of opponent, dopamine modulated D1/D2 plasticity in reinforcement learning

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The basal ganglia network is thought to be involved in adaptation of organism's behavior when facing its positive and negative consequences, that is, in reinforcement learning. It has been hypothesized that dopamine (DA) modulated plasticity of synapses projecting from different cortical areas to the input nuclei of the basal ganglia, the striatum, plays a central role in this form of learning, being responsible for updating future outcome expectations and action preferences. In this scheme, DA transmission is considered to convey a prediction error signal that is generated if internal expectations do not match the outcomes observed after action execution.

Aiming towards a model of a canonical circuit for learning task-conform behavior from both reward and punishment, we extended a previously introduced spiking actor-critic network model of the basal ganglia [1] to contain the segregation of both the dorsal (actor) and ventral (critic) striatum into populations of D1 and D2 medium spiny neurons (MSNs) [2]. This segregation allows explicit, separate representation of both positive and negative expected outcomes by the distinct populations in the ventral striatum. The positive and negative components of expected outcome were fed to dopamine (DA) neurons in SNc/VTA region, which compute and signal reward prediction error by DA release.

In the dorsal striatum, we implemented a winner-takes-all (WTA) circuit to choose between a number of possible actions. We show that plasticity, modulated by D1 and D2 receptors, combined with WTA mechanism results in a TD-Learning like funcional circuit.

This modeling approach can be extended in the future work to study how abnormal D1/D2 plasticity may lead to a reorganization of the basal ganglia network towards pathological, dysfunctional states, like for instance those observed in Parkinson disease under condition of progressive dopamine depletion.

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Genetic networks specifying the functional architecture of orientation domains in V1

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Although genetic information is critically important for brain development and structure, it is widely believed that neocortical functional architecture is largely shaped by activity dependent mechanisms. The information capacity of the genome simply appears way too small to contain a blueprint for hardwiring the cortex.

Here we show theoretically that genetic mechanisms can in principle circumvent this information bottleneck. We find in mathematical models of gene regulatory networks of principal neurons interacting by long range axonal morphogen transport that morphogen patterns can be generated that exactly prescribe the functional architecture of the primary visual cortex (V1) as experimentally observed. We analyze in detail a representative genetic network that encode the functional architecture of V1 in a dynamically generated morphogen pattern. We use analytical methods from weakly non-linear analysis [1] complemented by large scale numerical simulation to dissect the solutions of the model and to gain a comprehensive view onto the system. In particular we find that the pinwheel density variations, pinwheel nearest neighbor distances and most strikingly the pinwheel densities are in quantitative agreement with high precision experimental measurements [2,3].

We discuss that the intriguing hypothesis that genetic circuits coupled through axonal transport shape the complex architecture of V1 is in line with several biological findings. (1) Surprisingly, transcription factors have been found to be transported via axons and to be incorporated in the nucleus of the target cells [4]. (2) A molecular correlate was recently found for ocular dominance columns in V1 [5]. (3) We estimate that the speed of axonal transport is rapid enough to achieve appropriate timescales.

This theory opens a novel perspective on the experimentally observed robustness of V1's architecture against radically abnormal developmental conditions such a dark rearing [6]. Furthermore, it provides for the first time a scheme how the pattern of a complex cortical architecture can be specified using only a small genetic bandwidth.

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Hybrid scheme for modeling local field potentials from point-neuron networks

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While recordings of extracellular potentials in neural tissue are commonly used for monitoring neural activity, interpretation of the low frequency part, the local field potential (LFP), remains ambiguous in terms of the underlying network activity. Studies have shown that the LFP depends on electrode position, extracellular volume conductor model, neuronal morphology, synapse distributions and synaptic input correlations [1,2,3]. In order to relate spiking dynamics in point-neuron neuron network models to extracellular signals, e.g., the LFP, we have developed a hybrid scheme that uses the spiking activity (Fig. panel A) generated by a network of single-compartment leaky integrate-and-fire model neurons (implemented in NEST [4]). The network provides synaptic input to populations of detailed multi-compartmental neuron models (Fig. panel B) which are used to compute the spatiotemporal LFP pattern (Fig. panel C) based on biophysical principles behind extracellular electric signals using LFPy (http://compneuro.umb.no/LFPy) [5] and NEURON [6]. The hybrid scheme is incorporated in a new, publicly available Python package named hybridLFPy (http://github.com/espenhgn/hybridLFPy).

We here demonstrate an application of the method with the network model of [7] describing the local microcircuitry under 1 mm^2 surface of cat primary visual cortex. The point-neuron network includes ~77000 cells in total distributed across four layers, each composed of one excitatory and one inhibitory population representing layers 2/3 through 6, activated by external input (cortico-cortical, thalamo-cortical). For the LFP model, the same amount of neurons, subdivided into 16 cell types with passive membrane properties are used with cell-type and layer-specific connectivity derived from the point-neuron network description and additional anatomical data [8]. Our results show that both spontaneous and stimulus-evoked LFPs depend critically on the level of synchrony in the underlying network state. Besides, we show that full-scale simulations, i.e., simulations including all cells in the network, are required to address the effect of network correlations on the LFP. Furthermore, the hybrid scheme can be used to develop and verify simplified models for LFP generation from point-neuron network models. Given the widespread use of point-neuron network models and the previous lack of tractable methods to associate their activity to easy-to-measure signals (e.g., LFPs), the present method is a step toward gaining important insight into the link between experimental measurements and the underlying network activity.

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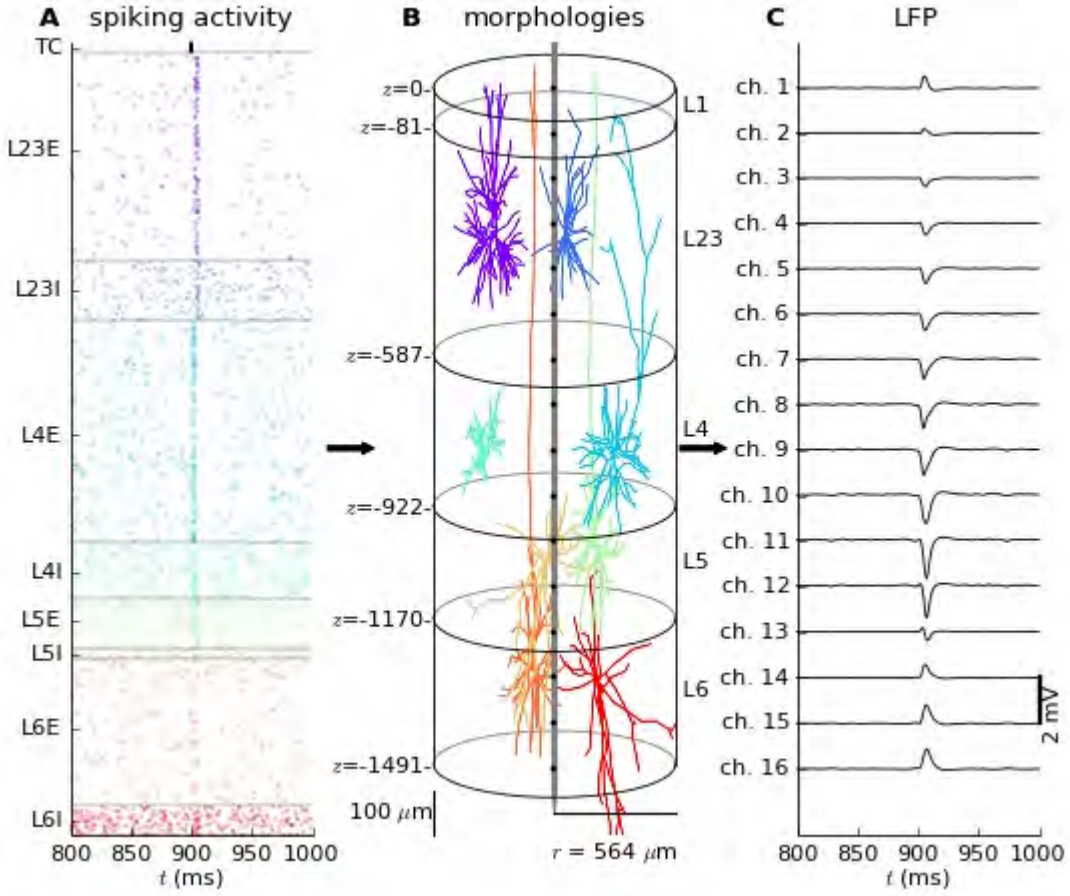

Impact of parametric uncertainties in computational models for deep brain stimulation

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Deep brain stimulation (DBS) has evolved as a widely employed procedure to treat the symptoms of motor skill disorders such as Parkinson's disease (PD), essential tremor and dystonia by applying electrical pulses to the stimulated target areas. Although the method is successfully employed and approved by the Food and Drug Administration across various clinical fields, the fundamental mechanisms of action of DBS remain uncertain. Starting in the last decade, several computational models for predicting the effects of DBS have been proposed. These models can be categorized into (1) neural network models examining the effects of the stimulation pulses on the neural network as well as the activation of neurons and (2) volume conductor models predicting the field distribution and volume of tissue activated (VTA) in the proximity of the electrode contacts. While the majority of these models consider only one set of values for the model parameters, large uncertainty, for example in the electrical properties of brain tissue, can be found reviewing literature values and knowledge on how this uncertainty influences the predicted neural activation is scarce. This additional information on the probability distribution of the extent of neural activation could help engineers as well as clinicians in evaluating the actual activated area and rating the likelihood of undesired activation.

To investigate the influence of uncertainty in the model parameters on the quantities of interest such as the required stimulation amplitude to obtain therapeutic effects during DBS, basic parameter studies as well as advanced probability sampling methods based on the generalized Polynomial Chaos technique are applied. The latter method allows for the computation of stochastic measures and tolerance intervals of the quantities of interest with a reasonable computational effort by approximating the deterministic model by a surrogate model based on a series expansion in multi-variate orthogonal polynomials. This deterministic is combined out of a volume conductor model of the DBS electrode and the brain to compute the time-dependent extracellular potential as well as a model to predict the resulting neural activation. The extracellular potential is computed using finite element brain models based on segmented magnetic resonance images, which allows for a consideration of the brain anatomy, the electrode geometry and position as well as effects at the electrode-tissue-interface. The influence of uncertainty in the parameters of the electrode-tissue-interface and the electrical properties of brain tissue on the predicted neural activation in single neuron and neural network models of the basal ganglia as well as on the time-dependent extracellular potential in the proximity of the DBS electrode are presented.

Impaired homeostatic regulation of feedback inhibition associated with system deficiency to detect fluctuation in stimulus intensity: a simulation study

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Information processing in animals‘ brain requires the balance between excitatory and inhibitory neural circuits. Feedback inhibition is involved in many sensory processes;however, the role of inhibition in system efficiency is not fully understood. Moreover, the regulation of inhibition intensity in response to different stimulus intensity is not fully studied in normal and pathological cases. In this work, a geometrical measure for
system efficiency is defined which measures the system ability to discriminate between similar stimulus intensities. For this purpose, we developed a simulation of a two-layer feed-forward neural system constrained by electrophysiological data. The
effect of inhibition on system efficiency was studied for different feedback inhibition parameter values. The simulations show that inhibition is critically required to detect fluctuations in stimulus intensity, especially for high stimulus intensities. Moreover, simulations demonstrate that incremental change of inhibition parameter value (by a hypothetical homeostatic regulation mechanism) to detect fluctuations in incremental
stimulus intensity is critically required to obtain high level of system efficiency. This work assigns a vital role for feedback inhibition in system efficiency of feed-forward neural systems.

Integrating Touch and Vision in Stick Insects and Insectoid Robots

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Using their compound eyes, various insect species can sense light intensity and polarization, recognize color patterns or extract optic flow. All insects also have antennae which bear numerous and diverse receptors for sensing touch, vibration, smell, taste and heat. To date, compared to human studies, little is known on how insects perform multimodal sensory integration in order to exhibit adaptive behaviors. Visuo-tactile integration, in particular, has received little attention, potentially because the discrepancy between spatial sampling properties of compound eyes and antennae is difficult to capture. Indeed, while the antennae sample near-range objects with sequential local hot-spots with high 3-D resolution, compound eyes provide simultaneous, omnidirectional 2-D images of the environment at relatively low resolution, though for a wide range of distances. Although some of these limitations may be overcome by active sensing strategies, e.g., by distance estimation via visual peering, direct evidence of visuo-tactile integration in insects is still lacking.

Here we report the measurement of two spatial resolution maps in the stick insect *Carausius morosus*, mapping the two spatial senses of vision and touch in the same species. Interestingly, we found that the zone of highest visual resolution overlaps with the preferred region for active tactile searching movements during unrestrained walking and climbing. This indicates that, albeit being nocturnal animals, stick insects may perform visuo-tactile integration possibly for finding foothold, or generally for improving locomotor efficiency.

Key properties of both the visual and the tactile sensory organs have been combined in a virtual 3D environment. This environment allows us to replicate representative natural locomotion sequences, including antennal movement, and to map the two antenna images onto the visual field of the compound eyes. The resulting animations suggest that the optics of the eye are not sufficient to resolve the movement of the own antennae.

In addition, we have designed a biomimetic robotic head equipped with two pan-tilt units actuating vibration-based antennae, and two panoramic cameras. The camera images can be down-sampled using the properties of the stick insect's visual field. Most morphological proportions of the standard animal head (average measures, N=10) were respected and scaled up by a factor of about 50, accounting for size constraints of the mechatronic components. Using a combination of behavioral, simulation and biorobotic experiments, we are now able to address research questions on real-world visuo-tactile integration that cannot be tackled otherwise. For example, robot experiments allow evaluating holistic models in real world scenarios, which are difficult to model in simulation because of the complex tactile interaction between compliant feelers and various substrates.

Lability and constancy of orientation tuning in the visual cortex depends on the functional architecture

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A fundamental question in the function of cortical sensory systems is how information is represented by neuronal ensembles to be processed by higher cortical areas. In the primary visual cortex the spatial arrangements of tuning properties show fundamental interspecies differences. While in primates and carnivores neurons of similar orientation preference are clustered in iso-orientation columns, in rodents and lagomorphs they are spatially interspersed. It is widely assumed that these representations are robust in time to allow for a proper decoding, but little is known about their dynamic stability under livelong plasticity and how it depends on the underlying functional layout.

Here, we study mathematical models of circuit dynamics where the tuning parameters evolve dependent on the current architecture and intra-cortical interactions of the network. Our previous work shows that columnar designs quantitatively matching the biological systems naturally emerge when orientation selective long range interactions are present while disordered layouts are stabilized when local circuits are predominantly inhibitory [1]. When subject to temporally random perturbations we find that receptive field properties in disordered layouts exhibit a pronounced lability compared to maps. This difference is maintained even near pinwheel centers, where neurons with very different orientation preferences are in close proximity.

An examination of the energy landscape of the model in the different interaction regimes explains this quantitative difference. In disordered layouts because of the high number of distinct disordered solutions the energy barriers between them seem shallow, such that small perturbations can cause transitions to a different organization. Maps, in contrast, have higher energy barriers between ordered solutions, such that a collective change in the tuning of many neurons is necessary to elicit a transition, keeping the representation of the pattern as a whole less labile.

We also find that the drift of orientation preferences in disordered layouts is reduced when biologically relevant conditions are implemented in the tuning dynamics, such as orientation selective excitatory interactions [2], dynamical matching of binocular orientation preferences after the emergence of selectivity [3] and orientation tuned input from the LGN [4].

Taken together our study indicates that maps and disordered layouts differ in the lability of orientation representations and lays the basis for an experimental assessment of this difference.

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Mesoscopic population dynamics of spiking neurons derived from single cell properties

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Mean-field theory has been very successful to link single neuron properties to the population dynamics of spiking neurons (e.g. [1,2]). However, this approach is limited to the mean activity that would be observed for an infintely large population. In realistic neural populations the number of neurons of a given type can be rather small (e.g. N~200), which gives gise to finite-size noise and qualitatively different dynamical regimes.

The statistical description of finite-size neural populations is however a challenging theoretical problem. Existing theories are either based on rather simplified neuron models or rely on certain heuristic assumptions. Here, we present stochastic population equations for the large class of generalized integrate-and-fire neurons with spike-frequency adaptation. The equations can be regarded as a generalization of existing mean-field equations ([2,3]) to incorparate the effects of a finite system size.

Using our theory, we study spontaneous transitions between up and down states in a bistable network purely induced by intrinsic finite-size noise. Furthermore, in the asynchronous cortical state, we analytically calculate the spectral properties of the fluctuations by linearizing the dynamics about the mean activity. This allows us to investigate the role of spike-frequency adaptation and recurrent connectivity on the spontaneous activity (noise shaping) [4].

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Neuronal morphology and spike onset rapidness modulate the dynamic gain in cultured hippocampal neurons

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Action potential (AP) generation begins with the activation of Na channels in the axon initial segment (AIS). The Na currents that flow during the first hundred microseconds in the proximal axon shape the onset of the somatic AP waveform, while the second phase of the AP upstroke is shaped by local, somatic Na currents. Na channel voltage dependence and activation kinetics in the AIS control the initial phase of the AP, as well as the exact timing of the AP in relation to the input current waveform. The latter can be characterized by the spike-triggered average of the input (STA). To which degree the early AP shape at the soma is also related to the STA is currently debated; multi-compartment models do not provide unambiguous answers as the actual values of key parameters, e.g. somatic and axonal Na channel density and voltage dependence, are controversially discussed in the community.
We studied the maturation of the AIS function in cultured hippocampal neurons, as revealed by developmental changes in the AP waveform, and the relationship of these changes to maturation of neuronal encoding properties, as revealed by the STA and the dynamic gain. The measures of the AP shape changed quickly within the first week of AP firing: V_{thresh} dropped by 4 mV, the onset rapidness, peak rate of rise and V_{peak} - increased. After 20 DIV the shape of APs was largely stable. Consistent with the increase in onset rapidness, Na channel fluorescence intensity increased in the AIS during the first 20 DIV. In parallel with the maturation of AP waveform, spike encoding properties also change: the STA became narrower and the slope of the dynamic gain curve increased. The dynamic gain curves for fast input fluctuations ($t_{corr} << t_m$) showed shallow slope for frequencies below 100 Hz, followed by a steep fall-off. While the shallow slope changed during maturation (from $f^{-0.4}$ to $f^{-0.2}$), the characteristic frequency of the steep fall-off remained around 200-300 Hz. Dynamic gain curves of single compartment models can show such power-law relationship, however conductance based models display slopes of -1, while leaky integrate and fire models produce power-law tails of -0.5 for colored noise and no fall-off for white noise input. Slower input fluctuations ($t_{corr} \sim t_m$) improved the dynamic gain in the frequency range of 10 to 200 Hz thus demonstrating the Brunel effect.
For both fast and slow input fluctuations, we found that the membrane time constant is a strong predictor of the slope of the gain. In the regime of slow input fluctuations we also obtained a positive correlation between the onset rapidness and the slope of the gain. This relation is expected from theory if onset rapidness was a proxy for axonal Na channel voltage dependence.

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Online Parameter Estimation using GPU Super-Computing

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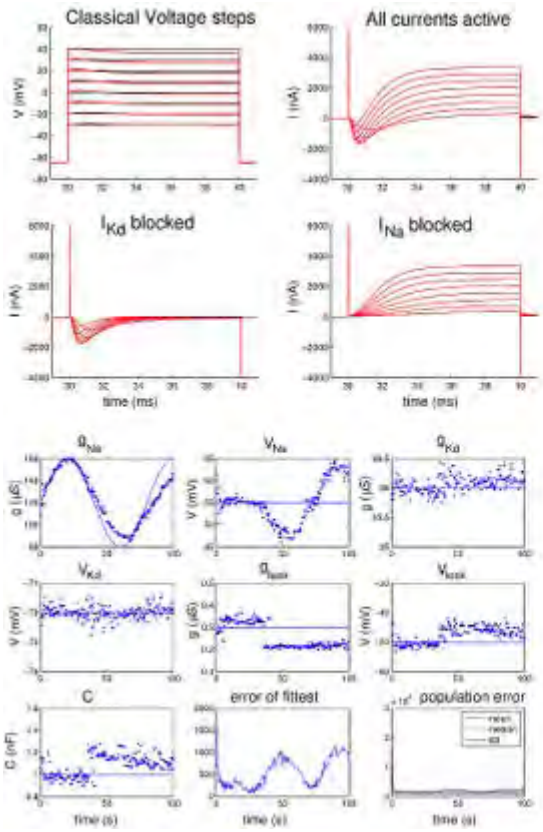
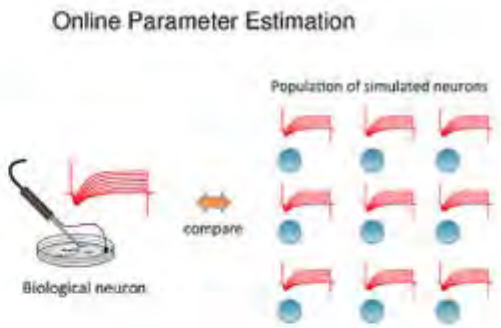
The standard method for characterising ion channels in neurons is voltage clamp. However, in the classical procedure, measurements are performed with constant voltage steps and chemical channel blockers are used to isolate individual ion channel types. Because chemical blockers can be irreversible, different ion channels have to be measured in different individual cells, sometimes even in preparations from different individual animals. Using this paradigm it has been observed in identified cells of invertebrates, previously believed to be prototypical across individual animals, that the parameters of channels, in particular the expression levels, can vary largely. Furthermore, modelling work has suggested that cells may be constructed with entirely disparate sets of parameters. While we have since found that this conclusion may have been premature, the questions how consistet the ion channel content of neurons is across individuals and how the nervous system can function reliably in the face of apparent large variability remain open.

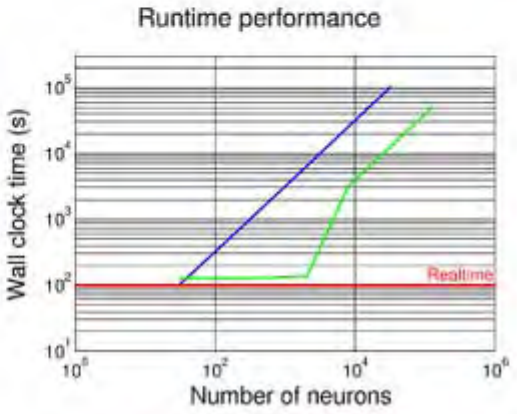
Here I present a proposal to go beyond classical constant voltage steps and design optimised stimulation patterns that are intended to isolate the effect of different ion channels without chemical blockers. Furthermore, I propose to use closed-loop online parameter estimation methods to build a model of all ionic currents in an individual neuron simultaneously. If successful this could help answering whether indeed neurons are constructed with disparate parameters.

I have obtained promising initial results that demonstrate in simulation that separating stimuli is possible and that online parameter fitting using random search methods like genetic algorithms is now feasible due to the advent of graphical processing unit (GPU) super-computing. I have been able to simulate up to 2048 Hodgkin-Huxley neurons in almost real time with our GeNN simulator and have observed that this number is sufficient to track the parameters of a (at this stage also simulated) neuron (see figure).


Classical Voltage steps
All currents active
I_{Kd} blocked
I_{Na} blocked
time (ms)
time (ms)

g_{Na}
V_{Na}
g_{Kd}
V_{Kd}
g_{leak}
V_{leak}
C
error of fitlest
population error
time (s)
time (s)
time (s)


Online Parameter Estimation
Population of simulated neurons
compare
Biological neuron


Runtime performance
Wall clock time (s)
Realtime
Number of neurons

Shaping phase space of neural networks via connectivity

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[*]*M. Schmidt and J. Schuecker contributed equally to this work.*

We present a phase-space method for the systematic modification of the connectivity of neuronal networks to obtain more realistic dynamics. Dynamical systems in general and neuronal networks in particular can exhibit bistable phase spaces governed by a low activity and a high activity attractor, the latter representing an unrealistic state of spontaneous brain activity. The separatrix between the basins of attraction limits the choice of parameters for fine-tuning of the low activity attractor. Furthermore, external perturbations such as transient stimuli might drive the system to the pathological high activity state.

Our method makes use of a reduction of the high-dimensional spiking model to a simpler system by replacing the dynamical variables of the neurons by mean firing rates of neuronal populations. The approach is based on mean-field techniques (Fourcaud et al., 2002; Wong et al., 2006) and enables us to theoretically predict the mean activity in the network model as well as its global properties. In particular we perform a stability analysis near the unstable fixed point on the separatrix of the system. Subsequently we subtract the projectors on the eigendirections associated with the exponential divergence between close-by trajectories in an iterative manner. This reveals mathematical rules for refining the structural connectivity. The procedure enlarges the basin of attraction of the low-activity fixed point.

We exemplify the method with a simple network model consisting just of an excitatory and an inhibitory population and an eight-population microcircuit model of early sensory cortex (Potjans et al., 2014). In addition, we demonstrate the practical usefulness by applying the method to a highly complex model, a multi-area model of macaque visual cortex comprising 32 cortical areas with eight populations each (Schmidt et al., 2013). We show how our method enables us to find a network state with asynchronous, irregular spiking activity and realistically low firing rates, whereby the modified connectivity is in agreement with the biological constraints.

Figure:
Illustration of stabilization procedure. Phase space of the model with the separatrices before (red dashed curve) and after one iteration (black dashed curve) of the procedure with red (original model) and black (after 1 iteration) curves showing trajectories starting at different points near the separatrix. The procedure enlarges the basin of attraction for the realistic low activity state so that all black trajectories converge to this state. Panel B shows the inset indicated in A.

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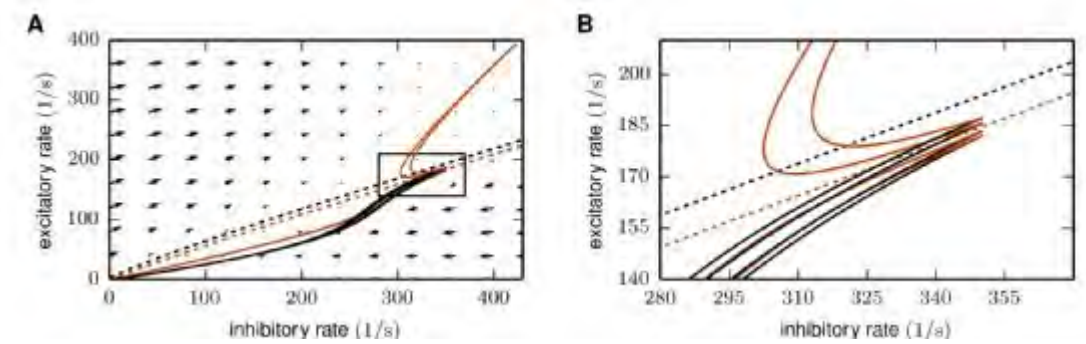

State dependent modulation of dopamine function in the striatum

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How dopamine affects the single neuron transfer function and thereby the network dynamics is of key important to understand the dynamics and function of the basal ganglia in both healthy and diseased states. There is extensive data available on how dopamine affects individual ion channels and synaptic transmission. Such detail is usually reduced to the simple rule which states that dopamine (DA) has excitatory effect on D1 MSN and inhibitory on D2 MSN.

Because dopamine affects the conductances of voltage dependent ion channels and synapses, it is straightforward to hypothesize that the effects of DA on a neuron must depend on the neuron's 'state', described by its firing rate or membrane potential statistics. Here we test this hypothesis using numerical simulations of a reduced single compartment conductance-based model of D1 MSN with three membrane ion currents (Gruber 2003). We examine the model responses under a range of balanced excitatory and inhibitory synaptic inputs (Kuhn 2004) and estimate the neuron transfer function. Our results support the initial hypothesis that in steady states, the compound effect of DA on the membrane and synaptic conductances could be either excitatory or inhibitory, depending on the outputfiring rate of the neuron: typically in D1 MSNs, DA has an excitatory (inhibitory) effect at low (high) output firing rates, however the exact effect may also depend on the baseline synaptic strength.
We expect similar results would hold for the D2 MSNs. Besides forming the basis of high level rules of the effect of DA on striatum dynamics, these results could also help us understand the observed complexity of DA function.

Acknowledgements

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Statistical Assessment and Neuronal Composition of Active Synfire Chains

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The synfire chain (SFC) model has been suggested [1] as a network model for cortical cell assemblies [2]. It is composed of consecutive groups of neurons connected in a feedforward fashion by a large number of convergent and divergent inputs. This connectivity structure enables stable propagation of packets of synchronous spikes after stimulation of the first group [3]. Electrophysiological recordings from hundreds of neurons in parallel increase the chance to detect active SFCs. A method to display repeated SFC activations by means of an intersection matrix was suggested in [4,5]. Each entry in the matrix contains the degree of overlap of identical neurons being active at two different time bins. If a particular SFC is activated twice, a diagonal structure appears in the matrix, composed of consecutive time bins of high intersection values. Further convolution of the matrix with a diagonal linear filter enhances diagonal structures compared to isolated high intersection values. Several features of the data, such as high neuronal firing rates, undersampling of the system or stochastic participation in assembly activation can make the diagonal structures wiggly or discontinuous, or may lower their contrast from individual pixels, thus affecting the analysis performance.

Here we introduce a quantitative statistical evaluation of the presence of SFCs which enables an automatic identification of the neurons participating in an SFC. Diagonal structures in the intersection matrix are enhanced by convolving the intersection matrix with a rectangular filter, which enables to cope with wiggly structures. The statistical significance of the diagonal structures is then assessed by filtered surrogate intersection matrices, generated by random permutation of the entries of the original matrix. This implements the null hypothesis that the repeated sets of synchronously firing neurons do not have temporal structure. The entries of all surrogate matrices form the distribution of matrix entries under the null hypothesis. By choosing an upper quantile we define a lower threshold to identify statistically significant entries in the filtered intersection matrix. Using a clustering algorithm we group the significant entries into single diagonal structures, interpreted as signatures of repeated SFC activation. Finally, we identify the neurons composing the SFC and their group membership.

We calibrated the method using stochastic simulations consisting of repeating consecutive synchronous spike patterns embedded in otherwise independent data. Our calibration shows that in most realistic scenarios, e.g. for realistic firing rates and downsampled networks, large portions (> 90%) of the embedded SFCs are identified at low false positive and false negative levels. We discuss future improvements and possible applications to electrophysiological data.

Acknowledgements

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Synaptic Consolidation of Competition

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Neurons have a numerous number of plastic synapses at their dendritic tree. Thereby, the changes at the synapses decay on a short time scale of several minutes [1]. Only the process of synaptic consolidation 'secures' these changes by an interaction between a synaptic tag and postsynaptic protein synthesis [2,4,5,7]. However, additionally, all synapses interact with each other and influence their plastic changes. This process of competition, that a positive change at one synapse induces negative changes at the others, is very important to explain several experimental data [8]. However, the mechanism, how competition is also consolidated and, therefore, secured from fast forgetting, is still unknown.
Here, we use an integrate-and-fire neuron model [6] with two plastic synapses, which are adapted by synaptic plasticity based on the calcium levels [3]. These changes decay on short time scales. To avoid this, we introduced a double-threshold mechanism for consolidation of the changes.
Using this model, we show how the two synapses 'talk' to each other mediated by the spiking activity of the shared postsynaptic neuron. Given inputs at the first synapse, the key factor that determines the second synapse strength is the post neuron's resistance and the detailed stimulation protocols. Importantly, we can recapture qualitatively similar synaptic consolidation results as in experimental observations [5, 7]. In addition, we see under which conditions competitive effects are induced at the second synapse and when they are consolidated, too.
In summary, this work combines important theoretical results of plasticity and competition with the idea of tagging and protein synthesis to bridge time scales from minutes to several hours.

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Synaptic plasticity maximizes information in recurrent neural circuits

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Maximum information has been proposed as a principle for neural coding. A few neural circuit models that implement maximum information have been proposed, but most of them are biologically implausible or they are unable to reproduce basic physiological observations.

Among the many facets of maximum information, we focus on decorrelation, the reduction of correlations across neuron pairs. Several mechanisms have been proposed for decorrelation in neural circuits. One mechanism is inhibitory feedback, which is well supported by experimental testing [1,2]. However, it does not explain the development of neural coding for sensory stimuli. Another mechanism is anti-Hebbian plasticity, which explains neural circuit development but is not experimentally supported [3] and it cannot maintain any memory of the input stimuli [4].

We introduce a biologically realistic synaptic plasticity rule based on STDP. We show that this rule can enable the system to sense both the spatial and temporal properties of inputs and decorrelate the sensory inputs. Furthermore, it maintains the memory of those inputs on arbitrary timescales. The model is able to account for a wide range of physiological observations, including balanced excitatory and inhibitory currents, the long-tailed and non-random distribution of synaptic strengths [5,6]. Interestingly, both the distribution of synapses and the balanced state have a strong influence on the decorrelation process. The simultaneous properties of maximum information and multi-scale memory make the model a candidate for efficient neural computation.

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The effect of heterogeneity on decorrelation mechanisms in spiking neural networks: a neuromorphic-hardware study

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Correlations in neural activity can severely impair the processing of information in neural networks.
In finite-size networks, correlations are however inevitable due to common presynaptic sources.
Recent theoretical studies have shown that inhibitory feedback, abundant in biological neural networks, can actively suppress these shared-input correlations and thereby enable neurons to fire nearly independently. [1,2]
For networks of spiking neurons, the decorrelating effect of inhibitory feedback has so far been explicitly demonstrated only for homogeneous networks of neurons with linear sub-threshold dynamics.
Theory, however, suggests that the effect is a general phenomenon, present in any system with inhibitory feedback,
irrespective of the details of the network structure and the neuron and synapse properties.
Here, we investigate the effect of network heterogeneity on correlations in sparse random networks of inhibitory neurons with conductance-based synapses.
Accelerated neuromorphic hardware [3] is used as a user-friendly stand-alone research tool to emulate these networks.
The configurability of the hardware substrate enables us to modulate the extent of network heterogeneity in a systematic manner.
We selectively study the effects of shared-input (light gray symbols in Fig.) and recurrent connections (black and dark gray symbols in Fig.) on correlations in synaptic inputs (Fig a) and spike trains (Fig b).
Our results confirm that shared-input correlations are actively suppressed by inhibitory feedback also in highly heterogeneous networks exhibiting broad, heavy-tailed firing-rate distributions.
However, while cell and synapse heterogeneities lead to a reduction of shared-input correlations (feedforward decorrelation),
feedback decorrelation is impaired as a consequence of diminished effective feedback (see Fig.).

Acknowledgments: Partially supported by the Helmholtz Association portfolio theme SMHB, the Jülich Aachen Research Alliance (JARA), EU Grant 269921 (BrainScaleS), and EU Grant 604102 (Human Brain Project, HBP).

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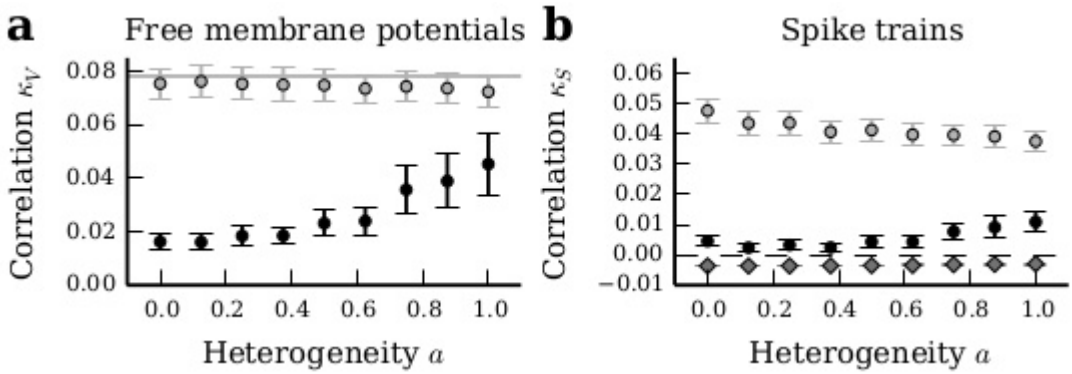
a
Free membrane potentials
Correlation κ_V
0.08
0.06
0.04
0.02
0.00
0.0
0.2
0.4
0.6
0.8
1.0
Heterogeneity a
b
Spike trains
Correlation κ_S
0.06
0.05
0.04
0.03
0.02
0.01
0.00
−0.01
0.0
0.2
0.4
0.6
0.8
1.0
Heterogeneity a

The rat connectome: All known connections of the rat nervous system in one database

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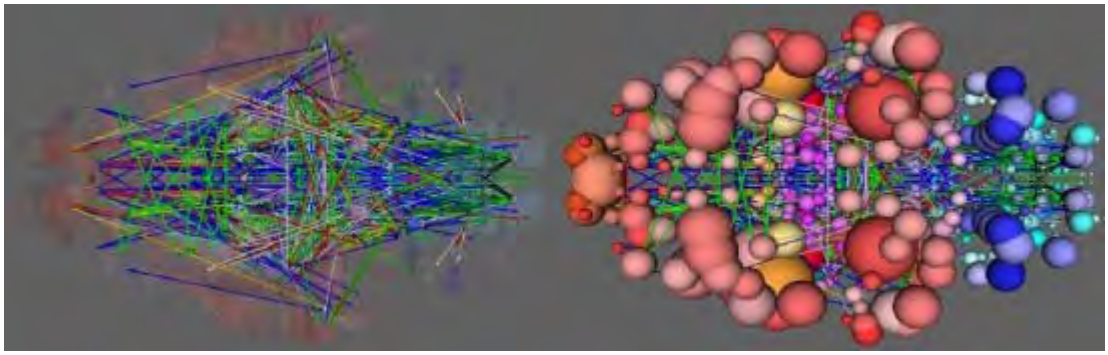
In an ongoing metastudy of tract tracing reports of the normal adult rat, connections from 5400 publications have been transferred to a database in neuroVIISAS. The connectome consists of 183616 ipsi- and 74700 contralateral connections of the central and peripheral nervous system. The interconnected regions are organized in a hierarchy of 21 levels from basic parts of the nervous system down to the level of cortical layers and in some cases down to the level of populations of neurons and individual neurons. The webpage http://neuroviisas.med.uni-rostock.de/connectome/index.php allows queries to the connectome database. Partial connectomes can be exported and analyzed offline using the generic framework neuroVIISAS which is available by download or a launching function. The connectome is integrated into the stereotaxic atlas of Paxinos and Watson and direct population based simulations using the NEST simulator are possible. The atlas data allows 3D visualization of the connectome within neuroVIISAS (See figure). To augment the raw data, recognized cells (computer vision) in serial virtual slides are imported into the connectome to obtain estimates of the total number of cells of all regions of the stereotaxic atlas. This high density of raw data will be the backbone of a more realistic computational model of the rat nervous system.



The transfer function of the LIF model: A reduction from colored to white noise

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The assessment of the stability of neuronal networks, the emergence of oscillations and correlated activity rely on the response properties of a neuron to a modulation of its input, i.e. the transfer function. For the leaky integrate-and-fire neuron model exposed to white noise the transfer function has been derived analytically [1,2]. The decay time of a few milliseconds for a postsynaptic current amounts to the synaptic noise not being white, but rather having higher power at low frequencies. The effect of such colored noise on the response properties has been studied intensively at the beginning of the last decade [3,4]. Analytical results were derived in the low as well as in the high frequency limit. The main finding is that the linear response amplitude of model neurons exposed to filtered synaptic noise does not decay to zero in the high frequency limit. A numerical method has also been developed to study the influence of synaptic noise on the response properties [5].
Here we first revisit the transfer function for neuron models without synaptic filtering and simplify the derivation exploiting analogies between the one dimensional Fokker-Planck equation and the quantum harmonic oscillator. Synaptic filtering comes along with considerable difficulties, since it adds a dimension to the governing Fokker-Planck equation. We overcome these complications by developing a general method of reduction to a lower dimensional effective system, respecting the details of the noise in the boundary conditions [6]. Static boundary conditions were derived earlier by a perturbative treatment of the arising boundary layer problem [4]. Here we extend this study to the dynamic case. Finally we compare the analytical results to direct simulations (Fig.1) and observe that the approximations are valid up to moderate frequencies. Deviations are explained by the nature of the made approximations.

Figure 1:
Transfer function. Absolute value (A) and phase (B) of the transfer function. Analytical prediction (solid curves), direct simulations (dots), and zero frequency limit (dashed horizontal line).

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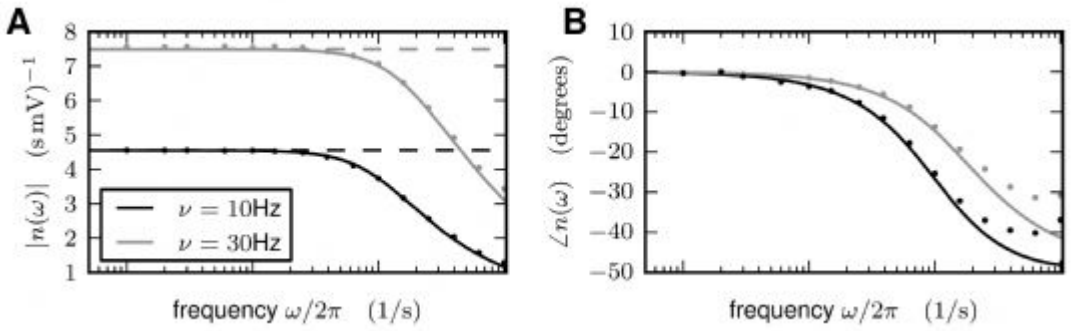

Tonic conductance changes in the Central Amygdala influence fear generalization

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Recent experimental studies (Ciocchi et al, 2010, Haubensak et al, 2010) have revealed an inhibitory microcircuit in the central amygdala, which is essential for the acquisition and expression of conditioned fear. Two physiologically distinct, mutually inhibiting subpopulations within the lateral part send inhibitory projections to the medial subdivision of the central amygdala, which drives conditioned fear responses. These subpopulations undergo plasticity of their phasic and tonic activity during fear conditioning, thereby gating fear expression. Notably there is a correlation between changes in tonic activity and the ability of the animal to discriminate between the conditioned stimulus and a neutral control stimulus (Ciocchi et al.).

These changes are associated with synaptic plasticity on afferent connections from the basolateral amygdala (Li et al., 2013) and subpopulation-specific modulation of tonic inhibition, caused by extrasynaptic GABA-receptors (Botta et al., in prep.). In order to understand how these two different types of plasticity affect fear generalization, we combined large scale spiking neural network simulations with a higher level functional model of the central amygdala. Specifically we addressed the effect of tonic conductance changes on the processing of incoming stimuli in the network. In our model stimulus-driven synaptic plasticity and changes of tonic inhibition reproduce the observed changes in tonic and phasic network activity. Furthermore our results suggest that tonic inhibition controls fear generalization in a manner similar to the process of regularization in regression models. Together with experimental work relating fear generalization to anxious behavior in mice (Botta et al.) these results provide a neuronal mechanism by which the central amygdala may acts as a link between fear learning and the emergence of anxiety disorders.

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Ultra-fast response to external electric pulses explained by neural morphology

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Neurons can be stimulated to fire an action potential by a transient stimulus, e.g. by a spatially homogeneous electric field that is switched on for about a millisecond. The duration that is needed to trigger a neural response depends on the strength of the field. This dependence is characterized by the *rheobase*, the field strength required in the limit of very long pulses, and the *chronaxie*, the minimal pulse duration required for a field strength of twice the rheobase.
We recently utilized patterned cell cultures to target the stimulation only to dendrites or to axons and dendrites together (Stern et al., in press). We showed that the previously reported short chronaxies of a few hundered microseconds occur only, when the axon is targeted. Stimulation of dendrites results in longer chronaxies and, surprisingly, a smaller rheobase, as if active elements in the dendrites were more voltage sensitive. The apparent high sensitivity of dendrites and the fact that axonal chronaxies are much shorter than the membrane time constant, could be connected to the properties of active elements, i.e. voltage dependent conductances or they could result from the passive properties of the spatially extended neuron.
Here we report, that both non-trivial effects can be well explained by passive properties. The short chronaxies of axonal stimulation can be explained by the fact that in the first tens of microseconds the axon initial segment is essentially voltage clamped due to the impedance mismatch of the large soma and the thin process. The longer chronaxie and smaller rheobase of dendrites can be attributed to slow charge accumulation effects in asymmetric, branched dendritic trees. These results suggest that pulse waveforms can be designed to differentially target morphologically distinct groups of neurons.

Poster Topic

T27: Techniques and Demonstrations

T27-1A A fluorescent molecule form marine sponges and its synthetic derivatives used for live imaging of neurons, tissues and organisms
Ulf Bickmeyer, Thorsten Mordhorst

T27-2A A large-scale method for establishing zebrafish neuronal cell cultures
Georg Welzel, Daniel Seitz, Stefan Schuster

T27-3A Analysis of Ca2+ handling properties: Perforated Patch Clamp Recordings Meet the Added Buffer Approach
Simon Hess, Martin E Hess, Christophe Pouzat, Jens C Brüning, Peter Kloppenburg

T27-4A Analysis of circadian regulation of gene expression in CNS microvascular endothelial cells
Jochen Ohnmacht, Markus Schwaninger

T27-5A Bayesian modelling of locust behaviour using BAYSIG
Peter Sutovsky, Swidbert R Ott, Tom Nielsen, Tom Matheson

T27-6A Characterization of the stimuli delivered by the Fly Mind-Altering Device (FlyMAD).
Dorothea Hörmann, John R. Stowers, Andreas Poehlmann, Andrew D. Straw

T27-7A Detecting Temporal Modulation of Higher-Order Correlations based on Pairwise Correlation Measures
Vahid Rostami, Junji Ito, Emiliano Torre, Pietro Quaglio, Moritz Helias, Sonja Grün

T27-8A Drug Exposure in Plasma, CSF and Brain: A Pharmacokinetic Comparison Study in Rats and Cynomolgus Monkey.
Marcel van Gaalen, Joost Folgering, Jane, S. Sutcliffe, Christina Schlumbohm, Bob E Stratford3, Thomas I Cremers

T27-1B Electrophysiological Characterization of Individual Neurons in Sparse Cortical Cultures
Anne Sinning , Keiko Weir , Oriane Blanquie , Werner Kilb , Heiko J Luhmann

T27-2B elephant: an Open-Source Tool for the Analysis of Electrophysiological Data Sets
Michael Denker, Alper Yegenoglu, Detlef Holstein, Emiliano Torre, Todd Jennings, Andrew Davison, Sonja Grün

T27-3B Estimating the position of active neurons with multichannel microelectrodes
Martin Nguyen, Christopher Doerr, Thomas Schanze

T27-4B Exceptionally well preserved central nervous system in an early Cambrian arthropod
Yu Liu, George S. Boyan, Jie Yang, Xiguang Zhang

T27-5B Freely vibrating nanostructured scaffolds as a novel assay to investigate mechanical properties of retinae
Saddam Mayazur Rahman, Andreas Reichenbach, Stefan G. Mayr, Mareike Zink

T27-6B Heptodes are superior for spike sorting to tetrodes: a simulation study
Christopher Doerr, Thomas Schanze

T27-7B High-resolution localization of synaptic proteins in social insect brains
Thomas S. Muenz, Vivien Bauer, Christian Stigloher, Wolfgang Rössler

T27-1C How to efficiently organize and exploit metadata of complex electrophysiological experiments
Lyuba Zehl, Michael Denker, Adrian Stoewer, Florent Jaillet, Thomas Brochier, Alexa Riehle, Thomas Wachtler, Sonja Grün

T27-2C Hybrid voltage sensor imaging of eGFP-F expressing neurons in chicken midbrain slices
Stefan Weigel, Tatiana Flisikowska , Angelika Schnieke, Harald Luksch

T27-3C Imaging of whole-mount samples with µm resolution using light-wedge- microscopy
Ulrich Leischner

T27-4C Implantable, yet adaptive multi-electrode positioning system for brain computer interface applications
Enrico Ferrea, Lalitta Suriya-Arunroj, Dirk Hoehl, Uwe Thomas, Alexander Gail

T27-5C Long-term decoding of continuous and discrete movement parameters with a wireless myoelectric implant
Pierre Morel, Enrico Ferrea, Bahareh Taghizadeh-Sarshouri , Sina Plümer, Josep Marcel Cardona Audí , Sören Lewis, Roman Ruff, Michael Russold, Lait Abu-Saleh, Dietmar Schroeder, Wolfgang Krautschneider, Thomas Meiners, Klaus-Peter Hoffmann, Alexander Gail

T27-6C Modelling Biological Signals with BAYSIG
Kamal Abu Hassan, Thomas Nielsen, Emma Chung, Ronney Panerai, Nazia Saeed, Angela Salinet, Thompson G Robinson, Thomas Matheson

T27-7C Neuroinformatics for efficient data management and reproducibility in electrophysiology
Thomas Wachtler, Adrian Stoewer, Andrey Sobolev, Christian Kellner, Yann Le Franc, Jan Grewe

T27-1D Obscured artifacts in multi-electrode recordings and their influence on correlation analysis
Julia Sprenger, Emiliano Torre, Vahid Rostami, Thomas Brochier, Alexa Riehle, Michael Denker, Sonja Grün

T27-2D Photonic modulation of membrane potential by flash-light illumination of metallic beads.
Navin K. Ojha, Jane C. Arifin, Martin Schink, Enrico Leipold, Stefan H. Heinemann

T27-3D *Post-mortem* magnetic resonance microscopy of the murine brain at 7 Tesla
Oliver von Bohlen und Halbach, Martin Lotze, Jörg Pfannmöller

T27-4D Simultaneous electrophysiological analysis of circadian rhythms of the circadian pacemaker center, of the electroretinogram, and of leg muscle activity in the cockroach *Rhyparobia maderae*
El-Sayed Baz, Marcel Heim, Ildefonso Atienza López, Monika Stengl

T27-5D Retracted

T27-6D Time-dependent cellular response profiling of an immortalized embryonic murine hippocampal cell-line (mHippoE-14) for the *in vitro* simulation of brain diseases
Apostolos Zarros, George S. Baillie

T27-7D Towards safe optogenetics with grafted cell implants: An alternative method to the direct virus delivery of the opsins
Lisa-Marie Selesnew, Paul Schlanstein, Luciano Furlanetti, Yijing Xie, Volker A. Coenen, Ulrich Hofmann, Máté D. Döbrössy

A fluorescent molecule form marine sponges and its synthetic derivatives used for live imaging of neurons, tissues and organisms

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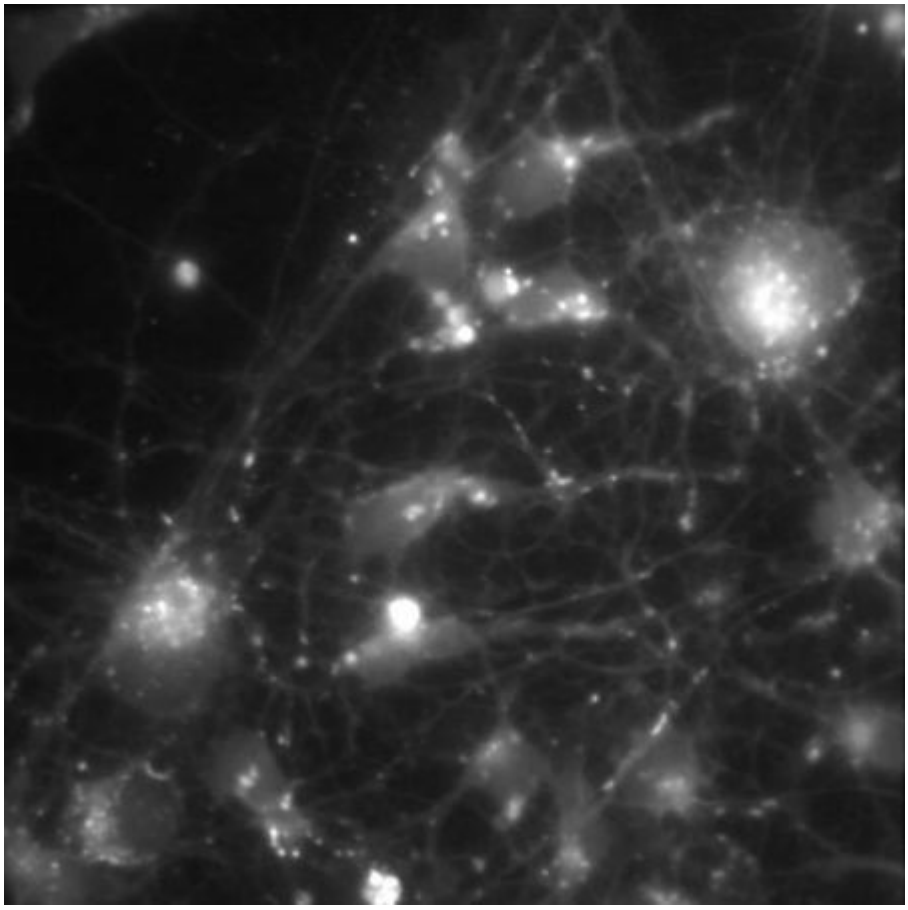
Marine sponges are known to produce pharmaceutical relevant secondary metabolites. One substance out of the group of the brominated pyrrole imidazole alkaloids showed anti-angiogenic activity and was later described to be a pH sensitive live imaging dye with little other side effects. This substance, Ageladine A, permeates membranes when uncharged and the two fold bromination seems to be a very important factor increasing its membrane permeability. In acidic cellular compartments or organs Ageladine A is up to two times protonated and in its charged status, the compound is trapped and not expelled by MDR and MRP transporters. Given the interesting properties of Ageladine A we synthetized twenty-five derivatives of which several are brightly fluorescent. Some compounds are as well pH sensitive and at least one allows ratiometric measurements over a wide pH range. Lysosomes can be best stained by LysoGlow84, whereas acidic transport vesicles are best stained with Ageladine A. As metabolic states like hypoxia or apoptosis are reflected by the cytosolic pH values, these dyes can be used to indicate the viability of cells. The major increase of fluorescence of Ageladine A and its sensitivity is in the pH range of pH 5 to pH 7, which is most interesting range for physiological measurements. Due to the non-toxic properties the dyes can be used to stain whole animals (transparent aquatic animals), tissues and e.g. astrocytes and cultured neuronal cells.

Fig. Hippocampal cells stained with Ageladine A (Image: M. Heine; U. Bickmeyer)



A large-scale method for establishing zebrafish neuronal cell cultures

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Neuronal cell cultures offer a crucial tool to address neurobiological issues on a cellular and molecular level *in vitro*. Despite the increasing importance of zebrafish (*Danio rerio*) as *in vivo* model in neurobiological and biomedical research, no method is currently available for establishing pure neuronal cell cultures. Here we describe the first large-scale generation of neuronal restricted progenitor (NRP) cultures from embryonic zebrafish by using a semi-automated dissociation procedure combined with magnetic activated cell sorting that could boost the use of neuronal cell cultures as a tool for the mechanistic dissection of key processes in neuronal regeneration and development.

Analysis of Ca2+ handling properties: Perforated Patch Clamp Recordings Meet the Added Buffer Approach

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The spatial and temporal Ca^{2+} dynamics in neurons are determined by a variety of cellular parameters including the calcium influx, calcium buffering and calcium extrusion. These neuronal Ca^{2+} handling properties of a neuron can be analyzed by using the 'added buffer approach' combined with whole cell patch clamp recordings and fast ratiometric Ca^{2+} imaging with fura-2 (Neher & Augustine, 1992). The 'added buffer approach' is based on a single compartment model of Ca^{2+} buffering. For measurements of intracellular Ca^{2+} concentrations with Ca^{2+} chelator based indicators, the amplitude and time course of the signals are dependent on the concentration of the Ca^{2+} indicator; in this case fura-2 that acts as an exogenous (added) Ca^{2+} buffer and competes with the endogenous buffer. With the added buffer approach, the capacity of endogenous Ca^{2+} buffer in a cell is determined by measuring the decay of the Ca^{2+} signal at different concentrations of 'added buffer', and by extrapolating to conditions in which only the endogenous buffer is present.

Originally the 'added buffer approach' is used in combination with whole cell patch clamp recordings, which allows convenient loading of the neuron with Ca^{2+} indicator via the patch pipette. However, since mobile Ca^{2+} buffers are washed out of the cell during whole cell recordings only the immobile fraction of the endogenous Ca^{2+} buffer can be determined. To circumvent this problem we successfully performed perforated patch clamp recordings combined with the 'added buffer approach' to determine the whole endogenous Ca^{2+} buffering capacity from adult (9 – 12 w) midbrain DA neurons and cerebellar Purkinje neurons. As ionophore we used ß-escin. In contrast to whole cell recordings, various basic cellular parameters (e.g. $[Ca^{2+}]_i$, membrane potential/conductance) remained stable over time during ß-escin perforated patch recordings. During the recordings we could achieve a controlled loading of the neurons with fura-2, while mobile Ca^{2+} binding proteins (e.g. calbindin D28k) was retained by the perforated patch. Our results show that the ß-escin perforated patch recordings combined with the 'added buffer approach' are useful for the stable and reliable determination of neuronal Ca^{2+} handling properties.

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Analysis of circadian regulation of gene expression in CNS microvascular endothelial cells

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Endothelial cells of the brain microvasculature and the integrity of the blood-brain barrier (BBB) have been shown to be critically involved in various diseases and pathological conditions[1]. Previously published and our own data show, that in the course of a normal 24-hour interval, the permeability of the BBB is subject to change[2]. It has been reported for mouse clock gene knockout strains, that endothelial cells display vascular dysfunction and remodelling, are subjected to increased stress[3,4], and that some of these changes can be attributed to cell-autonomous effects of clock gene knockout in vessels[5]. In the mouse gut, epithelial cells are also displaying circadian regulation of tight junction protein expression resulting in changed permeability[6]. Together, this data indicates that endothelial cells of the brain vasculature could be under circadian regulation, and that some aspects of the changes in BBB permeability could be attributed to changes in clock gene regulated gene expression.

We use gene expression analysis of microvascular endothelial cells to identify genes involved in these processes. While gene expression profiling on microvascular endothelial cells have been performed previously, most of these works rely on the isolation of blood vessel fragments or of endothelial cells themselves from brain tissue prior to RNA extraction. The changes introduced by these treatments could influence expression levels of genes involved in endothelial function, most importantly cell-cell contacts. We therefore apply a different approach: By using the Toxoplasma gondii derived enzyme UPRT newly synthesized RNA can be thio-labelled within a short time window after administration of thio-uracil[7,8]. Cell type specific expression of the enzyme under control of the endothelial specific promoter Slco1c1 allows us to label endothelial transcripts in vivo under physiological conditions. RNA of endothelial cells can be specifically labelled, extracted and subsequently analysed. Changes in gene expression profiles over a 24 hour time interval can then be determined using RT-qPCR, microarray or deep sequencing techniques.

Using this approach allows us to identify time-dependent regulation of candidate genes putatively involved in regulating endothelial function and BBB properties. The characterization of the chrono-physiology of vascular function and endothelial cell properties is essential to understanding the changes observed under pathological conditions. With a focus on microvascular endothelial cells in the central nervous system (CNS) and their properties contributing to the tightness of the BBB we aim to elucidate what role circadian regulation of gene expression has to play in physiological conditions. In addition, a deeper understanding of the physiological regulation of the tightness of the BBB will be crucial to develop better strategies for the delivery of pharmacological compounds to the CNS.

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Bayesian modelling of locust behaviour using BAYSIG

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A fundamental challenge in behavioural analyses is the gap between simple statistical tests based on calculated features, and detailed simulations obtained from stochastic physical models. Bayesian statistical analysis can provide a powerful way to model data for behavioural studies.

Bayesian approaches provide several advantages over classical statistical methods. One key advantage is that Bayesian inference via Markov Chain Monte Carlo (MCMC) methods allows us to use more complex models. Rather than explicitly extracting selected features from the data prior to statistical analysis, these methods learn parameters of the model representing observed behaviour. Another advantage is that they allow us to compute not only point estimates but also the distributions of the model's parameters. We are using BAYSIG (http://bayeshive.com/), a concise mathematical programming language for statistical analysis developed by OpenBrain (http://openbrain.co.uk/). BAYSIG is especially efficient for building, inference and verification of dynamic models represented either by stochastic differential equations or difference equations.

We are in the process of applying Bayesian models to the analysis of locust (Schistocerca gregaria) behaviour. In a classical assay setting a locust is released into arena where its movement trajectory is recorded. A key aim of the study is to characterise using Bayesian modelling hidden behavioural 'states' that drive observed differences in movement.

Characterization of the stimuli delivered by the Fly Mind-Altering Device (FlyMAD).

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Drosophila melanogaster is an important model system for understanding the neural basis of complex behavior. Its fast development cycle and the cutting edge genetic tools make the fruit fly an ideal candidate for experimental studies. Thermogenetic tools which exploit the properties of temperature gated ion channels or temperature sensitive mutant proteins to activate or silence specific subsets of neurons have been widely used in fly neurobehavioral research for several years. Recently, novel optogenetic tools which work by the means of light-gated ion channels and pumps have been developed for use in *Drosophila melanogaster*.[1,2] To study the neural basis of naturalistic behaviors, one strives to achieve high temporal and spatial resolution while providing a natural environment. Our earlier developed and recently described FlyMAD (Fly Mind-Altering Device) system[3] is capable of providing all of these by targeting a focused laser beam on freely walking flies. The heat transfer through an infrared beam can be used for activation and silencing with thermogentic tools or for generating aversive behavior in wild-type flies, whereas using a red laser beam provides a stimulus for activation with optogenetic tools. Here we further characterize the exact nature of the different stimuli provided by FlyMAD. Using FlyMAD's refined tracking capability we earlier showed that targeting different body parts of wild-type flies with an infrared beam leads to distinct aversion responses. We speculated that the difference can be attributed to the activation and inactivation of morphologically independent external or internal thermal sensors. We further test this by targeting flies mutant for individual known thermal channels in a body-part specific manner. Furthermore we extended our system to be capable of targeting flies depending on their position in the arena, thus we were able to simulate a step thermal gradient similar to a two-temperature choice assay.[4] Using this extension of our system we expect to link the earlier described difference in velocity upon targeting different body-parts to higher level behaviors and contexts.

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Detecting Temporal Modulation of Higher-Order Correlations based on Pairwise Correlation Measures

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The Unitary Event (UE) analysis method was designed to detect excess spike synchrony in parallel spike trains as an indicator of assembly activity [1]. The application of the method to simultaneous recordings from cortical neurons provided evidence for occurrence of excess synchrony related to behavior [2]. However, the UE analysis does not scale to arbitrary cell assemblies in massively parallel spike trains (MPST, e.g. 100 or more neurons) due to the combinatorial explosion of occurring spike patterns, and thus the consequent massive multiple testing problem.

Here we present an extended UE analysis that is applicable to MPST with acceptable computational effort. It provides indications of the presence of higher-order synchrony (HOS) in a time dependent manner. The extension is based on a population measure derived from pairwise measures, namely the sum of the empirical pairwise coincidences from all neuron pairs n_{emp}^{pop} and the expected number of coincidences n_{exp}^{pop}. The latter is calculated for all neuron pairs on the basis of their firing rates under assumption of mutual independence. The significance of the n_{emp}^{pop} given n_{exp}^{pop} is derived by the p-value p^{pop}, and accordingly the surprise $S^{pop}=\log(1-p^{pop})/p^{pop}$. Given recordings from multiple trials, we define the Fano factor FF^{pop} as the variance of S^{pop} across trials divided by its mean. For calibration of the measures, we use a compound Poisson process (CPP) [3] to model parallel spike trains that possess a) an arbitrary order of HOS, b) a specific average pairwise correlation, and c) a given firing rate distribution across the neurons.

We find that the average pairwise correlation is reflected by the mean of S^{pop} across trials, whereas the order of the synchrony by FF^{pop}. As the measures provide reliable statistics on a short time scale (~100 ms), we use them to track the temporal changes in correlation order and average pairwise correlation (Figure). Furthermore, our measures show robustness against the heterogeneity of experimental data, in particular non-homogeneous firing rates across neurons and trials, and non-stationary firing rates in time.

Our method thus suggests a way to detect temporal variation of the correlation order and the average pairwise correlation in MPST. We currently work on a comparison of our analysis to existing methods such as CuBIC [3], which infers the lower bound of the order of synchrony, in terms of reliability with respect to the amount of available data.

Figure caption: CPP parameters for data generation: ξ HOS order, ρ average pairwise correlation as a function of time (top panel). Mean S^{pop} (middle panel) and Fano factor of S^{pop} (bottom panel) derived within a sliding window (100 ms) analysis on simulated data of 90 neurons, 120 trials of 12 secs duration each. The pairwise correlation is well recovered by $E[S^{pop}]$, independent of the order of the correlation ξ. The latter is reflected by FF^{pop}.

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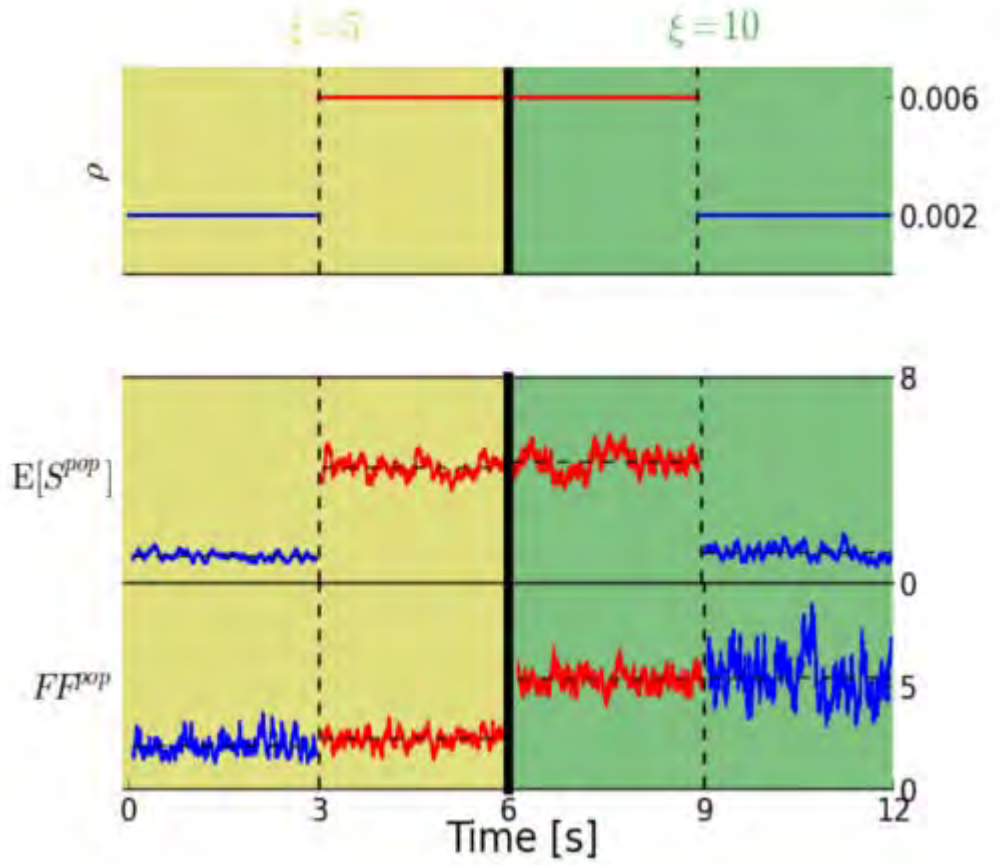

Drug Exposure in Plasma, CSF and Brain: A Pharmacokinetic Comparison Study in Rats and Cynomolgus Monkey.

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Rationale: Human pharmacokinetic prediction is essential for adequate dosing in early clinical trials. This is in particular challenging for drug development for psychiatric and neurological diseases, due to the blood-brain-barrier (BBB) and lack of methods to directly measure drug concentrations in the brain in humans.
Objectives: Comparing drug (amphetamine) distribution in plasma, CSF and brain in rats and cynomolgus monkey.
Methods: A single 0.1 mg/kg intravenous dose of amphetamine, a drug widely used in the treatment of Attention Deficit Hyperactivity Disorder (ADHD), was administered to male rats and cynomolgus monkeys. Microdialysis probes were inserted in the prefrontal cortex (PFC; in rat and cynomolgus monkey) and striatum (STR; in cynomolgus monkey) regions. Absolute amphetamine concentrations were measured simultaneously in the extracellular fluid (ECF) of these brain regions. Systemic exposure to amphetamine was also measured in the same animals via venous sampling. CSF amphetamine concentrations were determined from separate animals (in cynomolgus monkey) and in the same animals (in rat). In order to compare the time-dependent pharmacodynamic effect to drug exposure in cynomolgus monkey, dopamine concentration were measured in the dialysate in PFC and STR simultaneously to drug measurements. Sample analysis was performed using LC-MS/MS.
Results: Here we show, for the first time, that ECF dopamine concentrations in both the PFC and STR are closely correlated to the free concentration of amphetamine in these brain areas and plasma over time in cynomolgus monkey. Peak concentrations were observed around 1-hour following drug administration, indicating rapid uptake of amphetamine across the BBB. Relative to total plasma concentrations, however, exposure based on AUC ranged from 2.3 to 2.5 fold greater in brain ECF, a finding suggesting carrier-mediated transport of amphetamine across the cynomolgus monkey BBB. In response to the amphetamine dose, dopamine levels in PFC and STR increased an average of 2.3 ± 0.27 fold and 2.4 ± 0.34 fold over baseline in PFC and STR, respectively. Currently, additional analysis of samples is ongoing, which are needed to further compare the pharmacokinetic properties of amphetamine in both species.
Conclusion: Preliminary data support the notion that pharmacokinetic properties of compounds differ between species. Additional data will be presented allowing full analysis. It is suggested that in order to have the most reliable human pharmacokinetic prediction of CNS drug discovery candidates, drug exposure in plasma, CSF and brain in various species, including non-human primates, is advantageous.

Electrophysiological Characterization of Individual Neurons in Sparse Cortical Cultures

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Combined recordings of electrical activity and fluorescent microscopy can be powerful tools to correlate neuronal activity and cellular physiology. Multi-electrode arrays (MEAs) are commonly used for recordings of extracellular activity from a large population of neurons, but can in principle also be used to examine single-cell activity. Here, we use dissociated, cortical neurons from newborn mice which were sparsely cultured on 120-channel MEA (Multichannel Systems) in order to assign extracellularly recorded spikes to single neurons on the recording field. The proper assignment of spikes to individual, active neurons was confirmed by simultaneous calcium imaging. Spike waveforms and firing patterns at different developmental stages were analyzed in a Matlab routine and also correlated with the distance between recording electrode and the soma of the assigned neuron. Additional experiments with cultures from GAD67-GFP knock-in mice allowed the identification of GFP interneurons with the help of fluorescent microscopy. The results suggest that in our primary cortical culture systems spike waveform characteristics and firing patterns of neurons were fully developed after two weeks in culture. Analysis of spatial relationships revealed a positive correlation between spike amplitude and soma to recording electrode distance and provides a good estimate of the recording field of the MEA electrodes. Although excitatory and inhibitory neurons are present in our culture model at a similar ratio as described in vivo and extracellular signals could be attributed unambiguously to either neuronal class we could not detect significant differences neither in a large number of spike timing parameters nor in any spike waveform parameters. Our detailed characterization of spike waveforms and spike timing properties of cortical neurons in vitro emphasizes the need for direct confirmation of electrophysiological classifications on a single cell level before applying on a population-based level and demonstrates the strength of combined electrophysiological and optical recordings.

elephant: an Open-Source Tool for the Analysis of Electrophysiological Data Sets

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The need for reproducible research has become a topic of intense discussion in the neurosciences. Reproducibility rests on building workflows that may allow users to transparently trace their analysis steps from data acquisition to final publication. A key component of such a workflow is a set of defined analysis methods to perform the data processing. In recent years, an increasing number of software tools (e.g., Neurotools [1], spykeutils [2], OpenElectrophy [3], chronux [4], FIND [5]) have been made available to help experimental neuroscientists in analyzing electrophysiological data. However, many available tools tend to specialize in particular types of analysis and often do not use a common data model, making it cumbersome and error-prone to use multiple tools simultaneously or sequentially during an analysis. In addition, analysis functions are often not written in a modular fashion, thus making it hard to compare results obtained by different analysis methods.

To fill this gap and integrate the existing efforts, we introduce the Electrophysiology Analysis Toolkit (elephant) as a recently initiated community-centered initiative to develop an easy-to-use open-source software package written in Python that offers a broad range of functions for analyzing multi-scale data of brain dynamics from experiments and from brain simulations in a common framework. The focus is the analysis of recordings of electrical activity, such as single unit or massively parallel spike train data and local field potentials (LFP). In this presentation, we outline the scope of initial and future modules of the library, which each correspond to a specific type of analysis. These include signal based analysis (e.g., signal processing, spectral analysis), spike-based analysis (e.g., spike train statistics, rate estimation, spike train correlation, spike pattern analysis), and methods that combine both signal types (e.g., spike-triggered averaging). Lastly, utility modules for the generation of realizations of stochastic processes and of surrogate signals will be implemented, in the context of hypothesis testing.

While the development of the toolbox is an ongoing endeavor, we present here the conceptual framework and design decisions that we recently established for the toolkit specifications. In particular, we show how elephant is envisioned to connect to the “Neo” package [6], which serves as the data model supporting the toolbox functionality. This choice not only guarantees compatibility of the toolkit with a growing stack of software based on Neo, but also provides access to various file I/O modules to access raw data in a variety of open and proprietary formats. Finally, using example use cases we demonstrate hands-on how to analyze data using elephant.

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Estimating the position of active neurons with multichannel microelectrodes

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Introduction

Recordings of extracellular action potentials with multichannel microelectrodes like tetrodes and heptodes are often used to explore neuronal activities in an area of brain tissue in the vicinity of the electrode. The benefit of this recording method is its high spatial and temporal resolution. We are developing an algorithm to estimate the position of neuronal sources with recorded signal amplitudes for each recoding channel based on a simple model. The algorithm is tested with simulated multichannel microelectrode recordings and a simple signal propagation model.

Methods

The brain tissue is assumed to be an infinite, entirely ohmic and ideal homogeneous medium. The source and the multichannel microelectrode contacts are assumed as points in space. Further simplifications for the model are to neglect the influence of the quartz-glass isolation of the electrode and the electric properties of the electrode-tissue interface. To make the algorithm independent from the source model and its corresponding amplitude decay we established an amplitude decay coefficient estimation. With this coefficient it is irrelevant whether a neuronal source is described as a monopole, dipole or a complex multipole.

The amplitude decay for a real monopole was experimentally determined by analysing the recordings obtained for a 1 kHz sinusoidal microelectrode based current injection in an electrolytic trough. Furthermore the recorded signals were used to estimate the relative position of the source and to adjust the amplitude decay of our simulations.

We tested the algorithm with various electrode geometries, channel counts, noise and source-recording configurations.

Results

Preliminary results with simulated signals show that the precision of the position estimation depends on the configuration of the electrode's geometry, the noise contamination and the number of recording cannels. We found: the more signals are used simultaneously, the better the source position estimation performs. In addition, the spatial arrangement of the recording electrode contacts influences the spatial sensitivity considerably. Finally we would like to add that the estimated source positions depend on the signal to noise ratio (Fig. 1).

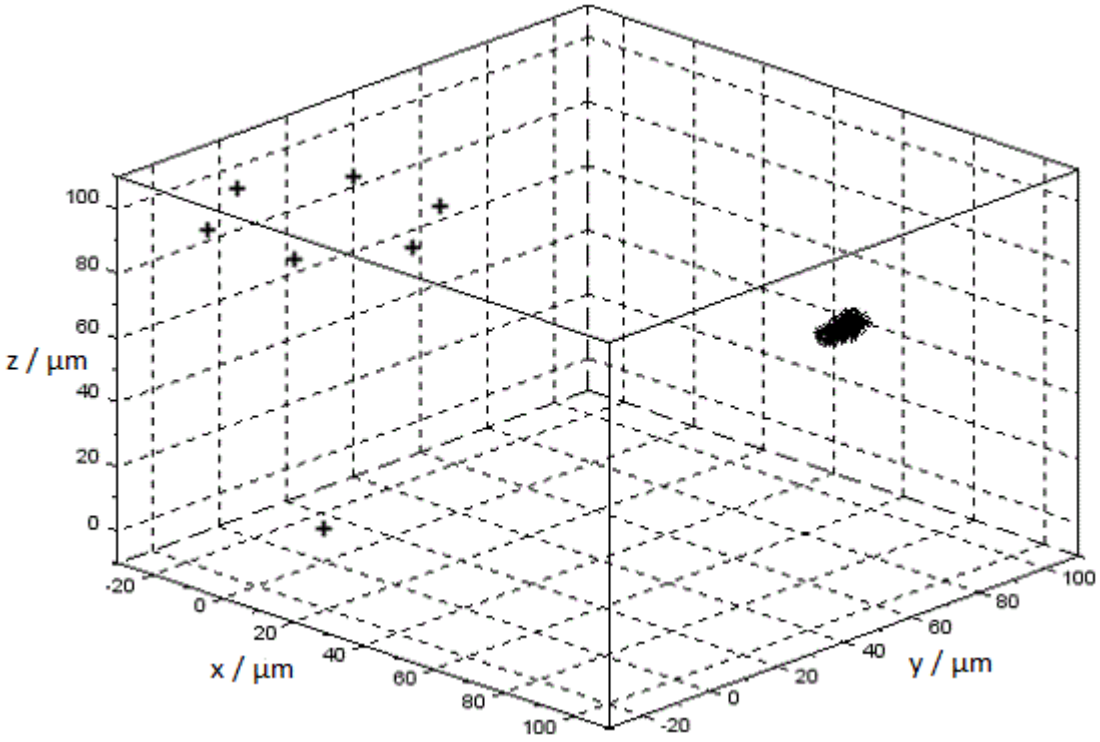
The estimated source positions in the electrolytic trough were reasonable, however the results differed slightly more than expected. This probably depends on the assumed spatial arrangement of the multichannel microelectrode and certainly on the model's simplicity.

Discussion

The developed algorithm allows the position estimation of different signal source types under ideal conditions. An important task for future work is to improve the modelling of the electrode contacts, especially the spatial arrangement. This may be used to estimate the spatial recording characteristics of a multi-channel fibre-electrode.

To adjust and verify the model and the estimation algorithm for in-vivo applications additional experiments using electrolytic troughs and in-vitro models are needed. With improved electrode models and simulations it should be possible to support the development of application specific multichannel microelectrodes.

Figure 1: *Estimation of a neuron's position by using a heptode. The crosses represent the heptode contacts. The dots (cloud) indicate the estimated source positions of 5000 injected signals that contain 10 percent Gaussian white noise. The expected position of the source, i.e. neuron, was (100, 50, 80) µm.*



Exceptionally well preserved central nervous system in an early Cambrian arthropod

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Morphological characters represent a powerful dataset for modern cladistic analyses and so provide valuable insights into our understanding of the evolution of the Arthropoda. However, due to the fragile nature of nervous tissue, the fossil record is sparse with respect to anatomical profiles of the central nervous system, particularly in early Cambrian Chengjiang arthropods. Although we previously identified a partially preserved ventral nerve cord-like structure in an early Cambrian fuxianhuiid, general details relating to the structure and development of the nervous system as evolved by early arthopods have remained unclear. Here we report for the first time an exceptionally well preserved central nervous system extending the length of *Chengjiangocaris kunmingensis* and displaying a ladder-like organization typical of modern arthropods. A pattern of alternating dark and light stripes is evident in the fossil. The most parsimonious interpretation is that the dark stripes represent synaptic neuropils within the cell body cortex of segmental hemiganglia, while the light stripes represent the longitudinal connectives. In addition, each dark stripe corresponds to the site at which a biramous appendage inserts into the exoskeleton. Four dark stripes are each covered by a thoracic tergite. Our findings demonstrate that the organization of the CNS of *C. kunmingensis* accords well with that of modern arthropods, and that the fundamental morphological characteristics of the modern arthropod nervous system are already evolved in the Cambrian.

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Freely vibrating nanostructured scaffolds as a novel assay to investigate mechanical properties of retinae

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Mechanical properties of the adult mammalian retina play a crucial role in many eye diseases such as retinoschisis where the retina swells, Müller cells are overstretched, become mechanical instable and rupture. Studying these properties offers new perspectives for novel therapeutic approached. However, measuring the elasticity of adult retina whole-mounts is difficult and hardly investigated until now. Recent studies use atomic force microscopy to probe local retinal tissue elasticity on the surface with a resolution of micrometers. Here we show a novel technique to study the global retinal mechanical properties in terms of elasticity and energy dissipation during mechanical probing. In a first step, titanium dioxide nanotube arrays are employed as scaffold to culture an adult guinea pig retina explant on. As we have shown recently, these scaffolds, which comprise parallel aligned nanometer-size tubes of TiO_2, can be employed for long-term culture of adult neuronal tissue such as the retina [1]. The scaffolds are produced by an anodization process, while the tube diameter that determines the interaction of the scaffold with the tissue is controlled by varying the applied voltage. For our assay the retina is first cultured on the scaffold. Afterwards the scaffold acts as a freely vibrating reed, viz. the solid rectangular scaffold is clamped to a holder on one end and excited with a motor to oscillate freely. During vibration the frequency and the damping of the reed with the retina on top is detected by a laser. From the eigenfrequency of the vibrating reed before and after retina culture, the elastic properties of the retina, i.e. the Young's modulus can directly be calculated [2]. From the damping of the oscillation amplitude, dissipated energy by the tissue, which can result from internal structural changes, is extracted. Moreover, the change in vibration frequency of the empty reed with the reed with retina offers the possibility to measure the interaction of the retina with the scaffold. Since proper adhesion of tissue to the scaffold material is a prerequisite for long-term culture, our assays also probes this interaction quantitatively. Together with Finite Element Simulations to further validate the obtained results, we gain new insides into the global mechanical properties of the retina with a quantitative new analysis method. Since we have already demonstrated that TiO_2 nanotube scaffolds are suitable for long-term culture of adult neuronal tissue of the brain as well, our new assay can directly be employed to study the mechanical properties of the brain.

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Heptodes are superior for spike sorting to tetrodes: a simulation study

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In neuroscience, simultaneous extracellular recordings from many neurons are used to get insights into the information processing of the brain. Spike sorting is applied to detect and separate the action potentials (spikes) of the different neurons recorded from. Multichannel microelectrodes like tetrodes and heptodes provide a powerful feature for spike sorting: the multitrode (or stereotrode) effect [1]. It describes the different amplitudes of the same action potential on the channels of the multichannel electrode that arise from the amplitude decay in the neural tissue and therefore encode the position of the neuron.

The focus of this work is to analyse the performance benefit due to increase of the number of channels used to record spikes. We automatically analyse simulated heptode and tetrode recordings with our spike sorting algorithm and evaluate the results quantitatively. Several recording conditions like different noise amplitudes and number of neurons are incorporated.

The signal simulation algorithm consists of a simple model of neurons and electrode contacts as point sources and point sinks [2]. The signal's amplitude V of one neuron recorded by one electrode channel is computed with $V(r)=V(0)\exp(r/28.42\mu m)$, which was found experimentally by Gray et al. [3]. Here, r is the distance in µm from the neuron to the electrode contact. The amplitude V(0), i.e. the neuron's amplitude, is set to one.

Spike detection is done by thresholding the instantaneous energy computed by the multiresolution energy filter (MEF). Features are extracted by a principal component analysis of the concatenated spike waveforms. Thereby we incorporate, implicitly, the multitrode effect and the waveforms of the spikes. Spikes are clustered with a heteroscedastic Gaussian mixture model.

As a measure for spike sorting performance we compute the fraction of correctly classified spikes. For this we assign every neuron to the classified cluster that contains the most of its spikes. Only spikes classified to the related cluster are counted as correctly classified.

Our results indicate that the spike sorting performance is better for heptodes than for tetrodes when many neurons are present and when the signals contain much noise (Fig. 1). In signals with few neurons and with little noise influence, both, tetrodes and heptodes, perform virtually equal.

However, we expect that the implicit exploitation of the multitrode effect by the PCA on the concatenated spike waveforms is not optimal. In future work we will focus on ways to exploit the multitrode effect to a higher degree. Finally, a more realistic simulation algorithm is expected to yield more lifelike results.

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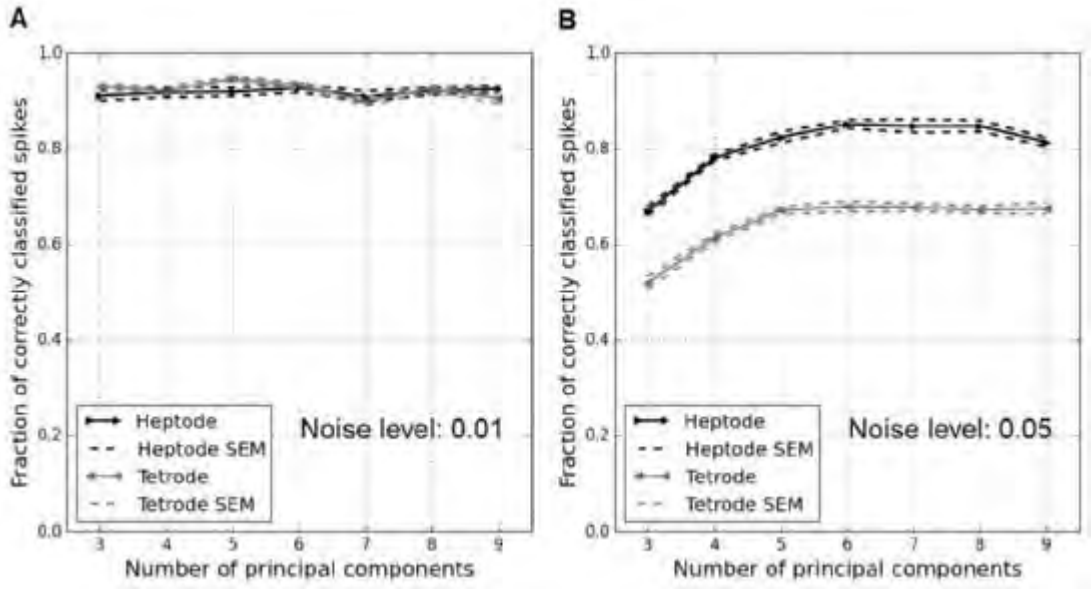
Figure 1: *Spike sorting performance in fraction of classified spikes for signals with noise level 0.01 and 0.05. 14 Neurons were simulated in the signal without overlapping spikes.*



High-resolution localization of synaptic proteins in social insect brains

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Social insect colonies of ants and bees consisting of thousands of individuals are ecologically extremely successful and have been described as superorganisms. The workers, as the main individual 'unit' for the functioning of the superorganism, show a highly adaptive behavioral repertoire enabling the colony to express emergent responses to varying environmental conditions. Therefore mechanisms of learning and memory as well as neuronal mechanisms of behavioral plasticity at the level of individual workers have become an important focus in neuroethological research. One important prerequisite for such studies is a clear understanding of the underlying neuronal architecture and localization of key proteins playing a role in neuronal plasticity and memory formation. Thus far, high-resolution information mainly comes from electron microscopy (EM) and confocal laserscanning microscopy. Both methods, however, have certain disadvantages: while EM in combination with immunogold labeling allows for precise protein detection in specific cell compartments, it clearly lacks possibilities for advanced colocalization analyzes. In contrast conventional confocal fluorescence microscopy offers the tools for multi-protein labeling, but lacks the high resolution of EM for subcellular detection levels. In the present study we applied and adapted, for the first time, array tomography for use in the brain of Hymenoptera (ants, bees). This rather new technique combines the advantages of two worlds by correlation of images taken from the same ultra-thin resin sections with high-resolution EM and fluorescence microscopy. We established a protocol using high-pressure freezing and LR-White embedding that preserves the tissue in a close to in-vivo status allowing for ultrastructural analyzes while leaving antigens intact. The obtained overlay of EM and fluorescent images of ultra-thin serial sectioned tissues provided high-resolution 3D models of synapses with the additional information of synaptic protein localization. This approach allowed us to push the limits of the so far achieved resolution in the analysis of pre- and postsynaptic structures and distribution of synaptic proteins (e.g. synapsin, pCaMKII) in the mushroom-body neuropil of the desert ant, *Cataglyphis fortis*. The method can be adapted for characterization of other neuropils and for other insect species. In future applications we will take advantage of the ultra-thin sectioned tissue by using super resolution microscopy like dSTORM instead of conventional fluorescent microscopy to achieve even higher resolution for protein localization in three dimensions. The combination of ultrastructural synapse morphology in combination with super-resolution protein localization will take neuroanatomical analyzes to new levels.

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How to efficiently organize and exploit metadata of complex electrophysiological experiments

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Technological progress in neuroscience allows recording from tens to hundreds of neurons simultaneously, both *in vitro* and *in vivo*, using various recording techniques (e.g., multi-electrode recordings) and stimulation methods (e.g., optogenetics). In addition, recordings can be performed under more or less natural conditions in (almost) freely behaving animals. Moreover, to disentangle the relationship to behavior, it is necessary to document animal training, experimental procedures, and details of the setup along with recorded neuronal and behavioral data. Consequently, electrophysiological experiments become increasingly complex. Given these various sources of complexity within an experiment, the availability of such information about the experiment, commonly referred to as metadata, is of extreme relevance for reproducible data analysis and correct interpretation of results.

Typically, experimenters have developed their own personal procedure to document their experiment, allowing at best other members of the lab to share data and metadata. However, at the latest when it comes to data sharing across labs, details may be missed. In particular if collaborating groups have different scientific backgrounds, implicit knowledge is often not communicated. In order to perform interpretable analysis of the data, each data set should therefore clearly link to metadata annotations about experimental conditions such as the performed task, quality of the data, or relevant preprocessing.

In order to provide metadata in an organized, human- and machine-readable way, an XML based file format, odML (open metadata Markup Language), was proposed [1]. We will here demonstrate the usefulness of odML for data handling and analysis in the context of a complex behavioral experiment with neuronal recordings from a large number of electrodes delivering massively parallel spike and LFP data [2]. We illustrate the conceptual design of an odML metadata structure and offer templates to facilitate the usage of odML across different laboratories and experimental contexts. In addition, we demonstrate hands-on the advantages of using odML to screen large numbers of data sets according to selection criteria relevant for subsequent analyses. Well organized metadata management is a key component to guarantee reproducibility of experiments and to track provenance of performed analyses.

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Hybrid voltage sensor imaging of eGFP-F expressing neurons in chicken midbrain slices

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Information processing in the brain relies on computation in single neurons and networks, which already starts at the level of dendrites [1, 2]. To understand cellular computation, the function and biophysical properties of cellular compartments like dendrites and spines has to be investigated at high spatial and temporal resolution. Genetic encoded voltage sensors [3, 4] are here most promising.
While such (opto-)genetic techniques are increasingly employed in mammalian neuroscience, they are nearly absent in avian neurophysiology. This is all the more surprising since genetic manipulations are broadly used in the study of avian embryonic development. Presumably, the lack of commercially available transgenic lines and adapted genetic constructs inflict a broader use of such techniques.
As an alternative technique, hybrid voltage-sensor (hVoS) systems allow optical recordings of neuronal activity at high spatial and temporal resolution without the need of genetic encoding of voltage sensors [5, 6]. A well-established hVoS consists of the molecule Dipicrylamine (DPA) and a membrane-bound green fluorescent probe i.e. GFP or DiO. The absorption spectrum of DPA partially overlaps with the emission of the probe, which leads to fluorescence quenching by FRET. The amount of quenching depends on the distance to the probe. Since DPA is shifted in the cellular membrane depending on the voltage, the amplitude of the fluorescence varies linearly with the membrane potential.
Here, we present data of the application of a hVoS system to the chicken midbrain [7]. We injected a plasmid encoding membrane-bound eGFP into the lumen of embryonic brain vesicles at embryonic day E2 followed by electroporation. This resulted in labelled neurons throughout the midbrain with a high level of structural detail at E21. Depending on the injection site, position of the electrodes, chosen promoter and age of the embryo, it is possible to achieve cell type specific expression of molecules at a particular location. By applying DPA, we were able to record neuronal activity with a high signal-to-noise ratio at high temporal and spatial resolution allowing subcellular analysis of neuronal activity.
We believe that hVoS imaging will become a major tool in avian neuroscience to perform multi-site recording of cellular compartments and study computational processes in single neurons. While it can be combined with genetic expression, single cell labelling with fluorescent membrane dyes, e.g. DiO by loose patch technique or retrograde labelling, is sufficient.

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Imaging of whole-mount samples with µm resolution using light-wedge- microscopy

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Imaging large objects with light-sheet microscopy is facing two major drawbacks: light sheets for a homogeneous illumination for millimeter-large fields of view are quite thick, resulting in a poor depth resolution of several micrometers(1, 2). This is about 5-10 times worse than the x-y resolution, making the overall data difficult to analyze and to display. The other disadvantage is a technical constrain: imaging deep inside large objects requires long working distances, both for the illumination- and for the imaging optics. These long working distances are very sensitive to aberrations due to refractive index mismatch, making the use of different embedding media(3-5) for increasing the transparency more difficult.

We addressed these problems by developing a custom optics for the generation of ultra-thin light-sheets that can be adapted to most of the refractive indices of the utilized embedding media. The illumination optics also includes a scanning technique that allows for placing the µm-thin focus line at any lateral position in the field of view, allowing for the acquisition of several light-sheet images with the thin illumination focus at different positions. These images can be combined to one overall image of high quality. In the imaging path, we compensated the image distortions due to aberration by a custom deconvolution.

These modifications of light-sheet microscopy allow for the generation of 3D fluorescence images in a different domain: It gathers data with an isotropic resolution of ~1µm in all 3 dimensions of objects with a cubic size of 2-3mm, not possible with other techniques. The micro-CT technique is imaging with a bit worse resolution and similar object size, but it does not allow for the use of fluorescent tags, nor is it suited for displaying non-absorbing substances like fat tissue. We show the advantages of this technique on image data cortex with YFP-labeled cells, as well as several insects (e.g. Drosophila melanogaster, and Zorotypus weideneri).

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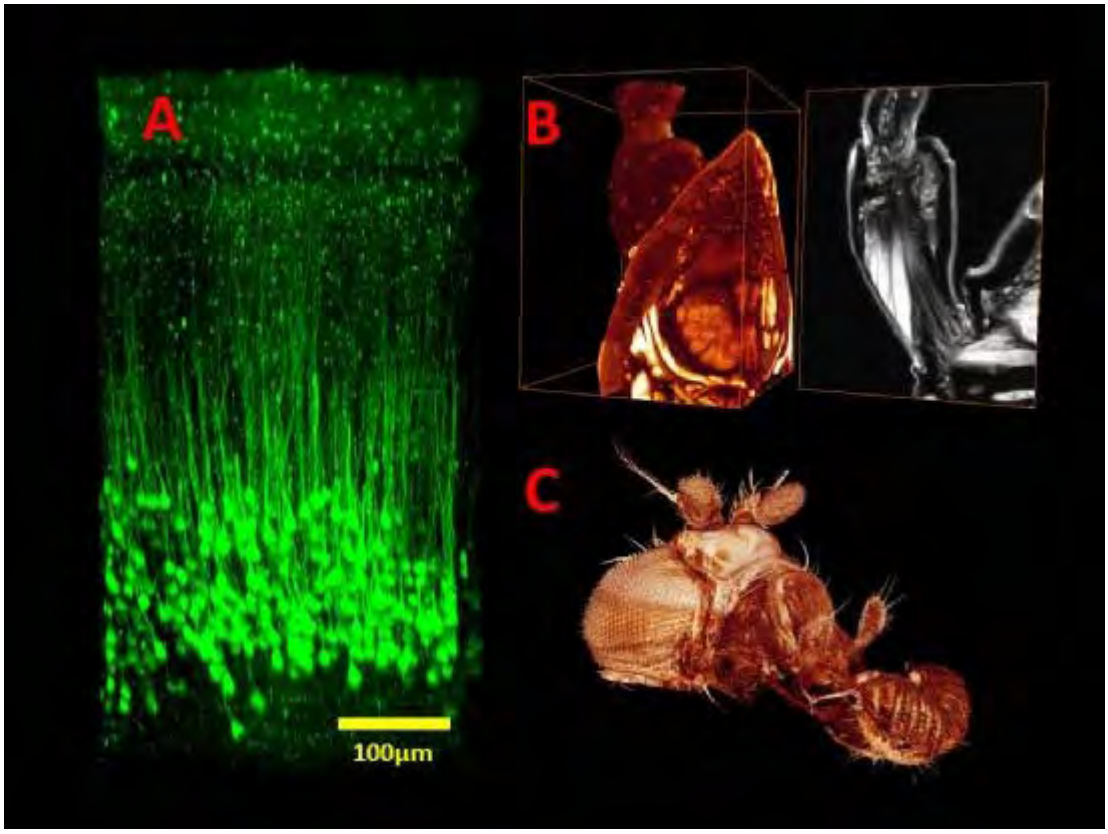
Figure 1: Images from this light-wedge microscopy. A: Cortex of a mouse with YFP-labeled cells. B: Antennal connection of the insect Zoraptera weideneri with olfactory glomeruli (160x210 µm). C: Head of Drosophila melanogaster (after deconvolution, length: 1100µm)


A
B
C
100µm

Implantable, yet adaptive multi-electrode positioning system for brain computer interface applications

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Chronically implanted intracortical multi electrode arrays provide opportunity for recording in non-human primates from many neurons simultaneously and for a prolonged amount of time. However, typically the transduced neuronal signal quality progressively degrades due to local tissue response at the electrode tips or mechanical implant deterioration (Barrese JC et al. 2013) which ultimately leads to an implant failure. When none or very few spikes are detectable, such implant would no longer be suited to control a neuroprosthetic device and re-implantation is normally not possible. Also, the most commonly used fixed-geometry implants are restricted to recording in surface brain areas, due to length constraints of the electrodes.

On the other hand, acute recordings with micro-drivable electrode matrixes guarantee a high signal-to-noise ratio which can be recovered after signal deterioration by re-positioning electrodes and typically cover a large depth-range. However, these systems do not usually allow to record from as many channels simultaneously and typically cannot be implanted for prosthetic control or long-term studies. Manually adjustable systems with high channel count allow a certain degree of adjustment but are time-consuming, less precise in positioning, and require repeated interaction with the implant-holding animal (Gray CM et al. 2007).

Here we present an implantable micro-controlled 16-channel adaptive multi-electrode positioning (AMEP) system. The system consists of two parts. An implantable array contains 16 glass-insulated tungsten-iridium microwire electrodes, which can be individually and bi-directionally adjusted in depth over a range of approximately 10mm, and the microelectronics for signal preprocessing EM-shielded in a metal chassis. The second part of the AMEP consists of a non-implantable xyz-micro-robot for repositioning the electrodes in depth attachable to the chronic implant.

In first tests, we were able to record from many neurons simultaneously at different depths (from 4mm to 6mm below dura) in the cerebral cortex of a behaving rhesus monkey. The AMEP system allowed recording of local field potential and single units from ensemble of neurons with high signal quality while being able to reposition the electrodes through the software-controlled robot. The AMEP system allowed us optimizing the signal quality for each channel. One can thereby compensate for potential signal non-stationarity either by manual software-guided control or with the help of autonomous control algorithms (Chakrabarti et al. 2011).

In conclusion, the AMEP system proofed highly suitable for acute daily recordings with currently 16 electrodes. Importantly, the AMEP can also stay implanted for a prolonged period of time thereby paving the way towards semi-chronic or chronic recordings with movable electrode arrays particularly suited for brain computer interface applications involving long-term neuro-prosthetic control.

Long-term decoding of continuous and discrete movement parameters with a wireless myoelectric implant

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Myoelectric activity can be recorded through surface electrodes, placed on the skin above the muscles of interest. These signals can be used for a variety of medical and biomedical applications. However, cross-talk limits the capacity to pick up signals from small or deep muscles [1], such as the finger muscles in the lower arm, which currently limits the degrees of freedom of myoelectric hand prostheses. Moreover, other factors limit the usefulness of surface electromyograms: sweating modifies electrode impedance, and movements change the electrode location relative to the muscles. These aspects particularly impair the reliability of EMG-based prosthetic devices, which require long-term stability of the conditioned signal. Conversely, intramuscular signals are more reliable, but require acute recording techniques that are typically unsuited for prosthetic use.

Here we show that signals recorded from a fully implanted, induction-powered wireless system allow decoding continuous as well as discrete movement parameters, including force. This implant, developed in the context of the Myoplant project [2], transmits up to four channels of EMG activity, using bipolar epimysial electrodes. Two Rhesus macaques received implants. Electrodes were placed in the upper arm (biceps, triceps, and over two fiber groups of the deltoid).

One of the monkeys was trained to do a cursor task via a haptic robot, which allowed us to control the forces exerted to/by the animal during arm movements. We used an artificial neural network (ANN) to decode time-varying force profiles from multiple features extracted from the raw EMG signals. The decoding performance was higher with the implanted system than with standard surface EMG recordings conducted simultaneously over the same muscles. Importantly, data from the implant allowed a decoder trained with data from a single day to maintain an accurate decoding performance during the following weeks, which was not the case for concurrent surface recordings.

The second animal was trained to do a center-out reaching task on a touchscreen, toward eight directions. Here, the discrete reach directions were classified using a GLM network trained on principal components extracted from the profiles of the EMG features during the movements. The performance of the decoder in this case was also higher using signals from the implant than from surface electrodes.

Results show that a fully implantable, intramuscular wireless system is particularly suited for demanding applications such as advanced forelimb prosthetic devices with multiple degrees of freedom.

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Modelling Biological Signals with BAYSIG

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This study aims to develop dynamical and statistical models for Electrogardiogram (ECG) recordings based on stochastic differential equations, and to use Bayesian inference to estimate parameters from real data. The study presents and utilises a reduced version of an established phenomenological ECG model [1] with added process noise from a Wiener process. Our general approach can be generalised to a wide range of time series data in neuroscience, biology and even in finance. One aim is to assess the differences in the estimated parameters between healthy subjects and patients with abnormal cardiovascular conditions. The study utilises a database of physiological recordings held by the Department of Cardiovascular Sciences and the Diagnostic Development Unit in the University of Leicester and uses the BAYSIG language for simulation and parameter estimation. Baysig code can be written and executed online by using BayesHive (http://www.bayeshive.com/).

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Neuroinformatics for efficient data management and reproducibility in electrophysiology

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Scientific progress depends increasingly on collaborative efforts that involve exchange of data and re-analysis of previously recorded data. The German Neuroinformatics Node (G-Node, www.g-node.org) aims at solutions for efficiently managing, accessing, and sharing electrophysiological data and metadata, so that the researcher can focus on the scientific questions rather than on problems of data management.

Here we present a suite of methods and tools to support data management across the scientific data workflow from acquisition and storage to analysis, collaborative data exchange, and publication. The variety of data formats is a challenge in neurophysiology, and the Neuroshare API (neuroshare.org) is an attempt to unify data access. The G-Node Neuroshare Tools provide Python utilities to access data in proprietary formats on various platforms [1]. To overcome the limitations of the Neuroshare standard in the long term, in the context of the Standards for Data Sharing Program of the International Neuroinformatics Coordinating Facility (INCF) we are developing a common data format capable of integrating various types of neuroscientific data and their metadata [2]. Efficient data selection for analysis requires that all metadata describing the experimental conditions are available with the data. The odML metadata format facilitates automated metadata capture and management and enables consistent data annotation [3]. odML tools include libraries, a graphical editor, and an app for mobile devices for convenient metadata entry during the experiment [4]. The G-Node GNData platform [5] achieves integration of neurophysiological data and metadata by combining data representation according to the NEO data model [6] with odML metadata, for efficient data organization, search and sharing. Client tools for popular frameworks (Matlab, Python) enable seamless integration with the scientist's data analysis [7].

The G-Node data sharing services enable researchers to access their data from anywhere in the world and to share data with colleagues or the public. In addition, G-Node supports scientists in making resources and data available, by hosting databases, services, and published data. Short courses offered on a regular basis contribute to training in neuroinformatics.

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Obscured artifacts in multi-electrode recordings and their influence on correlation analysis

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Many modern electrophysiological experiments focus on collecting an increasing amount of data by recording at high time resolution with large numbers of electrodes simultaneously. Therefore, commercial full package recording and data acquisition systems are often used, that provide the experimentalist with a compact data set, thus hiding the complexity of the system and any intermediate pre-preprocessing steps. This amounts to increasingly convoluted data that finally enter analysis to elucidate mechanisms of neuronal processes.

We investigated precise spike correlations in massively parallel cortical single-unit spike data recorded by a multi-electrode array implanted in motor cortex of awake behaving monkeys. Using higher-order correlation analysis methods we detected spurious synchrony of high order on a high time resolution (30kHz). These 'synchrofacts' occur with varying numbers of contributing spikes, including events of few synchronous spikes only. The occurrence probabilities of such highly precise events are typically higher than expected based on the firing rates, whereas an investigation with larger bin sizes of typical neuronal synchronization does not yield abundant coincidence counts (see population spike count histogram in Figure). Moreover, a biological origin of synchrofacts is unlikely, as cortical neurons seem not to have the physical ability to synchronize on such a short time scale that is about 2 orders of magnitude smaller than the spike duration. Despite their high precision, many synchrofacts are yet imprecise enough to evade classification as artifacts by various algorithms applied during the built-in preprocessing of the recording and spike sorting systems. Other synchrofacts go undetected due to intrinsic incompatibilities between recording system and the spike sorting systems. Therefore, a substantial fraction of synchrofacts typically remains undetected.

Next we investigated the raw electrode signals (at 30kHz sampling, wide band) that give rise to synchrofacts. When correlating sorted spikes of one electrode to the raw signals (spike triggered averages) at different electrodes we often observe a synchronized strong spike-related component across large portions of the array (up to 4 mm). We hypothesized that synchrofacts should be visible as a coherent signal deflection in the raw signal. When investigating the raw signal at the time of occurrence of each individual synchrofact, indeed some synchrofacts are found to reflect small, spike-like, coherent deflections. However, a part of the synchrofacts could not be related to such obvious coherent deviations in many channels, but were identified to relate to coherent signal fluctuations just above high pass cutoff frequency for spike extraction.

We demonstrate how the observed artifacts, though small in absolute number, may have a large impact on a population correlation analysis. Therefore, we investigate possible origins of the artifacts in our recording set-up and suggest approaches for artifact removal, including correction factors for correlation analyses to account for the removal process.

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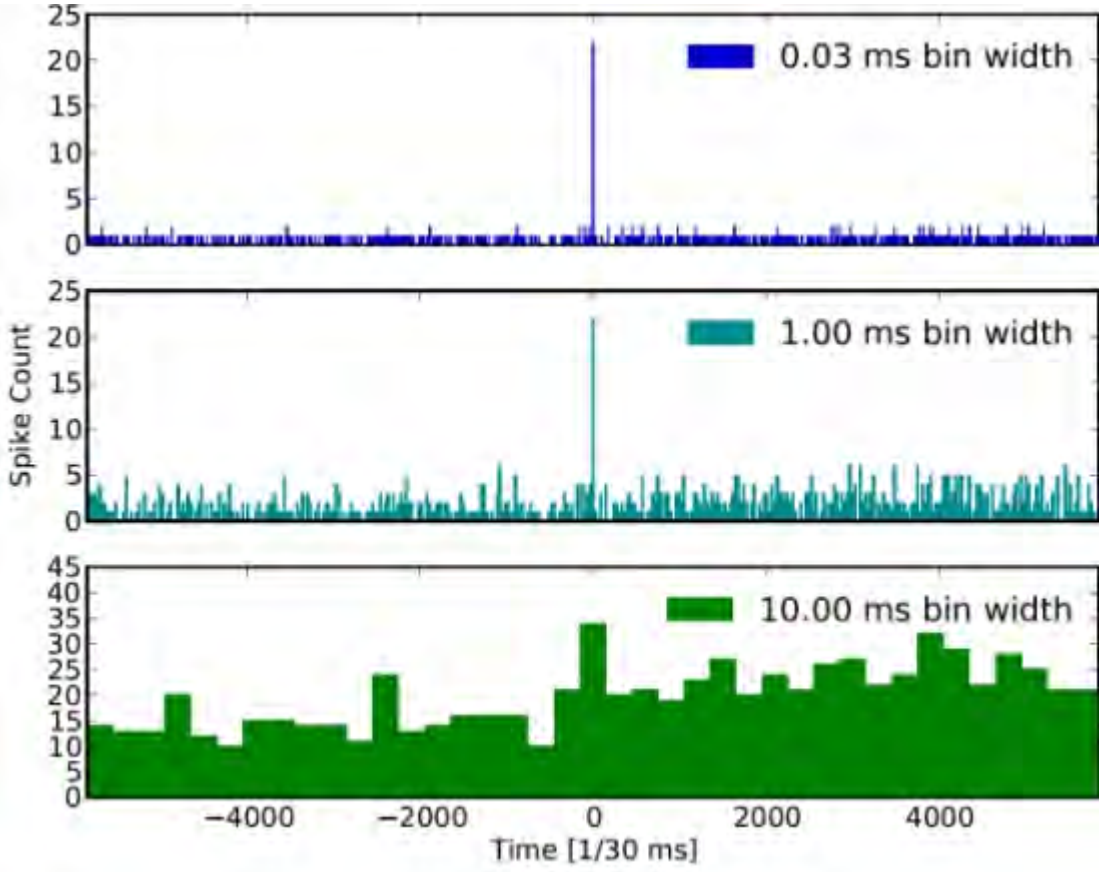

Photonic modulation of membrane potential by flash-light illumination of metallic beads.

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The electrical membrane potential (V_m) is a crucial parameter determining the physiological properties of cells – in particular of excitable cells. For studying cell function it is mandatory to manipulate V_m. Approaches involving genetically encoded proteins (optogenetics) are now widely used to manipulate V_m with optical means. Despite the success of such methods there are some shortcomings, such as the need to genetically modify cells or a limited time resolution, which ask for alternative/additional methods. Here we evaluated the use of transient illumination of metallic beads. We use superparamagnetic beads (polystyrene-coated iron-core beads, Dynabeads® 4.5 µm diameter, Life technologies AS, Oslo), targeted to the cell membrane by CD8-antibodies, to modulate V_m. Flash-light excitation via the epifluorescence channel of an inverted microscope (Xenon flash lamp, about 500 µs, $>10^{15}$ photon/m^2, up to 470 nm), was combined with whole-cell patch-clamp recordings. CD8 was expressed in HEK 293 cells. Flash illumination of a single cell-associated bead resulted in transient inward current of about 500 pA at -70 mV using a 40x objective; the waveform of the current reflected the time course of the excitation light. With a 100x objective inward currents of up to 20 nA were observed. Currents reversed at about 0 mV indicating a transient non-selective conductance. The current approximately doubled with two beads bound to the cell. Flash-light-induced currents decreased about exponentially with the distance to the cell membrane when the beads were placed using a separate patch pipette (half-width about 2-5 µm). Silver particles of approximately the same size resulted in similar signals. Measuring V_m of murine dorsal root ganglia neurons in the current-clamp mode, single-bead illumination was sufficient to elicit action potentials within 500 µs. Thus, transient illumination of superparamagnetic beads, targeted to specific cellular sites via antibody coating or direct placement, may provide means of rapid and spatially confined electrical cell stimulation.

Post-mortem magnetic resonance microscopy of the murine brain at 7 Tesla

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Small animal MRI with high field strength allows imaging of the living animal. However, the spatial resolution in in-vivo brain imaging is limited by the scanning time. Measurements of fixed mouse brains allow longer measurement time, but often introduces artifacts due to the fixation procedures. We present a quick and simple post-mortem approach without fixation that allows high-resolution MRI at 7 Tesla (T2-weighted MRI). The method was compared to in-vivo scans with optimized spatial resolution for the investigation of anaesthetized mice (T1-weighted MRI) as well as to ex-situ scans of fixed brains (T1- and T2-weighted scans) by using standard MRI-sequences, along with anatomic descriptions of areas observable in the MRI, analysis of tissue shrinkage and post-processing procedures. Post-mortem imaging quality was sufficient to determine small brain substructures on the morphological level. In addition, since no fixation was used, tissue shrinkage does not occur as it is, e.g., the case by using ex-vivo brains that have been kept in fixatives for several days. In summary, this post-mortem magnetic resonance imaging method results in a gain of resolution as compared to in-vivo MRI and is well-suited for comparative investigations, since it allows determining very quickly small structural alterations in the murine brain.

Simultaneous electrophysiological analysis of circadian rhythms of the circadian pacemaker center, of the electroretinogram, and of leg muscle activity in the cockroach *Rhyparobia maderae*

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The Madeira cockroach *Rhyparobia maderae* is an excellent insect model to study neural mechanisms underlying circadian rhythms in insects. Lesion and transplantation studies identified the accessory medulla (AME) ventro-laterally to the optic lobe´s medulla as circadian pacemaker center that controls circadian locomotor activity rhythms. It is still not well understood how the circadian network of the AME controls behavioral rhythms in response to dusk and dawn. To obtain information about mechanisms of gating and synchronization extracellular long-term recordings for up to 10 days of intact cockroaches were established. Stainless-steel electrodes were inserted into AME, compound eye, and leg muscles to search for synchronization between AME outputs to the eye (electroretinogram = ERG) and to leg muscles (electromyogram, EMG), respectively, in light-dark cycles and/or constant conditions. For comparison, locomotor activities were recorded as EMGs and with a video recording system. Moreover, the recording setup is supplemented by a microinjection system (i.e. Pneumatic PicoPump and micromanipulator) for automated series of neurotransmitter- and neuropeptide microinjection experiments to analyze the circadian network. We succeeded to record simultaneously neuronal activity of the AME, the EMG and ERG, combined with visual analysis of locomotor activity for several days. ERG amplitudes peaked during the subjective night in correlation with high locomotor activity. Preliminary data suggest that ensemble formation of pacemaker neurons in the AME gate and synchronize ERG- and EMG rhythms in intact cockroaches. [Supported by DFG grant STE531/18-1,2 and STE531/21-1 to MS]

Time-dependent cellular response profiling of an immortalized embryonic murine hippocampal cell-line (mHippoE-14) for the *in vitro* simulation of brain diseases

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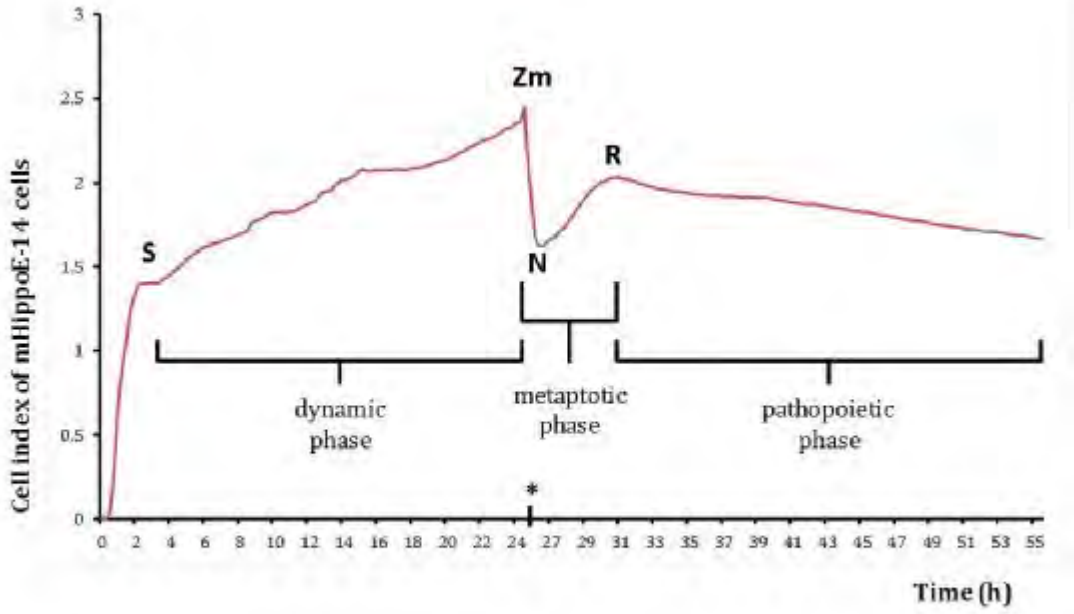
A new generation of embryonic murine hippocampal cell-lines has become commercially-available. These cell-lines will be of great neuroscientific interest as they have the advantage of being immortalized and maintaining distinctive phenotypes that closely resemble those of hippocampal neurons. These cells are ideal for the implementation of morphological batteries in neurotoxicological and/or high throughput drug-screening settings, but data regarding their *in vitro* characterization are still very limited. We herein present our findings regarding mHippoE-14 cells and their real-time cellular proliferation via the use of xCELLigence technology (that monitors cellular events without the incorporation of labels, by measuring electrical impedance across interdigitated micro-electrodes integrated on the bottom of tissue culture plates), in the presence and absence of foetal bovine serum (FBS). Our data provide a representative view of the xCELLigence information generated by the seeding of mHippoE-14 at an initial density of 7.5 x 10^3 cells/well (see provided Figure), and have required the introduction of the suggestive terms employed in order to define the real-time cellular response phases that these cells undergo under different conditions (presence and absence of FBS). Point “S” identifies the end of the settling period and the beginning of the “dynamic phase” (where mHippoE-14 have attached to the bottom of the well and start to proliferate in the presence of FBS). Point “Zm” identifies the replacement of the media with an FBS-deprived one and the beginning of the “metaptotic phase” (a phase where cells recover / adapt to the new conditions and where neuropathological or other assessments should be avoided). Finally, point “R” indicates the end of the “metaptotic phase” and the entry to the “pathopoietic phase” (a phase believed to represent a relatively steady-state form of the mHippoE-14 cells that more reliably represents the neuronal state in clinical reality). The asterisk (*) indicates the lost time needed for the FBS-removal and other treatments. The highly-reproducible xCELLigence data generated by the seeding of mHippoE-14 at an initial density of 7.5 x 10^3 cells/well have generated a number of further important observations of technical interest (equations and grey zones of evaluations) that could allow for a more reliable implementation of the specific cell-line in *in vitro* neurotoxicity and drug-screening assessment.


Zm
R
S
N
dynamic
phase
metaptotic
phase
pathopoietic
phase
*
Cell index of mHippoE-14 cells
Time (h)
0
0.5
1
1.5
2
2.5
3
0 2 4 6 8 10 12 14 16 18 20 22 24 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55

Towards safe optogenetics with grafted cell implants: An alternative method to the direct virus delivery of the opsins

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Optogenetic methods rely on the viral transduction of neurons leading to expression of transmembrane light sensitive ion channels. The novel ion channels, or opsins, build a new conductance through the membrane and permit the modulation of the neuron‘s excitability by light, and not only by electrical stimulation. This method offers a new, selective approach to study the function of neural circuits and has already accelerated our knowledge in many areas. However, as opsin expression in living brains requires viral infection, and given that there are yet no FDA approved viral gene-therapies, it is likely that this therapy will take some time to find its way into the clinic (Chow and Boyden, 2013).
The current study proposes an alternative strategy: not to inject an opsin-carrying virus into an animal‘s brain, but instead to transplant cells into target in vivo regions that have been transduced outside the brain prior to transplantation. This way, the risk of an unforeseen follow-up infection with free virus particles can be minimized and an extremely high level of specificity in cell types can be achieved as the grafted neurons can also integrate functionally under certain conditions.
Primary ventral mesencephalic (VM) tissue was dissected out from E14 rat embryos, prepared as a single cell suspension and transduced in vitro with Channel-rhodopsin-2 using a lentivirus carrying the plasmid EF1a-hChR2(H134R)-EYFP-WPRE. The cells were incubated with the virus particles for 6 hours in a 3:1 ratio which was shown to be a compromise between optimizing cell viability and efficacy of transduction. Transduced cells were used in two ways. Firstly, a proportion of the cells were plated in tissue culture for in vitro characterization of the opsin expression. Secondly, cells were also transplanted stereotactically into an animal model of Parkinson’s disease according to standard protocol and changes in the light induced electrical activity of the grafts were assessed 6 weeks post-transplantation.
The study identified the optimal parameters by which primary VM dopaminergic neurons can be transduced in vitro and prepared for transplantation. The grafts survived for at least 12 weeks, and the transduced cells continued expressing the reporter gene. To test in vivo functionality, the optimal recording/ light stimulation parameters are currently being identified and the data will be discussed at the German Neuroscience Meeting in Göttingen.

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